ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *ESCHERICHIA COLI* ISOLATED FROM URINARY TRACT INFECTED PATIENTS ATTENDING BIR HOSPITAL

A

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RECOMMENDATION

This is to certify that Mr. Amit Raj Sharma has completed this dissertation work entitled **"Antimicrobial susceptibility pattern of *Escherichia coli* isolated from urinary tract infected patients attending Bir Hospital"** as a partial fulfillment of the requirements M. Sc. degree in Microbiology (Medical) under our supervision. To our knowledge this work has not been submitted for any other degree.

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ABSTRACT

Antibiotic resistance among uropathogens is emerging public health problem. Bacteria may be innately resistant or may acquire resistance to antibiotics. Culture and antibiotics susceptibility testing of urine is important tool for diagnosis of infection and monitoring antibiotic resistance pattern of uropathogen. The objective of this study was to assess antibiotic resistance pattern of *Escherichia coli* (*E. coli*) and to describe MDR pattern of *E. coli*. This study was conducted in Bir Hospital, Kathmandu, among suspected UTI patients during January to March, 2011. Altogether, 739 urine samples were analyzed by semi-quantitative culture method and uropathogens were identified by conventional methods. A total of 109 *E. coli* were tested for antimicrobial susceptibility by Kirby Bauer disc diffusion method as per CLSI (Clinical and Laboratory Standards Institute) guidelines. Out of 739 samples, 27.3% gave significant growth while 3.1% and 29.2% samples gave mixed and non-significant growth, respectively. The distribution of UTI is the most common among age group 16-49 years. *E. coli* was found to be most predominant isolate (54.0%) followed by Cogulase negative Staphylococci (CoNS) (21.3%) and *Enterococcus fecalis* (7.3%). Nitrofurantoin was found to be the most effective antibiotic followed by ciprofloxacin and ofloxacin while cephalexin was least effective. Out of 109 *E. coli* isolates, 90.8% were MDR strains. *E. coli* showed higher rate of resistance towards commony used oral antibiotics. However, nitrofurantoin still active against *E. coli*. Thus, nitrofurantoin could be the choice for empirical therapy of UTI.

Key words: Urinary tract infection, *Escherichia coli*, antimicrobial susceptibility, multidrug resistant

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LIST OF ABBREVIATIONS

AMPs	Antimicrobial Peptides
CLSI	Clinical and Laboratory Standards Institute
CNF-1	Cytotoxic Necotrizing Factor-1
CoNS	Coagulase Negative Stapylococcci
EPEC	Enteropathogenic *E. coli*
ExPEC	Extraintestinal Pathogenic *E. coli*
HlyA	Alpha Hemolysin
IDAS	Infectious Diseases Society of America
MAR	Multiple Antibiotic Resistance
MDR	Multidrug Resistance
THP	Tam-Horsfall Protein
UPEC	Uropathogenic *E. coli*
UTI	Urinary Tract Infection

CHAPTER-I
INTRODUCTION AND OBJECTIVES

1.1 Background

Urinary tract infections (UTIs) are one of the most common infectious disease ranking next to upper respiratory tract infection (Ramesh et al., 2008; Hryniewicz et al., 2001). UTIs are often associated with significant morbidity and mortality (Ramesh et al., 2008; Hryniewicz et al., 2001; Raksha et al., 2003). Worldwide, about 150 million people are diagnosed with urinary tract infection each year, costing the global economy in excess of 6 billion dollars (Gonzalez and Schaeffer, 1999). Urinary tract infections occurs in every age and in both sexes, however it is more common in female due to short urethra and its closeness to the anus, absence of prostatic secretion. Also, sexual intercourse increases the chances of contamination of female urethra by fecal flora (Adedeji and Adbulkadir, 2009; Awaness et al., 2000).

The Enterobacteriaceae are the most frequently detected pathogen causing 84.3% of UTI (Wada et al., 2009). *E. coli* is the most common cause of UTI other organisms reported include member of the family Enterobacteriaceae (i.e., *Proteus*, *Klebsiella*, *Enterobacter* and *Citrobacter* spp.), *Pseudomonas* spp., *Enterococcus* spp., Streptococci, Staphylococci and *Candida albicans* (Ludwig, 2000). *Candida* is an increasing nosocomial problem; however, isolation of yeast from urine does not necessarily always indicate infection (Fidel et al., 1999).

E. coli, the most common member of the family Enterobacteriaceae, accounts for 75.0-90.0% of all UTIs in both inpatients and outpatients (Dromigny et al., 2005). *Escherichia coli* present in the gastrointestinal tract as a commensal provide the pool for initiation of UTI and certain serotypes of *E. coli* responsible for uropathogenicity were traditionally designated as Uropathogenic *E. coli* (UPEC) (Raksha et al., 2003). *E. coli* is predominant facultative aerobes of the human colinic microflora. Most *E. coli* strains are harmless to humans, but pathogenic strains cause gastroenterititis, urinary tract infection (UTI) and neonatal meningitis and rare cases, hemolytic uremic syndrome (HUS), peritonitis, mastitis, septicemia and gram negative pneumonia (Todar, 2012).

UTI has become the most common hospital acquired infection, accounting for as many as 35.0% of nosocomial infections, and they are the second most common cause of

bacteremia in hospitalized patient (Stamm, 2002; Weinstein et al., 1997). Nosocomial urinary tract infection usually associated with catheterization. The infection would be even more common but for the use of catheter system.

UTI is a serious problem for women and up to a third of all women experience UTI at some point in their life. If left untreated it may lead to pyelonephritis, preterm labor or group B streptococcal infection in the new born (Morgan, 2004). UTIs have a propensity to recur (Foxman, 2010). Up to 25.0% of women who present with an acute UTI will have recurrence within six months, despite receiving appropriate antibiotic treatment (Schilling et al., 2002). The recurrence of a UTI may be caused by several reasons. Unresolved bacteriuria is the most commonly caused by inadequate antimicrobial therapy. Sub–therapeutic levels of the antimicrobial agents may be a result of noncompliance, malabsorption, suboptimal drug metabolism, and resistant uropathgens unresponsive to attempted therapy (Pawitt and Schaeffer, 1997). Almost 10.0% of the pregnant women suffer from UTI (Bear, 1976).

Although UTI is infrequently associated with mortality, it is still a significant cause for morbidity. Delay in the treatment of UTI can lead to vesicoureteral reflux and renal scarring. Renal scarring has been cited as one of the most common causes of end stage renal disease in both adults and children (De Leon, 1997).

The cause of urinary tract infection are related to poor perianal hygiene, sexual intercourse pregnancy, urinary tract obstruction, urethral reflux, catheterization, instrumentation and neurogenical bladder but in many instance the pathogenesis is equivalence (Nahar et al., 2010).

Majority of UTIs are not life threating and do not cause any irreversible damage. However, when the kidneys are involved there is a risk of irreparable tissue damage with an increased risk of bacteremia (Hvidberg et al., 2000). UTI is the second leading cause of antibiotic consumption in the community (Kunin, 1994; Hooton and Stamm, 1997; Raz et al., 2000). About 15.0% of all antibiotic prescriptions are for the management of UTI (Mazzuli, 2001). Generally, UTI are treated empirically with antibiotic and laboratory testing was performed only when empirical treatment fails (Alos et al., 2004). The Infectious Diseases Society of America (IDAS) guidelines recommend therapy with co-

trimoxazole in setting where the prevalence of resistance is less than 10.0-20.0%, alternative therapy for uncomplicated UTI in setting with more than 10.0-20.0% resistance to co-trimoxazole may include a fluoroquinolone, nitrofurantoin and fosfomycin (Warran et al., 1999). Co-trimoxazole has been used widely as empirical therapy for UTIs caused by *E. coli*. However, resistance to co-trimoxazole has been increased with a prevalence of resistance which is reported 30 to 50 percent (Gupta et al., 2001). Ciprofloxacin belong to an important class of antibiotic for treatment of UTIs, it has been more active against *E. coli* strains other than commonly used agents such as co-trimoxazole and ampicillin (Moniri et al., 2003; Gupta et al., 1999). Resistance to co-trimoxaxole is generally associated with the resistance to ampicillin, cepatholin and tetracycline (Moniri et al., 2003).

The world health organization (WHO) has called antibiotic resistance an emerging disease. Bacteria may be innately resistant or may acquire resistance to antibiotics. The rapid spread of bacterial resistance to antimicrobial agents has led to the search for newer and more potent drugs. However, as soon as new drug appear, irrational use and abuse of them cause bacterial resistance (Omigieet et al., 2009). The emergence of antibiotic resistance in the management of UTIs is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance, and poor hygienic practices, there is also high prevalence of fake and spurious drug of questionable quality in circulation (Manikandan et al., 2011). The development of resistance to older agents such as ampicillin and co-trimoxazole, as well as the emerging problem of fluoroquinolone resistance may substantially limit our antibiotic choices (Karlowsky et al., 2002). Multidrug resistance (MDR) bacteria refer to those which are resistance to a vast range of antibiotics with structural independence (at least two or more antibiotics) (CDC, 2006). Nowadays, a big concern among the medical and clinical practitioners is the emerging MDR organism and their associated complications in developing world (Guyot et al., 1999). These conditions make the treatment more challenging and many even threaten the respective patients lives (Farshad et al., 2010b).

UTI is a common disease aliment among Nepalese population as well as one of the commonest nosocomial infection (Kattel et al., 2008). According to the annual report of fiscal year (2059/2060) published by the department of health services, morbidity of UTI in Nepal was 125,058. For the appropriate treatment of UTI, culture and sensitivity test is

essential although for most of the part of Nepal this facility in not available. In Nepal, there is limited information available on the prevalence and antibiotic susceptibility pattern of *E. coli* associated urinary tract infection. The present study was conducted to determine the prevalence and antibiotic susceptibility pattern of *E. coli* associated urinary tract infection among inpatients and outpatients attending Bir Hospital, Kathmandu, Nepal. The information will be useful for clinician and health care provider for treatment of *E. coli* associated urinary tract infection.

1.2 Objectives

1.2.1 General objective

To assess the antimicrobial resistance pattern of *E. coli* isolated from suspected urinary tract infection patients (inpatients and outpatients) attending to the Bir Hospital, Kathmandu, Nepal.

1.2.2 Specific objectives

a) To determine the prevalence of *E. coli* among suspected urinary tract infection patient (inpatients and outpatients).

b) To describe the demographic characteristics of UTI patients infected with *E. coli* associated UTI.

c) To describe the antimicrobial susceptibility pattern of *E. coli* to the commonly used antimicrobial agents.

CHAPTER-II
LITERATURE REVIEW

2.1 Urinary Tract Infection (UTI)

UTI is colonization of a pathogen occurring anywhere along the urinary tract: kidney, ureter, bladder, and urethra (Chang and Shortiffe, 2006). UTI is a condition in which bacteria are established and multiplying within urinary tract (Najar et al., 2009).

UTIs refer to the presence of microbial pathogens within the urinary tract and usually classified by the infection site: bladder (cystitis), Kidney (pyelonephritis) or urine (bacteriuria) and also can be asymptomatic or symptomatic. UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as “uncomplicated” whereas “complicated” infections are diagnosed in genitourinary tract that have structural or functional abnormalities, including instrumentation such as indwelling urethral catheters and are frequently asymptomaic (Gonzalez and Schaeffer, 1999; Stamn and Hooton, 1993).

2.2 Etiological Agents of UTI

Bacteria are the principal causative agents responsible for UTIs, accounts for more than 95.0% cases of UTI, although minor cases of UTI are caused by fungi and viruses (Bonadio et al., 2001). The causative agent varies based on age and associated morbidities (Chang and Shortliffe, 2006). Many different organisms can infect urinary tract, but by for most common agents are gram negative bacilli (Wilson and Gaido, 2004). The bacteriology of UTIs is very predictable. Although a number of different species can cause UTI, the majority of infections in all populations are caused by the gram negative facultative anaerobic, uropathogenic *E. coli* (UPEC) (Foxman, 2010).

For many years, pathogens associated with uncomplicated UTI have remained constant with *E. coli* identified as aetiologic agent in about 75.0 to 90.0% of infections (Nakhjavani et al., 2007; Omigie et al., 2009).

The remaining gram negative urinary pathogens are other Enterobacteria, especially, *Klebsiella* spp., *Proteus mirabilis* and *Pseudomonas aeruginosa*. Enterococci and

coagulase negative Staphylococci (CoNS) (e.g., *Staphylococcus saprophyticus*) are the most frequently implicated gram positive organisms (Shankel, 2007).

Escherichia coli is the principal organism responsible for nosocomial UTI; however, other gram-negative pathogens including *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp. and urease producing *Klebsiella* spp., *Proteus* spp., *Corynebacterium urealyticum*, and *Providencia* spp. are also involved (Wagenlehner et al., 2008). They are commonly involved in nosocomial UTI due to the inability of antibiotic to penetrate into the biofilm formed around and within the infectious stones (Marcus et al., 2008). Also, gram positive bacteria, including *Enterococus* spp. and *Staphylococcus* spp. can cause nosocomial UTI due to selective pressure from the antimicrobial agents used in hospitalized patients. Anaerobic bacteria are also described in UTI, but their role is not well-defined (Kauffman et al., 2000).

Klebsiella pneumoniae is the second leading cause of gram negative UTI but is a much less prevalent etiologic agent than UPEC (Rosen et al., 2008).

Staphylococcus saprophyticus is common causative agent of UTI in young women (Minardi et al., 2011). It is reported to colonize the rectum and, to lesser extent, the cervix and urethra in small proportion of women (Rupp et al., 1992). It is frequently causes lower UTIs and has been isolated in 3.0% of non-pregnant, sexually active, reproductive aged women with pyelonephritis (Scholes et al., 2005). *Staphylococcus sapropyticus* is notable uropathogen without involvement of indwelling catheter, whereas as two other Staphylococci (*S. aureus* and *S.epidermidis*) are often clinically isolated from hospitalized patients who have indwelling catheter rather than from outpatients (Von Eiff et al., 2002).

Proteus mirabilis is a common cause of UTI in individual with long term urinary tract catheters in place or individuals with complicated urinary tracts. *Proteus mirabilis*, despite its antibiotics sensitivity, can be difficult to clear by antibiotic treatment. It has been hypothesized that bacteria with in a stone matrix are protected from antibiotic treatment (Li et al., 2002).

Enterococci is usually associated with complicated UTI, in patients with indwelling urethral catheter or in patients receiving broad spectrum antibiotics for another infection (Dimitrov et al., 2004).

The UTI causing micro-organism in young men are similar to the organisms that cause uncomplicated infections in women. Enterococci and coagulase negative Staphylococci (CoNS) more common in elderly men, most likely representing recent instrumentation or catheterization (Najer et al., 2009).

The majority of fungal UTI, caused by *Candida* spp., and to a lesser extent by *Aspergillus* spp. and *Cryptococcus neoformans* (Minardi et al., 2011). In one study, positivity for *Candida* spp. was found in 5.0% of urine speciemens from a general hospital and in 10.0% from tertiary level care center (Rivett et al., 1986). Most UTI caused by *Candida* spp. are associated with the use of fole catheters, internal stents, and percuataneous nephrostomy drains and diebetics are particulary prone to fungal UTI (Minardi et al., 2011). Diabetics are particulary prone to fungal UTI (Sobel and Vazquer, 1999).

Cathter associated UTIs are usually caused by microorganism derived from fecal flora of native patient or originate in the hospital environment. *E. coli*, *Enterococcus* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Candida albicans* are usually associated with cathter associated UTIs (Emoris and Gaynes, 1993).

Escherichia coli

The scientific history of *E. coli* started with its first description in 1885 by Thedor Von Escherich, a pediatrician and scientist, who in a series of pioneering studies of the intestinal flora of infants discovered a normal microbial inhabitant of healthy individuals that he named *Bacterium coli commune*. It actual name became used and officially accept in 1956, in his honor (Gross and Rowe, 1985; Escherich, 1988; Kuhert et al., 2000).

The species of *E. coli* comprises gram negative, rod shaped, non spore forming, motile bacteria which are about 2 µm long and 0.6 µm in diameter, with a cell volume of 0.6-0.7 μm^3 (Darton et al., 2007; Kubistscheek, 1990). They are facultative anaerobes, oxidase negative, glucose, lactose and sucrose fermenting, with a optimum growth pH of

6.0-7.0 and temperature of 37^{o}C. However, some laboratory strains can multiply at temperature upto 49^{o}C (Fortadar et al., 2005).

Phylogenetic analysis showed that most *E. coli* strains fall into 4 phylogenetic groups, designated A, B1, B2 and D (Herzer et al., 1990). *E. coli* strains that cause extra intestinal infections derived prominently from group B2 and to a lesser extent, group D. Strains of group A and B1 represent most commensal strains and largely devoid of virulence determinants (Picard et al., 1999). The intestinal pathogenic strains are usually assigned to group A, B1 and D (Pupo et al., 1997).

The pathogenic strains are broadly classified as either diarrheagenic *E. coli* or Extraintestinal pathogenic *E. coli* (ExPEC) (Kaper et al., 2004; Russo and Johnson, 2000). Within each of these broad groups are sets of strains known as pathotypes that share common virulence factors and elicit similar pathogenic outcomes (Marrs et al., 2005).

E. coli that causes disease of the intestinal tract is referred as diarrheogenic *E. coli* (Nataro and Kapaer, 1998). Diarrhaeogenic *E. coli* strains rarely translocate the gastrointestinal epithelium and their pathogenic affect is mostly restricted to pathophysiological changes of the intestinal cells. The major pathotypes of diarrhhoegenic *E. coli* are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAggEC) , and Diffusely adherent *E. coli* (DAEC) (Natro and Kapper et al., 1998). Recently EPEC has been divided in typical EPEC (t-EPEC) and atypical EPEC (a-EPEC) (Trabulsi et al., 2002). Several pathotypes of diarrheagenic *E. coli* give rise to gastroenteritis, but rarely cause disease outside the intestinal tract (Wiles et al., 2008).

ExPEC have maintained the ability to exist in the gut without consequence, but have the capacity to disseminate and colonize other host niches including the blood, central nervous system, and urinary tract, resulting in disease (Wiles et al., 2008). ExPEC more frequent includes the strains associated with urinary tract infections (UPEC), neonatal meningitis (MAEC), and bacteremia (Campos et al., 2004). Among ExPEC, strains of uropathogenic *E. coli* (UPEC) are most commonly associated with human disease (Wiles et al., 2008). These bacteria are primarily cause of community acquired urinary tract

infections (UTIs) (75.0-95.0%) and a large proportion of nosocomial urinary tract infections (UTIs) (50.0%), accounting for substantial medical costs and morbidity worldwide (Foxman and Brown, 2003).

UPEC are different from the *E. coli* strains that normally inhabit the gastrointestinal tract in that they are better adapted to living with in the urinary tract and evading the host's immune response (Foxman, 2010).

UPEC serotypes

Traditional classification of *E. coli* strains is based on the presence of certain O (somatic), K (capsular polysaccharide), and H (flagellar) antigens (Wiles et al., 2008).

The O antigen, which defines >176 serogroups, is a polysaccharide consisting of approximately 10-25 repeating sugar subunits anchored in the outer core of the lipopolysaccharide component of the bacterial membrane (Stenutz et al., 2006).

Among the various serotype of *E. coli*, 01, 02, 04, 06, 07, 08, 016, 018, 025, and 075 are commonly recovered from patients with UTI (Yamamoto et al., 1997). However, specific K and H antigens have a less defined pattern (Lloyd et al., 2007). This O antigen trend is reiterated with the sequenced UPEC isolates CFT073 (O6), 536 (O6), UTI89 (O18), as well as with two other often used UPEC strains, J96 (O4) and F11 (O6) (Wiles et al., 2008).

The K1 capsular antigen is typically associated with ExPEC strains that caused neonatal meningitis (Wiles et al., 2008). However, there is no clear evidence for the involvement of specific K antigen in UPEC pathogenesis, it has been noted that UPEC isolates bearing K1 or O18 antigen encode more virulence associated factors than other ExPEC isolates (Ewers et al., 2007).

2.3 Epidemiology of UTI

The incidence of UTIs differs greatly between the sexes, with infection occurring fourteen times more often in females than males (Presscott et al., 2002). The prevalence of bacteriuria has a J shaped distribution, with a higher frequency among the very young and a gradual increase with age in both men and women. Until the age of 60 years and

older, the prevalence is significantly higher for women than men (Johnson, 1991a). The distribution of symptomatic infection has a somewhat different shape: women aged 15–29 years have the highest frequency (approaching 20.0%) (Foxman and Brown, 2003) Among women aged 18 years and older, the estimated incidence was 12.6%; for men, this incidence was only 3.0% (Johnson, 1991a).

The epidemiology of UTI in children varies by age, gender and other factors. The incidence of UTI is highest in the first year of life for all children (1.0%), but decrease substantially among boys after infancy (Jakobsson et al., 1999).

UTI is less prevalent in male population. Males are less affected by UTI due to the greater distance between anus (the usual source of uropathogens) and the urethral meatus, the drier environment surrounding the male urethra, the greater length of the male urethra, and the antibacterial activity of prostatic fluid (Lipsky, 1989).

The incidence is increased in elderly men and women, particulary among those living institutions where it can be upto 53.0% and 37.0% in women and men, respectively (Neild, 2003).

It has been estimated that nearly 10.0% of the human population will experience a UTI during their life time (Hryniewize et al., 2001).

The prevalence of UTI varies according to season also. The prevalence of UTI caused by *E. coli* is more in summer season than winter season because in summer season increased temperature causes sweating more than winter, therefore urine production become less and concentrated which produces the opportunity to multiply the bacteria. Conversely in winter season less sweating causes more dilute urine production, which washes away any multiplying bacteria (Nahar et al., 2010).

2.5 Host defense

The urinary tract has a number of specialized defenses against bacteria colonization, keeping urine sterile.

The urinary tract (i.e., kidney, ureter, bladder, and urethra) is a closed, normally sterile space lined with mucosa composed of epithelium known as transitional cells. The main

defense mechanism against UTI is constant antegrade flow of urine from the kidneys to the bladder with intermittent complete emptying of the bladder via the urethra. This washout effect of the urinary flow usually clears the urinary tract of pathogens (Cox and Hinman, 1961).

The urine itself also has specific antimicrobial characteristics and this is thought to result from its relatively acidic pH, high osmolality, high urea concentration, polymorphonuclear cells, and Tamm-Horsfall protein (THP), which inhibits bacterial adherence to the bladder mucosal wall (McCormick et al., 2008; Sobel, 1997).

The epithelial cells lining of urinary tract system produce and secret a number of antimicrobial peptides (AMPs) and proteins, which help to maintain the sterility of the urine through their antimicrobial action. Some AMPs are constitutively expressed, and others are only expressed when the organism (or a tissue) has been injured or exposed to microbes. Defensins, cathelicidin, THP, lactoferrin, and lipocalin are antimicrobial proteins implicated in bacterial defense of the urinary tract (Zasloff, 2007).

THP is the most abundant protein in normal human urine, synthesized in the epithelial cells of the ascending limb of Henle's loop and in the proximal part of the distal tubules (Jelakovic et al., 1996). THP coats uroepithleial cells with the main role to bind type 1 fimbriae to prevent bacteria from adhering to epithelia cells (Pak et al., 2001).

The secretions that protect the mucosal surface of renal epithelia contain an array of host defense factors, including immunoglobulins, of which immunoglobulin A (IgA) is the major class (Rice et al., 2005).

Exfoliation of the infected superficial facet cell layer of urinary tract begins within hours of interaction and facilitates the elimination of the infected cells into the urine (Mulvey et al., 1998).

The bladder epithelium produces cytokines primarily through stimulation of the host toll like receptor 4 (TLR4) by bacterial lipopolysaccharides (LPS), that recruit polymorphonuclear neutrophils (PMNs) to assist in the elimination of bacteria (Hunstad et al., 2005).

Lactobacilli in the vagina are protective because they prevent initial colonization with uropathogens (Gupta et al., 1998).

2.6 Risk factors

Womens are at elevated risk due to the short distance between the anus and urethra as well as a short urethra emptying the bladder. UTI risk factors include extreme age, female gender, bladder catheterizations, nephrostomy tubes, prior antibiotic administration, diabetes, mechanical obstruction and anatomical abnormalities that promote urinary statis (e.g., Neurogenic bladder, pregnancy, kidney stones) (Foxman and Brown, 2003; Nicolle, 2009).

Risk factors for UTI among women vary by age groups. In school-aged girls, common risk factors include congenital abnormalities and new onset of sexual activity. Risk factors for premenopausal women are history of urinary tract infection, frequent or recurrent sexual activity, diaphragm contraception use, use of spermicidal agents, increasing parity, diabetes mellitus, obesity, sickle cell trait, anatomic congenital abnormalities, urinary tract calculi, neurologic disorders or medical conditions requiring indwelling or repetitive bladder catheterization. In postmenopausal women, common risk factors include viginal atrophy, incomplete bladder emptying, poor perineal hygiene, rectocele, cystocele, urethrocele, or uterovaginal prolapse, lifetime history of urinary tract infection and type1 diabetes mellitus (ACOG practice bulletin, 2008). In women rate of UTI increases with advancing age, likely because of the hypoestrogenic state and vaginal epithelium atrophy, impaired voiding, and changes in hygiene (Scholes et al., 2005; Sheffield and Cunningham, 2005).

Risk factor associated with UTI in healthy men including intercourse with an infected female partner, homosexuality and lack of circumcision although of them none of these factors is present in men with UTI (Hooton and Stamm, 1997; Mehnert-Kay, 2005). Uropathogenic strains infecting healthy young men tend to be highly urovirulent (Hooton Stamm, 1997).

2.4 Virulence factor of *Escherichia coli*

Bacteria that cause UTI in otherwise healthy hosts often exhibit distinctive properties known as virulence factors-to overcome the normal defenses of the urinary system.

UPEC strains possess specialized virulence factors, enabling them to colonize and invade the host, disrupt the host defense mechanism, injure host tissues and/or stimulate a noxious host inflammatory response. The abilities of UPEC to grow extraintestinally may enable them to cause a variety of diseases, not just UTIs (Zhao et al., 2009).

In *E. coli* virulence results from the cumulative impact of several properties or virulence factors (VFs), which serve to distinguish potential pathogens from harmless intestinal strains (Johnson 1991b). The virulence of individual of individual strains in a given infection is determined by the presence and actual expression of the virulence genes present in them and also by the environmental conditions in the host (Sharma et al., 2007).

Bacterial adherence to host mucosal surfaces is often an important first step in the infection process. This is especially true in the case of urinary tract infection (UTIs) (Svanborg et al., 1983). The ability of UPEC to establish infection in the urinary tract is most closely linked to the expression of adhesive organelles called pili or fimbriae that interact with the proteins on urinary epithelial cells (Hunstad et al., 2005). Common adhesive organelles elaborated by UPEC are type 1, P, S and F1C pili encoded by the fim, pap, sfa and foc operons, respectively (Wiles et al., 2008). The expression of different fimbriae during UTI seems to be a coordinated process and *E. coli* express mainly one fimbriae type at a time (Nowicki et al., 1984; Synder et al., 2005). Two of the most studied adhesive organelles are type 1 and p pili which are encoded by many UPEC strains (Lane and Mobley, 2008). About 80.0% of UPEC express p pili which anchor to the glycolipid of the outer membrane of urothelia cells localized in the kidney (Poliot et al., 1987; Plos et al., 1991). P pili are produced by pyelonephritis strains and are frequently associated with cases of acute pyelonephritis (Roberts et al., 1994; Minardi et al., 2011). In the strain that cause cystitis, type 1 fimbriae are continually expressed and the infection is confined to the bladder (Connell et al., 1996). S fimbriae and F1c fimbriae

have also been shown to bind to epithelial and endotheila cells from the kidney and lower urinary tract (Emody et al., 2003; Marre et al., 1990).

Some uropathogenic *E. coli* strains have been shown to express flagella. Flagella are up to 15 μm long complex organelles, which contribute to bacterial motility (Romos et al., 2004). Flagellar motility and swarm cell differentiation contribute to the virulence of another urinary pathogen, *Proteus mirabilis* (Allison et al., 1994; Harmon et al., 1989). In contrast, the role of flagella in the colonization of urinary tract by *E. coli* seems to be of a subordinate importance (Wright et al., 2005; Yamamato et al., 1990).

UEPC may also express Dr. adhesion, which are associated with cystitis and recurrent UTI in young adults and pyelonephritis in pregnant women. Dr. adhesion binds to type IV collagen and decay accelerating factor (DAF, also CD55) in the kidney. Dr. adhesion are critical in the development of chronic pyelonephritis in an experimental model (Goluszko et al., 1997).

Most UPEC strains produce an acidic polysaccharide capsule, which protects the bacteria from phagocytosis by human polymorphonuclear neutrophils (PMNs) and inhibits complement activation (Johnson, 2003).

Alpha hemolysin (HlyA) is a pore forming toxin secreted by *E. coli* (Cavalieri and Snyder, 1982). It is principally responsible for the lysis of erythrocytes. In addition to lysing erythrocytes, hemolysin is toxic to range of host cells, in ways that probably contribute to inflammation, tissue injury, and impaired host defence. Such toxic actitivity may contribute to kidney damage seen in pyelonephritis. Approximately half of UPEC stains that cause upper UTIs, about a third of those that cuase lower UTIs, and only about 10.0% of fecal isolates produce HlyA (Slavchev et al., 2008-2009).

Cytotoxic necrotizing factor (CNF-1) is encoded by chromosomally *cnf1* (Johnson, 1991). CNF-1 targets the Rho family GTP-binding proteins and induces actin cytoskeleton reorganization, leading to apoptosis, which facilitate bacterial invasion into deeper tissue layer of urinary tract (Fiorentini et al., 1995; Mills et al., 2000). This process enables bacteria to persist within the urinary tract (Rippere-Lampe et al., 2001).

Aerobactin, a bacterial siderophore has recently been shown to be associated With *E. coli* strains which cause pyelonephritis and cystitis (Carbonetti et al., 1986). It is an iron sequestration and transport system which enables *E. coli* to grow in iron poor environments such as dilute urine and complement depleted serum. The aerobactin system is associated with *E. coli* isolates from serious UTI and other serious infections in humans and animals probably because it promotes bacterial growth in the limiting iron concentrations encountered during infection (Slavchev et al., 2008-2009).

Urease have been has been implicated as a major virulence factor for UTI. Urease of *Proteus mirbalis* and *Klebsieall* spp. has been also implicated as a uropathogenic factor that promotes the persistent growth and renal stone formation (Jones et al., 1990; Podschun et al., 1993).

Other Enterobacteriaceae, including *Klebsiella* spp. and *Proteus* spp. as well as *Providencia stuartii* have been shown to express fimbriae that are important in both uroepthelial adherence and attachment to urinary catheters (Mobley et al., 1987).

Adherence to the uroepithelial cell and production of urease has been reported to play a important role in the persistent growth and invasiveness of *Staphylococcus saprophyticus* in the bladder (Gatermann et al., 1989; Hell et al., 1998).

2.7 Pathogenesis

UTI is frequently caused by organisms which are normal commensals in the distal urethra and adjacent sites. The most common route of infection is by ascension. Uropathogens are part of the normal fecal flora. These bacteria colonize the perianal region and then ascend in females to the introitus vaginae which are reservoir for several uropathogns. Colonization spreads to the periuretheical area, urethera and bladder, strongly depending on sexual activity (Franz and Horl, 1999). The ability of UPEC to bind host tissue is one of the important factors that facilitate UPEC colonization of urinary tract, allowing the bacteria to withstand the bulk flow of urine and promoting UPEC invasion of urothelia cell. Once inside the urinary tract, UPEC preferentially colonizes the bladder and causes cystitis; however, can ascend through the ureters into the kidney, causing pyelonephritis. UPEC have evolved a number of strategies to evade these innate immune responses,

enabling the pathogens to more effectively colonize the urinary tract and persist (Wiles et al., 2008).

2.8 Routes of Infection

Microorganism can reach the urinary tract by way of ascending, hematogenous, or lympatic routes.

2.8.1 Ascending route of infection

In general, bacteria infect the urinary tract by ascending route from the urethra. Ascending infection of the urinary tract is complex process that has been associated with bacterial adhesion, virulence, and motility properties as well as host anatomic, humoral, and genetic factor (Zorc et al., 2005; Svanborg and Godaly, 1997). The ascending route accounts for almost 95.0% of cases for UTI (Goldman et al., 2000). This is particularly common for *E. coli* and other Enterobacteriaceae (Nadi et al., 2006).

2.8.2 Hematogenous route of infection

Hematogenous infection of the urinary tract is restricted to a few relative uncommon uropathogen, such as yeast (usually *Candida albicans*), *Mycobacterium tuberculosis*, *Salmonella* spp. and *Staphylococcus aureus*, which cause primary infection elsewhere in the body. *Candia albicans* readily causes a clinical UTI via the haematogenous route, but is also an infrequent cause of an ascending infection if an indwelling catheter is present or following antibiotic therapy (Grabe et al., 2008). Hematogenous spread usually occurs as a result of bacteremia and accounts for only less than 5.0% of UTIs (Forbes et al., 2002).

2.8.3 Lymphatic route of infection

The importance of lymphatic spread of uropathogens to the urinary tract in the pathogenesis is not known (Hooton, 2000).

2.9 Clinical presentation

Urinary tract infections have traditionally been viewed as acute and often self-limiting infections. However, this concept has been challenged by recent findings demonstrating that an acute bladder infection results from a complex series of host pathogen interactions that can lead to bacterial invasion and persistence and that ultimately can determine the course of the infectious disease (Schilling et al., 2002). In general, UTIs can be classified as asymptomatic bacteriuria, cystitis, or acute pyelonephritis. Cystitis predominantly involves colonization of the bladder (Gunther et al., 2001).

The symptoms of UTIs include fever, burning sensations while urinating, lower abdominal pain, itching, formation of blisters and ulcers in the genital area, genital and suprapubic pain, and pyuria generally depend on the age of the person infected and the location of the urinary tract infected (Amali et al., 2009).

In asymptomatic UTI, significant bacteriuria is often not associated with symptoms and does not require treatment except in pregnant women, in infants and before urological surgery (Neild, 2003).

About 95.0% of all UTIs are cystitis, occurring in anatomically normal individuals and characterized by burning sensations, frequent micturition, incomplete voiding, and suprapubic pain (Muhldorfer et al., 2001).

Patients with cystitis usually report dysuria, frequency, urgency and tenderness and pain over the area of the bladder. In some individuals, the urine often grossly cloudy and malodorous, and it is is bloody (Forbes et al., 2002).

The more severe upper urinary tract disease acute pyelonephritis involves colonization of kidneys and represents an infection capable of progressing to bacteremia (Gunther et al., 2001). The typical clinical presentation includes fever and flank pain and, frequently, lower tract symptoms (frequency, urgency, and dysuria). Patients can also exhibit systemic signs of infection such as vomiting, diarrhea, chills, increased heart rate, and lower abdominal pain. Of significance, 40.0% patients with acute pyelonephritis are bacterimic (Forbes et al., 2002). If pyelonephritis left untreated can result in renal failure, bacteremia and sepsis (Mehnert-Kay, 2005).

2.10 Laboratory diagnosis

Urine specimens make up a large proportion of the samples submitted to a routine diagnostic laboratory. The frequency of UTI generates a significant workload for the laboratory, with large laboratories analyzing from 200 to 300 urine specimens per day (Graham and Gallowy, 2001). The diagnosis of UTI based on urinary sampling and testing with reagent sticks and/or laboratory culture, both of which require a high quality specimen free of perineal, fecal or vaginal contamination organisms and inflammatory cells (Franz and Horl, 1999). UTI is diagnosed by either clinical observation or isolation of the causative microorganisms from pathologic urine (Weiss et al., 2002).

2.10.1 Method of urine collection

Urine, which should be obtained before the initiation of antimicrobial therapy (Chang and Shortliffe, 2006). Urine can be sampled by suprapubic aspiration, straight catheter, indwelling catheter or mid-stream urine (MSU) collection.

2.10.1.1 Suprapubic aspiration

Suprapubic aspiration is the best method to avoid contamination of specimen with commensal bacteria in distal urethra and still considered the “gold standard” (Wilson and Giado, 2004; Graham and Gallowy, 2001). This collection method is used infrequently because it is invasive and uncomfortable and it requires too much time and too many resources to be practical (Wilson and Gaido, 2004). Suprapubic aspirate were, often obtain from babies and young children and limited to a few specific clinical situations (Graham and Gallowy, 2001).

2.10.1.2 Straight catheter

Straight catheter is the second most best method after suprapubic aspiration in term of minimal contamination, but again, it is not indicated clinically for most patients because it is too labor intensive and costly for routine use and it is invasive. There is also risk of introduction of bacteria into urethra (and thereby cause UTI) during catheter insertion and rare complication have been reported (Wilson and Gaido, 2004).

2.10.1.3 Indewlling catheter

Indewlling catheter specimen obtained from patient with long term indwelling catheter. Urine should be aspirated directly from, the catheter using sterile needle and syringe and then placed in sterile container. Bacteria are frequently recovered but only a few are important and samples should be taken when signs and symptoms of UTI suggest. Bacteria multiply in catheter bags so specimens from this site are unsuitable (Graham and Gallowy, 2001).

2.10.1.4 Mid-stream urine (MSU)

Most urine specimens are obtained from adult patients via the clean catch midstream method. This method is the most appropriate because it is neither invasive nor uncomfortable, it is simple and inexpensive, it can be performed in almost any clinical setting, there is no risk of introducing bacteria in the bladder by catheterization, and there is no risk of complication (Wilson and Gaido, 2004). Although it requires good control of micturition and an adequate volume of urine in the bladder. It may prove difficult to get such a sample in the elderly or those with hip joint problem (Graham and Gallowy, 2001). The specimen may be contaminated with the commensal bacteria passing through the distal urethra (Wilson and Gaido, 2004). Contamination rates as high as 30.0% have been reported (Lifshitz and Kramer, 2000). Such contamination obscure interpretation of the urine culture and may mask underlying bacteriuria (Jackson et al., 2005).

2.10.2 Microscopy

2.10.2.1 Detection of pyuria

Pyuria can be detected and quantified microscopically by measuring the urinary leukocyte excretion rate, counting leukocytes with a hematocytometere, counting leukocytes in urine specimens using Gram staining, or counting leukocytes in a centrifuged specimen (Wilson and Gaido, 2004). The most accurate microscopic method for quantitating pyuria is to measure the urinary leukocyte excretion rate (Pappas, 1991). Patients with symptomatic UTIs have urinary leukocyte excretion rates of >/ 400,000 leukocytes/h (Pappas, 1991). However, it should be noted that pyuria only indicates inflammation and does not always always mean infection (Graham and Gallowy, 2001). A simple and inexpensive alternative is to count urine leukocytes with a

hematocytometer. Hematocytometer count of >/ 10 leukocytes /mm3 correlates with a urinary leukocytes excretion rate of >/40,000 leukocytes/h (Pappas, 1991). Pyuria and / or bacteria on microscopy are highly suggestive of UTI and are useful criteria to select specimens for direct sensititvity testing. However, the absence of pyuria does not exclude infection because patients with neutropenia may have an inadequate white cell response to infection. Alkanline urine, such as that encountered with *Proteus* spp. Infection, results in the white cells disintegreatin before microscopy being performed (Graham and Gallowy, 2001). Pyuria with a negative urine culture may also be found when there is infection with *Chlamydia trachomatis*, *Ureaplasma*, or *Neisseria gonorrhea*, or when a patient has taken antimicrobials (Cheesbrough, 2006).

2.10.2.2 Detection of hematuria

Hematuria (red cells in urine) is commonly seen in acute cystitis but is not diagnostic of that condition. It is rarely seen in other dysuria syndromes but is often seen in non-infective renal disease (Graham and Gallowy, 2001). Microscopic hematuria may be present in 40 to 60.0 % of patients with UTI (Faro and Fenner, 1998). Hematuria may be found in urinary schistosomiasis (usually with proteinuria), bacterial infections, acute glomerulonephritis (inflammation of the glomeruli of the kidneys), sickle cell disease, leptospirosis, infective endocarditis, calculi (stones) in the urinary tract, malignancy of the urinary tract, and haemorrhagic conditions (Chessbrough, 2006).

2.10.2.3 Detection of bacteriura

Bacteriuria can be detected microscopically using gram staining of uncentrifuged urine specimens, gram staining of centrifuged specimens, or direct observation of bacteria in urine specimens (Wilson and Gaido, 2004). Microscopy of uncentrifuged, unstained urine will detected more than 104 bacteria/ml of urine (Graham and Gallowy, 2001). Centrifugation and gram staining of urine increases the sensitivity to detect pyuria and provide the information of nature of infecting bacteria or yeast and thereby guiding the physician in selecting empiric antimicrobial therapy (Wilson and Gaido, 2004; Graham and Gallowy, 2001). However, these methods are labour intensive and impractical for routine specimens (Graham and Gallowy, 2001).

2.10.3 Nitrate reductase

Nitrate reductase reduces nitrate to nitrites and associated with members of the family Enterobacteriaceae (the pathogens most commonly responsible for UTIs) but not other bacteria such as *Staphylococcus saprophyticus*, *Pseudomonas* spp., or enterococci. The test is best performed on early morning urine, as >/ 4 h are required for bacteria to convert nitrate to nitrite at levels that are reliably detectable (Wilson and Gaido, 2004; Graham and Gallowy, 2001). Nitrite test can be false negative if the urine specimen is too diluted (Faro and Fenner, 1998).

2.10.4 Leukocyte esterase

Leukocyte esterase is specific for polymorphonuclear neutrophils (pus cells). It detects the enzyme from both active and lyzed white cells. Leukocyte esterase testing is an alternative method of detecting pyuria when it is not possible to examine fresh urine microscopically for white cells, or when urine is not fresh and likely to cotain mostly lyzed white cells (Chessbrough, 2006). Leukocyte esterase test can yield false positive test results when urine is contaminated with vaginal fluid containing bacteria, when the specimen contain eosinophils or *Trichomonas* spp., both of which can act as source of esterase, and when oxidizing agents or formalin react with the test strips to generate false positive results (Wilson and Gaido, 2004). Leukocyte esterase test show false negative or decrease in positivity test in the presence of ascorbic acid, boric acid, dexycclyline, cephalexin, gentamicin, nitrofurantoin, glycosuria, urobilinogen or high concentration of proteins (Grahma and Gallowy, 2001). Leukocyte esterase is specific (94.0 to 98.0%) and reliabley sensitive (75.0 to 96.0%) for detecting uropathogens equivalent to 100,000 colony forming unit (CFU) per ml of urine (Hooton and Stamm, 1997).

2.10.5 Urine culture

The gold standard for diagnosis of UTI is growth of pathogenic bacteria in a urine culture (Zorc et al., 2005; Mar, 2010). However, diagnosis is complicated by contamination from fecal bacteria that colonize the perineal area and distal urethra (Zorc et al., 2005). Several tests are available for the detection of urinary tract infection. However, culture remains he preferred methods of detecting and quantifying bacterial growth.When urine is microscopically and biochemically normal, except when screening for asymptomatic

bacteriuria in pregnancy. Culture required when the urine contains bacteria (as indicated by the Gram smear), cells, casts, protein, and nitrite or has a markedly alkaline or acidic reaction (Chessbrough, 2006).

The major indications for urine culture are (Najar et al., 2009)

a. Patients with symptoms or signs for UTIs,
b. Follow-up recently treated UTI,
c. Removal of indwelling urinary catheters,
d. Screening for asymptomatic bacteriuria during pregnancy; and
e. Patients with obstructive uropathy and statis before instrumentation.

Urine specimens must be cultured promptly within 2 hours or can be preserved by refrigeration or a suitable chemical additive (boric acid) (Najor et al., 2009). Boric acid is often used to retain the bacterial count, but its antimicrobial activity can reduce the number of organisms present, especially if an inadequate volume of urine is dispensed into the container (Graham and Gallowy, 2001).

Routine urine culture should be plated using calibrated loops for the semi-quantitative method. This method has the advantage of providing information regarding the number of CFU/ml, as well as providing isolated colonies for identification and susceptibility testing (Wilson and Gaido, 2004). Moreover, semi-quantitative urine culture is the reference standard for diagnosis of urinary tract infection, but it has several practical problems. At least, 18 hours are need for the detection of bacterial growth on culture media by standard microbiological technique, leading to delay or inappropriate treatment. Urine culture is also expensive and needs a microbiological laboratory with skilled technicians. Urine culture is necessary to identify the causal pathogen and establish antibiotic sensititives that guide the choice of antibiotics (Willianms et al., 2010).

The type of media used for routine culture should be limited to blood agar and MacConkey agar (Wilson and Gaido, 2004; Manges et al., 2001). For urine culture specimen obtained from out patients, it is not necessary to routinely inoculated a medium that is selective for gram positive bacteria (Wilson and Gaido, 2004; Hryniewicz et al., 2001).

Blood agar should continue to be a part of the urine culture workup, as isolation and discrimination of different gram-positive bacterial species are much easier with this medium (Aspevall et al., 2002). However, it lacks the ability for primary differentiation between the gram negative colonies, thereby necessitating further identification tests and causing delay in the final result. It also fails to prevent swarming of the colonies of *Proteus* spp. (Lakshami et al., 2004).

Several choromgenic media have been compared to traditional urine culture media (i.e., blood and MacConkey agars) and were found to be at least good as traditional media for the isolation of uropathgens (Lakshami et al., 2004; Aspevall et al., 2002; Samra et al., 1998; D'souza et al., 2004). The first selective chromogenic medium used by Kilina and Bulow in 1976 for the direct detection of *E. coli* in primary culture of urine (Perry and Freydiere, 2007).

Chromogenic media differentiate pathogens based on colony colors produced by interaction of specific bacterial enzymes with chromogenic substrates. It allows the growth and primary identification of the predominant uropathogens like *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. This media also supports the growth and differentiation of gram positive organisms like *Staphylococcus aureus* and *Enterococcus* (Lakshami et al., 2004).

The greatest advantages of chromogenic media over traditional media are easier recognition of mixed growth, shorter analysis duration, workload reduction and higher detection rates. However, chromogenic media from different manufacturers vary in performance and cost. In particular, the cost of chromogenic media presents a major obstacle for their use in routine practice (Retelj and Harlander, 2007).

Blood cultures are warranted only in case of pyelonephritis severe enough for hospitalization or where there is suspicion of sepsis (Linda, 2006).

The most commonly used criteria for defining significant bacteriuria is the presence of $>/10^5$ CFU/ml of urine (Graham and Gallowy, 2001).

2.11 Treatment

The treatment of UTI may varies according to patient age, sex, associated disease,infectious agent and whether the problem is in upper or lower urinary tract (Arslan et al., 2005). UTIs are often treated with different broad spectrum antibiotics when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organism (Dimitrov et al., 2004). The antimicrobial agents most commonly used to treat uncomplicated urinary tract infection include the combination drug trimethoprim and sulfamethxazole, trimpehotprim, beta lactamas, fluoroquinolone, nitrofurantoin and fosfomycin trimethamine (Jancel and Dudas, 2002). These agents are primarily because of their tolerability, spectrum of activity against suspected uropathogens, and favorable phamacoketntic profile (Neu, 1992).

a. Beta lactams

In the past, beta lactam antibiotic such as first generation cephalosporins (Cephalexin) and the aminopenicillins (ampicillin, amoxicillin) were routinely used to treat urinary tract infections (Jancel and Dudas, 2002). Although the first generation cephalosphorins and aminopenicillins achieve high urinary concentrations, they are no longer recommended as first line therapy for UTIs because of resistance and high recurrence rates compared to with other agents. However, in certain setting, such as during pregnancy or when enterococci are suspected, ampicillin or amoxicillin may still be an appropriate choice for acute UTI (Warren et al., 1999). These agents should be used only if a urine culture documents susceptibility. However, third-generation cephalosporins such as cefixime and cefpodoxime offer the advantage of longer half-lives, which allows for less frequent dosing. Also, lower resistance rates to *E. coli* have been observed with these agents than with the first-generation cephalosporins and aminopenicillins. These agents may be options for patients who are intolerant to trimethoprim-sulfamethoxazole or who have resistant infections (Jancel and Dudas, 2002).

b. Trimethoprim-sulfamethoxazole

Trimethoprim-sulfamethoxazole has long been considered the standard of therapy for acute and recurrent urinarytract infections because of its activity against the most common uropathogens and its low cost and tolerability. The synergistic combination of

trimethoprim and sulfamethoxazoleworks at two separate steps of the bacterial folate metabolism, resulting in the inhibition of DNA synthesis (Jancel and Dudas, 2002). Use of trimethoprim-sulfamethoxaxole is nowadays limited because of widespread microbial resistance and should only be used in regions with a known low resistance rate (<20.0%) and after culture and sensitivity testing (Bean et al., 2008). However, in countries where resistance is low, trimethoprim-sulfamethoxazole can still be a valid first-line antibiotic (Minardi et al., 2011).

c. Nitrofurans

Nitrofurans are a group of compounds characterized by the presence of one or more nitrogroups on a nitroaromatic or nitro-heterocyclic backbone. Furanzolidone, nitrofurazone and nitrofurantoins are compounds belonging to this group and they all display antimicrobial activity and used clinically to treat different types of infections (Sandegren et al., 2008). Nitrofurantoin was the first truly effective and safe antimicrobial therapy for UTI but its spectrum of activity is limited (Nickel, 2005). On the other hand, another study by Hooton (2003) indicated that used of nitrofurantoin does not share cross resistance with more commonly prescribed antimicrobials and its wider spread use is justified from public health perspective as a fluoroquinolone-sparing agent. Nitrofurantoin is taken orally, rapidly absorbed and excreted in urine to generate high therapeutic concentaration (Conlink, 1978). Nitrofurantoin, including nitrofurantoin macrocrystals and nitrofurantoin monohydrate macrocrystal, remains effective after 50 years of wide spread use, for the treatment of urinary infection (Warren et al., 1999). Nitrofurantoin is safe for use in pregnancy and is not tetragenic (Christensen, 2000). However, it should avoided late in pregnancy, given the risk of fetal hemolytic anemia in a patients with G6PD deficiency (Sivick and Mobley, 2010). Occasionally, nitrofurantoin may cause acute and choronic pulmonary side effects. It is not recommended for the treatment of upper urinary tract infection, and is contraindicated in individuals with renal failure, as metabolites which accumulate may cause neuropathy (Spring et al., 2001). In comparative studies, nitrofurantoin has a somewhat lower cure rate than trimethoprim/sulfomethxozole or fluoroquinolone (Iraveniet et al., 1999). Nitrofurantoin is less active against non *E. coli* gram negative rods and inactive against *Proteus* and *Pseudomonas* spp. (Gupta et al., 2001).

d. Fluoroquinolones

The quinolones are a group of synthetic antimicrobial agents that includes nalidixic acid and the fluorinated quinolones (Tavio et al., 1999). Fluoroquinolones are potent broad spectrum antibiotics that are structurally related to nalidixic acid and used for treatment of infections caused by gram–negative bacilli. A broad spectrum of activity, good oral absorbability, and overall tolerability have resulted in extensive clinical use of fluoro fluoroquinolonequinolone. The introduction of fluoroquinolone in clinical practice has been associated with an increasing incidence of quinolones resistant bacteria, especially among *S. aureus* and gram negative bacilli such as *Pseudomonas aeruginosa*, *Citrobacter* spp., *Salmonella* spp., *Klebsiella pneumoniae*, and *Serratia marcescens* (Eom et al., 2002). However, the resistance of *E. coli* to quinolones remain rare until 1990; since then the wide spread use of fluoroquinolone (such as ciprofloxacin and norfloxacin) for the treatment of UTIs has been implicated with emergence of resistant strains (Ena et al.1998; Garau et al 1999; Pena et al., 1995; Perez-Trallen et al.,1993). The first fluoroquinolone widely used for treatment of UTI, namely norfloxacin, ciprofloxacin, ofloxacin and levofloxacin (Gupta et al., 2001). Fluoroquinolone are contraindicated in pregnancy because of potential adverse effects of fetal cartilage (Christensen, 2000). Although quinolones have a high efficacy against the main genitourinary tract microorganisms, particularly in vitro, clinical traits have failed to demonstrate their superiority in eradicating infections in comparison with other drugs. Moreover, higher rate of side effects in comparison with the other drugs and their lower tolerability limit the use of quinolones as a second-line (Minardi et al., 2011). Furthermore, surveys conducted in many European countries have already shown higher resistance rates in *E. coli* strains for nalidixic acid and its derivatives (over 10.0% and upto 32.6% in Hungary) (Schito et al., 2009).

Higher urinary concentration (>100 times the peak plasma level) enable these antimicrobial agents to penetrate the renal parenchyma effectively, thus making fluoroquinolone very effective agen for both empiric and culture specific treatment of both uncomplicated and complicated UTIs. Another advantage of fluoroquinolone over other antibiotic agent is their tissue lipophilic properties. These physochemical properties allow excellent drug penetration into prostate gland (2-4 times the serum level) (Emo et al., 2002).

e. Fosfomycin trimethamine

Fosfomycin trimethamine is a phosphoric acid derivative that is newly licensed for treating uncomplicated cystitis caused by *E. coli* or *Enterococous feaclis* (Patel et al 1997). However, it is not approved for used for cystitis caused by *Staphylocccus saprophyticus* or for treatment of pyelonephritis. It is achieved very high concentrations in the urine and persists in the urine for moer than 24 hours (Gupta et al., 2001).

2.12 Prevention of UTI

Patients to be considered for preventive measures include women, experiencing recurrent UTIs, children with structural abnormalities of the urinary tract or recurrent UTI, patients with spinal cord injury or neurogenic bladder, and patients after renal transplant (Stapletom, 2003).

a. Cranberry juice

Cranberry juices have been used to prevent UTI (Mohsin and Siddiquie, 2010). Cranberry juice contains tannin called A-type proanthocyanidin, a compound found in many plants that serve as antimicrobial defense. It is likely act by decreasing microbial adherence of the P-fimbriae to the urothelium. Additionally, it is acidic, including a precursor of a known bacteriostatic agent (hippuric acid) (Duplessis et al., 2011). No definite mechanism of action has been established for cranberry in the prevention or treatment of UTI (Jepson and Craig, 2008).

b. Lactobacilli probiotic

Probiotic is the group of microbes that may help directly for enhancing resistance against intestinal pathogens and in the prevention of disease. Probiotics are available in many forms, such as capsules, liquid/gel and powders. Probiotic bacteria may produce many compounds, which are inhibitory to pathogens growth, which include organic acids (lactic and acetic acids), bacteriocins, and reuterin. The organic acid not only lowers the pH, thereby affecting the growth of the pathogen, but can also be toxic to microbes (Bhutada et al., 2011).

Lactobacilli probiotic administration has many proposed mechanisms efficacious at decreasing UTIs, including elaboration of hydrogen peroxide, production of bacteriocidins and biosurfactants (Duplessis et al., 2011).

c. Topical estrogen

Topical estrogen is a reasonable treatment for postmenopausal patients with recurrent UTIs (Duplessis et al., 2011). Theoretically, it is thought to reestablish a vaginal acidic milieu and to reconstitute premenopausal flora, including lactobacilli (Sivick, 2010; Falagas and Karageorgopoulous, 2009). However, studies are conflicting on the efficacy of oral or topical estrogen therapy in preventing recurrent UTIs (Duplessis et al., 2011).

d. Methenamine salt

Methenamine salts are often used as an alternative to antibiotics for the prevention of urinary tract infection (Mohsin and Siddiquie, 2010). Methenamine hippurate is hydrolyzed to formaldehyde and ammonia in acidic urine. Formaldehyde has nonspecific bactericidal action, and hippuric or mandelic acid aids in maintaining the requisite urine acidity, in addition to suppressing bacteria. To ensure acidified urine, it may be combined with cranberry juice or vitamin C. Methenamine hippurate appears to be ineffective when used in patients with neurogenic bladder or renal tract abnormalities (Duplessis et al., 2011).

e. Acupuncture

Acupuncture has been shown to be successful in the preventing frequent UTI episodes in one randomized, single blind controlled trial in 67 patients with recurrent UTI (Aune et al., 1998).

2.13 Antibiotic resistance

Drug resistance is one of nature's never ending process by which the organisms develop tolerance to new environmental condition. It may be due to a preexisting factor in the organisms or result from the acquired factor(s) (Manikandan et al., 2010).

The human gastrointestinal tract is an important reservoir of antibiotic resistance genes, which contribute to the maintenance and distribution of resistance in the environment

(Shoemaker et al., 2001). The enteric bacteria in fecal flora are often reported to be highly resistant and *E. coli* is reported to be main carrier of antimicrobial resistance (Osterblad et al., 2000).

Antimicrobial resistance is now recognized as an increasingly global problem which was observed for the first time in *E. coli* in 1940 (Ahmed et al., 2000; Tenvor and Hughes, 1996).

The prevalence of antimicrobial resistance with UTI is increasing and varies according to geographical and regional location (Khan and Zaman, 2006).

There is a gradual increasing antibiotic resistance in both community and nosocomially acquired UTI causing uropathogens. Even in women with acute uncomplicated UTI increasing resistance to ampicillin (30.0-40.0%), cephalothin (20.0-30.0%) and co-trimoxazole (15.0-20.0%) has been demonstrated in causative *E. coli* (Gupta et al., 1999).

Most of *E. coli* strains were normally sensitive to most of antibiotics and chemotherapeutic agents, but in recent years resistance has been encountered in many cases. Most of the isolates show resistance towards cephaloridine, cephradine, cephalizolin, fluoroquinolone, gentamycin and tetracycline (Hameed et al., 1995)

MDR bacteria refer to those which are resistance to a vast range of antibiotics with structural independence (at least two or more antibiotics) (CDC, 2006). Nowadays, a big concern among the medical and clinical practitioners is the emerging MDR organism and their associated complications in developing world. These conditions make the treatment more challenging and many even threaten the respective patients lives (Guyot et al., 1999; Farshad et al., 2010b). *Shigella* was the first organism observed to show resistance to multidrugs (Tenover and Hughes, 1996). MDR has been demonstrated in *E. coli, Salmonella enteria serovar* Typhimurium, *Shigella dysenteriae, Enterococcus faecium, Staphylococcus aureus, Mycobacterium tuberculosis, Haemophilius influenza, Pseudomans aeruginosa, Klebsiella pneumoniae, Acinetobcter baumannii, Stenotrophomonas maltophilia, Xanthomonas and Burkholderia* (Dizidic et al., 2008).

MAR index is a tool that reveals the spread of bacteria resistance in a given population (Krumpermann, 1983). An MAR index greater than 0.2 implies that the strains of such

bacteria originate from an environment where several antibiotics are used (Ehinmidu, 2003).

Multiple factors have had led to the prevalence of antibiotic resistance in micro-organisms (Ahmed et al., 2000)

a. The wide use of antibiotics due to high prevalence of infectious diseases
b. A shortage of physician
c. Selective prescribing due to cost constraints and the pressure of pharmaceutical companies promotional activities
d. Lack of laboratory support in rural areas
e. The difficulties in distributing information regarding antibiotic resistance.

Mechanism of antibiotic resistance

There are several different mechanisms responsible for development of antibiotic resistance.

Biochemical aspect for antibiotic resistance mechanism

a. Alterning the target protein to which the antimicrobial agents bind by modifying or eliminating the binding site (e.g., Change in penicillin-binding protein 2b in pneumoncocci, which result in penicillin resistance)
b. Upregulating the production of enzymes that inactivate the antimicrobial agent (e.g., erythromycin ribosomal methylase in staphyloccoi)
c. Down regulating or altering an outer membrane protein channel which the drug requires for cell entry(e.g., OMPF in *E. coli*)
d. Up-regulating the pumps that expel the drug from the cell (e.g., efflux of fluoroquinolones in *S. aureus*) (Tenover, 2006).

Genetic aspect for antibiotic resistance mechanism

Resistance can be an intrinsic property of the bacteria themselves or it can be acquired. Acquired bacterial antibiotic resistance can result from a mutation of cellular genes, the acquisition of foreign resistance gene or a combination of these two mechanisms. Thus resistance there is two main way of acquiring antibiotic resistance (Dizidic et al., 2008)

a. Through mutation in different chromosomal loci and
b. Through horizontal gene transfer(i.e., acquisition of resistance genes form other microorganism)

CHAPTER-III
MATERIALS AND METHODS

3.1 Materials

The materials required for this study are listed in Appendix-I.

3.2 Methods

A descriptive cross sectional study was conducted during January-March 2011 at Bir Hospital located in Kathmandu, Nepal. The patients referred by doctors as suspected UTI for culture to the laboratory were the target population in this study.

3.2.1 Data collection

Data on age, sex and type of patient (inpatient and outpatient) were collected from culture and sensitivity request form.

3.2.2 Sample collection and handling

A freshly voided midstream urine samples (10-20 ml) were collected in a sterile wide mouth container. The urine specimens were the delivered to Microbiology Laboratory immediately and processed within one hour.

3.2.3 Culture and identification

Urine specimens mixed well and aseptically inoculated on Blood and MacConkey Agar (HiMedia, India) using calibrated nichrome loop of internal diameter of 4 mm (HiMedia, India). The culture plates were incubated aerobically at 37^{o} C for 24 hours. A positive culture was defined as colony count $>10^{5}$ cfu/ml. All positive cultures were further identified by their cultural characteristic, gram stain and battery of biochemical reaction. *E. coli* colonies were identified initially morphologically and species was verified by biochemical tests such as catalase, oxidase, Triple sugar iron (TSI), Sulphur indole motility (SIM), citrate and urease tests.

3.2.4 Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of *E. coli* isolate was done by modified Kirby Bauer disc diffusion method using commercial disk (HiMedia, India).

When pure culture was obtained, a loopful bacteria was taken from a colony and was transferred to the tube containing 5 ml peptone broth and mixed gently to obtain homogenous suspension. The turbidity of the suspension was then adjusted to density of McFarland 0.5 in order to standardize inoculum size.

A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Muller Hinton agar (HiMedia, India). The inoculated plates were left for 3-5 minutes at room temperature and with the aid of sterile forceps the following concentration of antibiotics discs were put on the surface of Muller Hinton Agar (HiMedia, India): ampicillin (10 µg), cephalexin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), norfloxacin (10 µg), nitrofurantoin (300 µg) and co-trimoxazole (1.25/23.75 µg) and were incubated for 18-24 hrs at 37^{o} C. Zone of inhibition after incubation were observed and the diameter of inhibitory zones were measured in millimetres (mm). The interpretation of the measurement as Sensitive (S) and Resistant (R) was made according to the manufacture's standard zone size interpretative manual.

The percentage resistance was calculated using the formula PR= a/b×100, where 'PR' was percentage resistance, 'a' was the number of resistant isolates and 'b' was the number of isolates tested with the antibiotic. The percentage sensitivity was calculated using the formula PS= c/d ×100, where 'PS' was percentage sensitivity, 'c' was the number of sensitive isolates and 'd' was the number of isolates tested with the antibiotic.

The result was interpreted according to the most recent version of CLSI.

MDR was defined as resistance to two or more class of the antimicrobial tested.

Determination of multiple antibiotic resistance index (MAR)

MAR index was determined using the formula MAR=x/y, where x was number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for sensitivity (Akinjogunla and Enabulele, 2010).

3.2.5 Qualit control for test

In this study, quality and accuracy of all test was maintained by following standard procedures of collection, isolation and identification. For identification and standardization of the Kirby-Bauer test, standard culture of *E. coli* ATCC 25922 was used as a reference strain.

For quality control, media, antibiotics and reagents were prepared, stored and utilized as recommended by the manufacturing company. Antibiotics discs were stored at refrigerator temperature. For each batch of test, a positive and negative known culture was used for colour reaction, biochemical test and antibiotic sensitivity test.

3.2.6 Data management and analysis

All raw data obtained from laboratory investigation were tabulated and presented in defined tables to explore the major findings. The data were statistically analyzed by Chi-square (χ^2) test at 5.0 % level of significance by entering the data in the computer based PASW (Predictive Analytical Soft Ware), version 18.0, the premier vendor for (Statistical Package for the Social Sciences) program. A p-value of less than or equal to 0.05 is considered to be statistically significant ($p \leq 0.05$).

CHAPTER –IV
RESULTS

4.1 Characteristics of patients requesting for urine culture

A total of 554 outpatients, 209 were male and 345 were female, whereas out of 185 inpatients, 77 were male and 108 were female.

Table 1: Type of patients (inpatients and outpatients) requesting for urine culture

Type of patients	Male (%)	Female (%)	Total
Outpatients	209(37.7)	345(62.3)	554
Inpatients	77(41.6)	108(58.4)	185
Total	**286**	**453**	**739**

4.2 Culture positive rate in urine samples

A total of 739 urine samples, 202 (27.3%) samples showed significant growth, whereas majority of samples i.e., 298 (40.3%) showed no growth, 216 showed non-significant growth, and out of total only 23 samples showed mixed growths.

Table 2: Culture positive rate in urine samples

Culture result	Number of samples	Percent
Significant growth	202	27.3
Non-significant growth	216	29.2
No growth	298	40.3
Mixed growth	23	3.1
Total	**739**	**100.0**

In case of outpatients among total of 141 isolates, 44 (31.2%) were from males and 97 (68.8%) from females. Whereas from inpatients, among 61 isolates, 26 (42.6%) from males and 35 (57.4%) from females.

Table 3: Sex wise distribution of significant growth of uropathogens

Sample	Male (%)	Female (%)	Total (%)	p-value
Outpatients	44(31.2)	97(68.8)	141(69.9)	0.117
Inpatients	26(42.6)	35(57.4)	61(32.1)	
Total	**70(34.7)**	**132(65.3)**	**202(100.0)**	

A total of 141 isolates in outpatients, maximum isolates 97 (68.8%) were found to be gram negative bacilli and the remaining 44 (31.2%) were found to be gram positive cocci. Similarly, in case of inpatients, the gram negative bacilli were predominant i.e., 37 (60.7%) followed by gram positive cocci i.e., 19 (31.1%). And remaining were yeast cell i.e., 5 (8.2%).

Table 4: Percentage of isolated pathogens in culture positive patients

Uropathogens	Outpatients (%)	Inpatients (%)	Total (%)
Gram negative bacilli	97(68.8)	37(60.7)	134(66.3)
Gram positive cocci	44(31.2)	19(31.1)	63(31.2)
Yeast cells	0(0.0)	5(8.2)	5(2.5)
Total	**141(100.0)**	**61(100.0)**	**202(100.0)**

E. coli was the most predominant uropathogen followed by CoNS (21.3%), *Enterococcus fecalis* (7.3%), *Pseudomonans aeruginosa* (4.0%) and *Klebsiella* spp. (2.7%). *Acinetobacter* spp. and *Candida* spp. were 2.2% each. Other bacterial isolates were 1.0% or less than 1.0%.

Table 5: Spectrum of uropathogens isolate from urine sample

Uropathgens	Total isolates	Percent
Escherichia coli	109	54.0
Coagulase negative staphylococci(CoNS)	43	21.3
Enterococcus fecalis	16	7.3
Pseudomonas aeruginosa	10	4.0
Acinetobacter spp.	5	2.5
Candida spp.	5	2.5
Klebsiella pneumoniae	6	2.7
Staphylococcus aureus	4	1.0
Citrobacter spp.	2	1.0
Enterobacter spp.	1	0.5
Salmonella Typhi	1	0.5
Total	**202**	**100.0**

The distribution of UTIs in both genders was found to be maximum in age group 16-49 years i.e., 61.4% and 71.0% in male and female, respectively. Similarly, in both gender the distribution of UTIs in least in age group 1-56 years i.e., 2.9% and 3.0% in male and female, respectively.

Table 6: Distribution of UTIs in males and females of different age groups

Age groups(Years)	Male	Percent	Female	Percent
1-15	2[a]	2.9	4	3.03
16-49	43	61.4	95	71.0
50-64	11	15.7	19	14.4
>65	14	20.0	14	10.6
Total	**70**	**100**	**132**	**100.0**

[a] Number of cases representing a given age group out of total 202 cases.

4.3 Distribution of uropathogens

The distribution of *E. coli* were found to be the most frequent in age group 16-49 years in both sexes i.e., 13 and 60 in male and female, respectively. In age group 1-15 years only two *E. coli* isolated from female.

Table 7: Age wise distribution of *E. coli* isolates form urine samples

Age group(years)	Male	Female	Total
1-15	0	2	2
16-49	13	60	73
50-64	6	15	21
>65	5	8	13
Total	**24**	**85**	**109**

E. coli the most common uropthogen isolated from female patients comparatively to the male patients and it is statistically siginificant ($p<0.05$). Isolation of CoNS is more common among male patient; however, it is statistically insignificant ($p>005$).

Table 8: Distribution of Uropathogens among males and females

Uropathogens	Male (%)	Female (%)	p- value
Escherichia coli	24(34.3)	85(64.4)	0.0001
Coagulase negative staphylococcus(CoNS)	22(31.4)	21(15.9)	0.084
Enterococcus fecalis	9(12.9)	7(5.3)	0.145
Pseudomonas aeruginosa	4(5.7)	6(4.5)	1.0
Acinetobacter spp.	1(1.4)	4(3.0)	0.654
Candida spp.	2(2.9)	3(2.3)	1.0
Klebsiella pneumoniae	3(4.3)	3(2.3)	0.682
Staphylococcus aureus	2(2.9)	2(1.5)	0.643
Citrobacter spp.	2(2.9)	0(0.0)	0.149
Enterbacter spp.	0(0.0)	1(0.8)	1.0
Salmonella Typhi	1(1.4)	0(0.0)	0.387
Total	**70(100.0)**	**132(100.0)**	

E. coli is frequently encounterd among outpatients. However, the distribution of *E. coli* among outpatient and inpatients are statically insignificant ($p>0.05$). Similarly, CoNS and *Enterococcus fecalis* also frequently encountered among outpatients and distribution among outpatients and inpatients are statically insignificant ($p>0.05$).

Table 9: Distribution of uropathogens among inpatients and outpatients

Uropathogens	Outpatients (%)	Inpatients (%)	p- value
Escherichia coli	85(60.3)	24(39.3)	0.431
Coagulase negative staphylococcus(CoNS)	33(23.4)	10(16.4)	0.782
Enterococcus fecalis	10(7.1)	6(9.8)	0.249
Pseudomonas aeruginosa	4(2.8)	6(9.8)	0.019
Acinetobacter spp.	5(3.5)	0(0.0)	0.339
Candida spp.	0(0.0)	5(8.2)	0.001
Klebsiella pneumoniae	2(1.4)	4(6.6)	0.037
Staphylococcus aureus	1(0.7)	3(4.9)	0.05
Citrobacter spp.	0(0.0)	2(3.3)	0.062
Enterbacter spp.	0(0.0)	1(0.0)	0.25
Salmonella Typhi	1(0.7)	0(0.0)	1.0
Total	**141(100.0)**	**61(100.0)**	

Of the 24 male *E. coli* UTI cases, 5 (20.8%) were from inpatients, whereas 19 (19.2%) were from outpatients. Similarly, in 85 female *E. coli* UTI cases, 19 (22.4%) were from male and 66 (77.7%) were from outpatients.

Table 10: Sex wise and inpatients and outpatients distribution of *E. coli*

Sex	No. of patients	Inpatients	Outpatients	p-value
Male	24	5(20.8%)	19(22.4%)	0.874
Female	85	19(19.2%)	66(77.7%)	
Total	**109**	**24**	**85**	

4.4 Antibiotic susceptibility pattern of *E. coli*

Majority of *E. coli* showed susceptibility towards nitrofurantoin (94.5%) followed by ciprofloxacin and ofloxacin with the susceptibility of 50.5% for each drug. Cephalexin (7.3%) were found least effective drug followed by ampicillin (18.3%). Norfloxacin, nalidixic acid and co-trimoxazole were found effective only for less than half of the isolates of *E. coli.*

Table 11: Antibiotic susceptibility pattern of *E. coli*

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	20(18.3)	89(81.7)
Cephalexin	8(7.3)	101(92.7)
Nalidixic acid	23(21.1)	86(78.9)
Ciprofloxacin	55(50.5)	54(49.5)
Ofloxacin	55(50.5)	54(49.5)
Norfloxacin	51(46.8)	58(53.2)
Co-trimoxazole	50(45.9)	59(54.1)
Nitrofurantoin	103(94.5)	6(5.5)

Table 12: Antibiotic susceptibility pattern of *E. coli* isolates

S.No	Amp	Na	Cp	Cip	Of	Nx	Nit	Cot
1	S	R	R	S	S	S	S	R
2	R	R	R	S	S	S	S	R
3	R	R	R	R	R	R	S	R
4	S	S	R	S	S	S	S	S
5	R	R	R	R	R	R	R	R
6	R	R	R	S	S	S	S	S
7	S	R	S	S	S	S	S	R

S.No	Amp	Na	Cp	Cip	Of	Nx	Nit	Cot
8	R	R	R	R	R	R	S	S
9	S	R	S	R	R	R	S	R
10	S	S	R	S	S	S	S	S
11	S	S	R	S	S	S	S	S
12	S	S	R	S	S	S	S	S
13	R	R	R	R	R	R	S	R
14	S	R	S	S	S	S	S	R
15	S	R	S	S	S	S	S	R
16	S	R	S	S	S	S	S	R
17	R	R	R	R	R	R	S	R
18	R	R	R	S	S	S	S	R
19	R	R	R	R	R	R	S	S
20	R	R	R	R	R	R	S	R
21	R	R	R	R	R	R	R	R
22	R	R	R	S	S	S	S	R
23	R	R	R	S	S	S	S	R
24	R	R	R	R	R	R	S	R
25	S	S	R	S	S	S	S	S
26	S	R	R	S	S	S	S	S
27	R	R	R	R	R	R	S	S
28	R	R	R	R	R	R	S	S
29	R	R	R	R	R	R	S	R
30	R	R	R	S	S	S	S	R
31	S	S	S	R	S	S	S	S
32	R	R	R	R	R	R	S	S
33	R	R	R	S	S	S	S	S
34	R	R	R	R	R	R	S	R
35	R	R	R	S	S	S	S	S
36	R	R	R	R	R	R	S	R
37	R	R	R	R	R	R	S	R
38	R	R	R	R	R	R	S	R
39	R	R	R	R	R	R	S	S
40	S	R	R	S	S	S	S	S
41	R	R	R	R	R	R	S	R
42	S	R	R	S	S	S	S	S
43	R	R	R	S	S	R	S	S
44	S	R	S	S	S	S	S	S
45	S	R	S	S	S	S	S	S
46	R	R	R	R	R	R	S	R
47	R	R	R	R	R	R	R	R
48	R	R	R	R	R	R	S	R
49	R	S	R	S	S	S	S	S

S.No	Amp	Na	Cp	Cip	Of	Nx	Nit	Cot
50	R	R	R	R	R	R	S	R
51	R	R	R	R	R	R	S	R
52	R	R	R	R	S	R	S	S
53	R	R	R	R	R	R	S	R
54	R	S	R	S	S	S	S	S
55	R	R	R	S	S	R	S	R
56	R	R	R	S	S	S	S	S
57	R	R	R	R	R	R	S	S
58	R	R	R	S	R	R	S	R
59	R	R	R	S	S	S	S	R
60	S	R	R	S	S	S	S	S
61	R	R	R	R	R	R	S	R
62	R	R	R	S	S	S	S	R
63	R	R	R	R	R	R	R	R
64	R	R	R	R	R	R	R	R
65	R	R	R	S	S	S	S	R
66	R	S	R	S	S	S	S	S
67	R	R	R	R	R	R	R	S
68	R	R	R	R	R	R	S	R
69	R	R	R	R	R	R	S	R
70	R	S	R	S	S	S	S	S
71	R	R	R	S	S	S	S	S
72	R	S	R	S	S	S	S	S
73	S	S	R	S	S	S	S	S
74	R	R	R	S	S	S	S	R
75	R	R	R	R	R	R	S	R
76	R	R	R	R	R	R	S	R
77	R	R	R	R	R	R	S	R
78	R	R	R	R	R	R	S	S
79	R	S	R	S	S	S	S	S
80	R	R	R	R	R	R	S	S
81	R	R	R	R	R	R	S	R
82	R	R	R	R	R	R	S	R
83	R	S	R	S	S	S	S	S
84	R	S	R	S	S	S	S	S
85	R	R	R	S	S	S	S	R
86	R	R	R	S	R	R	S	R
87	R	S	R	S	S	S	S	S
88	R	S	R	S	S	S	S	S
89	R	S	R	S	S	S	S	S
90	R	R	R	R	R	R	S	S
91	S	S	R	S	S	S	S	S

S.No	Amp	Na	Cp	Cip	Of	Nx	Nit	Cot
92	R	R	R	R	R	R	S	S
93	R	R	R	R	R	R	S	R
94	R	R	R	R	R	R	S	R
95	R	R	R	R	R	R	S	R
96	R	S	R	S	S	S	S	S
97	R	R	R	S	S	R	S	R
98	R	R	R	R	R	R	S	S
99	R	R	R	R	R	R	S	S
100	R	R	R	S	S	S	S	R
101	R	R	R	R	R	R	S	S
102	R	R	R	R	R	R	S	R
103	R	R	R	R	R	R	S	R
104	R	S	R	S	S	S	S	R
105	R	S	R	S	S	S	S	S
106	R	S	R	S	S	S	S	R
107	R	R	R	S	S	S	S	R
108	R	R	R	R	R	R	S	R
109	R	R	R	R	R	R	S	S

Note: Amp-Ampicillin, Cp-Cephalexin, Na-Nalidixic acid, Cot-Co-trimoxazole, Nx-Norfloxacin, Cip-Ciprofloxacin, Of-Ofloxacin, Nit-Nitrofurantoin, S-Sensitive, R-Resistant

E. coli isolates from females showed relatively less resistance than males. Nitrofurantoin is the most effective drug in both sexes, whereas cephalexin is the least effective antibiotic in both male and female.

Table 13: Sex-wise antibiotic resistance pattern of *E. coli*

Antibiotic	Male		Female	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ampicillin	4(16.7)	20(83.3)	16(18.8)	69(81.2)
Cephalexin	1(4.2)	23(95.8)	7(8.2)	78(91.8)
Nalidixic acid	2(8.3)	22(91.7)	24(24.7)	64(75.3)
Ciprofloxacin	12(50.0)	12(50)	43(50.6)	42(49.4)
Ofloxacin	13(50.0)	12(50)	43(50.6)	42(49.4)
Norfloxacin	11(45.8)	13(54.2)	40(47.1)	45(52.9)
Co-trimoxazole	11(45.8)	13(54.2)	39(45.9)	46(54.1)
Nitrofurantoin	22(91.7)	2(8.3)	81(95.3)	4(4.7)

E. coli isolated from the outpatients showed less resistance than in inpatients. Nitrofurantoin is the most effective, whereas cephalexin in least effect in both patients. All of the isolates showed resistance towards cephalexin in inpatients.

Table 14: Type of patient wise antibiotic resistance pattern of *E. coli*

Antibiotic	Outpatients		Inpatients	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ampicillin	19(22.4)	66(77.6)	1(4.2)	23(95.8)
Cephalexin	8(9.4)	77(90.6)	0(0.0)	24(100)
Nalidixic acid	22(25.9)	63(74.1)	1(4.2)	32(95.8)
Ciprofloxacin	51(60.0)	34(40)	4(16.7)	20(83.3)
Ofloxacin	51(60.0)	34(40.0)	4(16.7)	20(83.3)
Norfloxacin	48(56.5)	37(43.5)	3(12.5)	21(87.5)
Co-trimoxazole	44(51.8)	41(41.2)	6(25)	18(75)
Nitrofurantoin	83(97.6)	2(2.4)	20(83.3)	4(16.7)

MDR was defined as resitance to 2 or more classes of antibiotics. MDR was detected in 90.8% isolates. Among the 99 (90.8%) MDR strains, 21(19.3%) were resistant to two antibiotics and 78 (71.6%) were resistant to three or more antibiotics.

Table 15: Multidrug resistance (MDR) pattern of *E. coli*

Bacterial isolate	No. of isolates	Resistance to Antibiotic					
		MDR Strains				Total	Percent
		0 Drug	1 Drug	2 Drug	>2 Drug		
Escherichia coli	109	0	10	21	78	99	90.8

Sixteen multidrug resistance atterns were observed in *E. coli* for the eight antimicrobial agents tested. Resistance to Amp-Na-Cp-Cip-Of-Nx-Nit was the most frequent pattern observed in 30.3% of *E. coli* isolates.Whereas Na-Cp-Cot, Amp-Na-Cp-Nx, Amp-Na-Cp-Cip-Nx, Na-Cip-Of-Nx-Cot and Amp-Na-Cp-Cip-Of-Nx-Nit were the least frequent pattern observed in 1.0% of *E. coli* for each.

Table 16: Antibiotic resistance pattern of MDR *E. coli* strains

Antibiotic resistant pattern	**Number (%)**
Amp-Cp	13(13.1)
Na-Cp	4(4.0)
Na-Cot	4(4.0)
Amp-Cp-Cot	2(2.0)
Amp-Na-Cp	5(5.0)
Na-Cp-Cot	1(1.0)
Amp-Na-Cp-Nx	1(1.0)
Amp-Na-Cp-Cot	12(12.0)
Amp-Na-Cp-Nx-Cot	2(2.0)
Amp-Na-Cp-Cip-Nx	1(1.0)
Na-Cip-Of-Nx-Cot	1(1.0)
Amp-Na-Cp-Cip-Of-Nx	15(15.0)
Amp-Na-Cp-Of-Nx-Cot	2(2.0)
Amp-Na-Cp-Cip-Of-Nx-Cot	30(30.3)
Amp-Na-Cp-Cip-Of-Nx-Nit	1(1.0)
Amp-Na-Cp-Cip-Of-Nx-Nit-Cot	5(5.0)
Total	**99(100.0)**

Note: Amp-Ampicillin, Cp-Cephalexin, Na-Nalidixic acid, Cot-Co-trimoxazole, Nx-Norfloxacin, Cip-Ciprofloxacin, Of-Ofloxacin, Nit-Nitrofurantoin

Out of 109 *E. coli* isolates only 10 isolates showed MAR index of 0.1 i.e., these isolates were only resistance to only one antibiotic. However, 5 isolates showed MAR index of 1 i.e., these isolates were resistance to all antibiotic used in antibioitic susceptibility testing.

Table 17: Multiple antibiotic resistance (MAR) indices of *E. coli*

MAR index	Frequency of MAR index
	***E. coli* (n=109)**
0	0(0.0)
0.1	10(9.2)
0.2	21(19.3)
0.3	8(7.3)
0.4	0(0.0)
0.5	13(11.9)
0.6	4(3.7)
0.7	17(15.6)
0.8	31(28.4)
0.9	0(0.0)
1.0	5(4.6)

CHAPTER-V
DISCUSSION

This study was conducted among outpatients and inpatients suspected of urinary tract infection, attending Bir Hospital, Kathmandu, Nepal. Seven hundred thirty nine midstream urine samples subjected to culture and one hundred nine were *E. coli. E. coli* were further subjected to antimicrobial susceptibitiy testing.

In this study, 27.3% urine specimens from suspected UTI patients gave significant growth. The result is an agreement with that reported by other investigators from Nepal (Chhetri et al., 2001; Rai et al., 2008; Kumari et al., 2005; Karki et al., 2004) and rest of the world (Bashar et al., 2009; Levitt, 1993; Obi et al., 1990). The majority of urine specimens showed no growth (40.3%). This may be due to that patients were already undergoing antibiotics therapy which have inhibited or destroyed bacterial growth (Okonofua and Okonofu, 1989), or slow growing organisms and those organisms that were not able to grow on the routine culture media used (Kattel et al., 2008). Dimitrov et al., 2003 found that only 11.4% urine specimens yielded significant growth of uropathogens. Similarily, Akram et al., 2007; Beyene and Tsegaye, 2011 and Gupta et al., 2007 reported low rate of significant growth of uropathogens from urine samples. However, Saeed and Mohameed, 2010; Das et al., 2006 and Arjunan et al., 2010 found 70.0%, 71.7% and 55.2% significant growth of uropathogens, respectively.

In this study, the most predominant organism isolated among UTIs patients attending Bir Hospital were *E. coli* (54.0%). This study shows that commonest isolates were *E. coli* (54.0%), CoNS (21.3%), *Enterococcus fecalis* (7.3%), *Pseudomonas aeruginosa* (4.0%) and *Klebsiella* spp. (2.7%). In study from Kathamnadu, in combined group, outpatients as well as inpatients *E. coli* (59.6%) is commonest followed by *Staphylococcus aureus* (12.5%), *Klebsiella* spp. (10.8), *Enterococcus fecalis* (7.9%) and *Pseudomonas aeruginosa* (5.0%) (Kattel et al., 2008).

In our study, *E. coli* was isolated as the most predominant isolate and accounted for 54.0% of the total uropathogens. This finding agreed with other study done by Kattel et al., 2008, Jha and Bapat, 2005, Das et al., 2006 and Chhetri et al., 2001 in Nepal. The result is also agreed with the study done at international context, which indicated that the

gram negative bacteria mostly *E. coli* was the commonest bacteria isolated in patients with UTI (Ahmed and Imran, 2008; Amin et al., 2009; Hasen et al., 2007; Manjunath et al., 2011; Biadglegue and Abera, 2009; Omigie et al., 2009; Tambekar et al., 2006; Alzohairy and Khadri, 2011; Astal, 2005). However, differ from the report of Ehinmidu, 2003 and Aboderin et al., 2009 which reported *Pseudomonas aeruginosa* and *Klebsiella* spp., respectively as the predominant bacteria. Whereas, Aypak et al., 2009; Bobos et al., 2010 and Hryniewicz et al., 2001 has reported still higher incidence of *E. coli* 71.3%, 73.0% and 76.8% respectively in urine sample.

In the present study 54.0% of the microorganisms were *E. coli*, but there was a significant difference between males and females: among males only 34.3% of the pathogens were *E. coli* compared to 64.4% among females ($p<0.05$). The incidence of CoNS in our study is high 21.3%, with predominance in men at 31.4% vs15.9% in women ($p<0.05$). However, the incidence of *Klebsiella* spp. in our study is low 2.7%, with predominance in men at 4.4% vs 2.3% in women ($p>0.05$).

In contrary to other study finding where second reported isolates was *Klebsiella* spp. (Kumari et al., 2005; Bahandin et al., 2011; Ramesh et al., 2008; Amin et al., 2009; Nwadioha et al., 2010), in this study was CoNS which is in agreement with the finding of Enayat et al., 2008 and Zia and Hassanshahi, 2010. Our findings demonstrated that these pathogens play important role in UTI and 21.3% of UTI in our study were caused by coagulase negative staphylococcus (CoNS) and there was no significant difference between male and female ($p>0.005$). The frequency of this type of UTI in our study was not sex dependent.

Coagulase negative Staphylcocci (CoNS) were second most common pathogen in our study, whereas *Enterococcus* spp. were the third most cause of UTI. Al Benwen et al., 2010 has reported *Streptococcus agalactiae* as second most common isolate after *E. coli* in their study on 56,506 urine samples. Khan and Ahmed, 2001 has reported *Candida* spp., the second most common isolate after *E. coli*. Factors such as the changing patient population, extensive use and misue of antimicrobial agents could all contribute to changes in the bacterial profile of UTI (Brosnema et al., 1993). Adedeji and Adbulkadir, 2009 reported *Staphyloccus saprophyticus* as the second most common cause of UTI with isolation rate of 23.8%. *Staphylococcus saprophyticus* is usually found in infection

among sexually active young women (Chessbrough, 2006). However, in our study in present study the incidence of CoNS was higher in male as compared to female, however, isolation of CoNS with sex of patients is statistically insignificant ($p>0.05$). The reason for the higher prevalence of CoNS in males is not clear, though lack of circumcision, receptive anal intercourse and HIV infection recogniszed risk factor for in males (Orrett and Davis, 2006).

Enterococcus spp. is the thirs most common pathogen in our study. Similar reporting is done by Alzohairy and khadir, 2011; Vasquez and Hand, 2004 and Manjunath et al., 2011 showing that now gram positive bacteria are becoming one of the main uropathogens of UTI. *Enterococcus* often is a problem in complicate UTI, in patients with indwelling uretheral Cathers, or patients receiving broad spectrum antibiotics for another infection (Dimitrov et al., 2003). The higher prevalence of *Enterococcus* (7.3%) in this study is consistent with the fact the patient in this study were with indwelling cather or treated with broad spectrum antibiotic for another infection.

In our study, out of total 202 uropathogens, 66.3% were gram negative bacilli, 31.2% were gram positive cocci and 2.5% were yeast cells. This study is similar to study done by Khattak et al., 2006, 66.7% isolates were gram negative bacilli, 27.8% were gram positive cocci, and 5.6% were yeast cells. Van Norstrand et al., 2000, also found gram negative bacilli in 67.0%, gram positive cocci in 25.0% and yeast cells in 8.0%. Astal, 2005, found 88.8% gram negative bacilli, 8.7% gram positive cocci and 2.4% yeast cells.

The study revealed that females (63.3%) were more susceptible to UTI than males (34.7%), which is also similar to other studies (Manjunath et al., 2011; Akram et al., 2007; Dimitrov et al., 2003; Amin et al., 2009; Arjunan et al., 2010; Bashir et al., 2008; Alzohairy and Khadri, 2011). The increased incidence of the urinary tract infection in women is conditioned by favoring anatomic factors, by hormonal changes and by the urodynamic disturbance occurring with age (Bobos et al., 2010).

The activities of the antibiotics investigated in this study against *E. coli* in female patients are as follows, in decreasing order: nitrofurantoin > ciprofloxacin = ofloxacin > norfloxacin > co-trimoxazole > nalidixic acid > ampicillin > cephalexin. For isolates from male patients, as in order is: nitrofurantoin > ciprofloxacin = ofloxacin>norfloxacin=co-

trimoxazole > ampicillin > nalidixic acid > cephalexin. The higher rate of antibiotic resistance to all agents observed in male as compared to female. This is may be due to the complicated nature of UTI in male (Lipsky et al, 1989). Also, UTI in male patient may be associated with more antimicrobial resistant pathogens (Sahm et al., 2001). The similar higer rates of antibiotic resistance to male patients were observed by Bean et al., 2008 and Sahm et al., 2001.

UTI affects all age groups, many study proved that sexually active person have more chance having UTI (Foxman et al., 1997). The present study also supports this likelihood as more than 50.0% UTI positive individual were between 16 and 49 years. It is also found that women aged greater than 49 years were more affected than age group 1-15 years but less than 16-49 years. This is might because during post menopause their significance change occurs in urethra when *E. coli* can outnumber the Lactobacilli (normal flora) and may easily cause UTI (Schaeff, 2001).

Isolation of *E. coli* from females and outpatients were higher than that of males and inpatients. However, isolation of *E. coli* according to sex of patient and type of patients were statistically insignificant ($p>0.05$). As for the patients status, this study revealed that there was higher rate of community based than nosocomial *E. coli* associated UTI cases. There were 85 (78.0%) outpatients as compared to 24 (22.0%) inpatients cases of *E. coli* associated UTI. This is similar to finding of Rafique et al., 2002, 141 (70.5%) were outpatients and 59 (29.5%) were inpatients cases of *E. coli* associated UTI. This discrepancy may be due to poor hygienic conditions within our community, our house hold, a lack of education and proper personal hygienic practice. Similarly, 24 (22.0%) were male, whereas 85 (78.0%) were female. This is comparable to the study done by Khan and Zaman, 2006, where 18 (17.6%) were males and 84 (82.4%) were females. As far for the inpatients cases, it is well documented that numerous factors, including poor patients care in the hospitals, catherization and other surgical procedures related to lower abdomen, bowel region are highly associate with UTI in inpatients (Stamm and Norby, 2001; Raz, 2001; Sottoo et al., 2001).

MAR index is a tool that reveals the spread of bacteria resistance in a given population (Krumpermann, 1983). An MAR index greater than 0.2 implies that the strains of such bacteria originate from an environment where several antibiotics are used (Ehinmidu,

2003). The MAR indices of *E. coli* obtained in this study is a possible indication that a very large proportion of the bacteria isolates have been exposed to several antibiotics.

Most *E. coli* isolates in our study were resistant to ampicillin (81.7%) which is resembles other studies (Rashedmarandie et al., 2008; Farshad et al., 2010a; Farshad et al., 2011; Khorshidi et al., 2003; Behroozi et al., 2010).

Overall quinolone resistance of *E. coli* were 57.7%. Increased resistance in Quilones against *E. coli* may reflect the overuse of quilones for treatment of UTI (Saleh et al., 2009). Another factor could be the generalized use of fluoroquinolone in animal feed (especially in poultry), and the subsequent transmission of resistant strains from animals to humans (Miller and Tang, 2004).

Acar and Goldsein, 1997 found that quinolone resistance is higher in developing countries than in developed nations because the use of less active quinolones, such as nalidixic acid, and the use of low dosages of more potent compounds such as ciprofloxacin, resulting in selection of mutant isolates.

MDR was defined as resistance to two or more classes of antibiotics (CDC, 2006). To assess the current breadth of MDR among urinary isolates of *E. coli*, the antibiotic susceptibility was carried out using 8 antibiotics. Most of the *E. coli* isolates showed the MDR (90.8%) in agreement with other studied that found MDR *E. coli* ranging from 67 to 100.0% (Moyo et al., 2010; Bashar et al., 2009; Farshad et al., 2008; Farshad et al., 2010b; Gupta et al., 2007; Hassan et al., 2011). Such high MDR has serious implications for the empirical therapy of infection caused by *E. coli* (Farshad et al., 2008). The higher MDR may be due to large portion of *E. coli* isolates being previously exposed to several antibiotics.

Another study conducted in Poland revealed that the resistance pattern of hospital *E. coli* was similar to that of community isolated except for those found to produce extended spectrum B-lactamsese (ESBL). Other species of Enterobacteriaceae were more resistant when isolated from the hospital setting (Hryniewicz et al., 2001). According to this study, MDR was usually related to production of ESBL, in both community and hospital isolates.

Nitrofurantoin is an effective urinary tract antiseptic that is not used for other kinds of infection. It does not affect antibiotic use in any other infection (Bosch et al., 2011). In our study only 5.5% isolates were resistance to nitrofurantoin. This data agreement with Bean et al., 2008; Biadglegne and Abera, 2009; Moniri et al., 2003 and Bahadin et al., 2011. Nitrofurantoin was found the most effective drug in our study. Nitrofuarantion was also found the most effective antimicrobial in UTI caused by *E. coli* from studies in Nepal (Karki et al., 2003; Sharma et al., 2011), USA (Jamie et al., 2002; Sahm et al., 2001), Nigeria (Okonko et al., 2009); Iran (Behrooozi et al., 2010); Spain (Alos et al., 2004); Turkey (Eryimaz et al. 2010). However, Akram et al., 2007, Kausar et al., 2009 and Arjunan et al., 2010 were found 80.0%, 76.0% and 61.1% *E. coli* resistance to nitrofurantoin, respectively. However, in present study, Nitrofurantoin was found to be the most effective antimicrobial.

Nitrofurantoin is an effective urinary antiseptic that is not used for other kind of infection. It doesn't affect antibiotic use in any other infection (Bosh et al., 2011). Nitrofurantoin has a resistance rate of less than 20.0% in present study. Therefore it should be used as a first choice treatment in our institution.

Nitrofurantoin as an option for empirical therapy has been considered by many authors (Karlowsky et al, 2002). Resistance to nitrofurantoin among *E. coli* isolates from UTIs remains low despite more than 50 year's widespread use of the drug (Mazzulli et al., 2001; Kahlmeter, 2000). Reason for the lack of emerging resistance are not fully understood, but likely include restricting use to indication for urinary infection, limited systemic absorption, and the need for multiple genetic mutations for the bacteria to develop resistance (Nicolle et al., 2006). However, nitrofurantoin demonstrated poor in vitro activity against Enterobacterieacea other than *E. coli* (Farrell et al., 2003). Moreover, this antibiotic doesn't penetrate into tissue and couldn't be used to treat infections with suspected tissue involvement (such as pyelonephritis).

Antimicrobial resistance is a natural biological phenomenon of response of microbes to the selective pressure of antimicrobial drugs. Resistance may be inherent (Ahmed and Imran, 2008). In this study the antimicrobial agents shows that *E. coli* are hightly resistant to commonly used antibiotics i.e., cephalexin, amipicllin, nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin, and co-trimoxazole. The resistance rate of *E. coli* to commonly

used antibiotics was: ampicillin (81.7%), cephalexin (92.7%), nalidixic acid (78.9%), ciprofloxacin (49.5%), ofloxacin (49.5%), norfloxacin (53.2%), co-trimoxazole (54.1%) and Nitrofurantoin (5.5%). Similary, in a study conducted in India in 200 *E. coli* isolates from symptomatic cases of UTI attending outpatient department of Karnatake institute of medical sciences (KIMS) hospital, the resistance rate reported were: ampicillin (91.5%), cephalexin(76.0%), nalidixic acid (93.0%), ciprofloxacin (83.0%), ofloxacin (83), norfloxacin (85.0%), co-trimoxazole (72.0%) and nitrofurantoin (76.0%) (Kausar et al., 2009). The resistance rate was higher than present study except for cephalexin. Similarly, study conducted in England, the resistance rates reported were: ampicillin 55.2%, ciprofloxacin 11.9%, cephalexin 10.0% and nitrofurantoin (5.9%) (Bean et al., 2008). These resistance rates were lower for first three antibiotics than those obtained in present study. Whereas for nitrofurantoin the resistance rate is almost same. The higher values found in this study can be explained by the widespread, frequent and uncontrolled use of antimicrobials.

A North American UTI collaborative Alliance study determined the susceptibility of antibiotics commonly used for the treatment of UTIs, to *E. coli.* Urinary isolates obtained from outpatients in various geographic regions in the USA and Canada, overall resistance to ampicillin was 37.7% followed by co-trimoxazole 21.3%, ciprofloxacin 5.5% and nitrofurantoin 1.1% (Zhanel et al., 2006). The resistance for all these antibiotics found in our study was higher than those in the North American study.

Since the resistance rate of UPEC strains to antimicrobials has been gradually increasing, in order to minimize resistance development, prior to antimicrobial therapy it is important to investigate antimicrobial susceptibilities of pathogens (Eryimaz et al., 2010).

The increasing resistant of co-trimoxazole to *E. coli* has been reported in other studies from Nepal and other countries (Kattel et al., 2008; Rai et al., 2008; Bashar et al., 2009; Haghi-Ashteiani et al., 2006; Biadglegne and Abera, 2009; Abou-Dobara et al., 2010; Jadhave et al., 2011). They were higher than the rate reported in our study. Ampicillin resistant among *E. coli* was 81.7%, which is comparable with other studies (Biadglegne and Abera, 2009; Beheroozi et al, 2011; Rafique et al., 2002; Alzohairy and Khadri, 2011). The ampicillin resistance among urinary tract pathogens is probably due to continuous use of it for many years (Hasan et al., 2011). Earlier it has been reported that

ampicillin has no more effect on urinary tract pathogens (Sahm et al., 2001). On the basis of our finding, antimicrobials such as ampicillin and co-trimoxazole should no longer be recommended for initial empirical therapies for UTIs.

Higher resistance rate to all antibiotics used in this study except for nitrofurantoin may be explained by high and uncontrolled use of these antibiotics in our institute. Morever, all antimicrobial are available in medical shop without requiring the doctor prescriptions in our country. Many factors have contribute to such high rates of resistance, including misuse of antibiotics by health professionals, unskilled practitioners and laypersons, misuse of antibiotics by public (antibiotics can be purchased without prescription), poor drug quality, unhygienic condition accounting for the spread of resistance bacteria, and inadequate surveillance (lack of information from routine antimicrobial susceptibility testing of bacterial isolates and surveillance testing of bacterial isolates and surveillance of antibiotic resistance, all of which are crucial for good clinical practice and for rational policies against antibiotic resistance (Okeke et al.,1999).

The degree of resistance to routine antibiotics by *E. coli* isolates from inpatients was higher than shown by outpatients. This is maybe due to hospital uropathogens are exposed to broad spectrum antibiotics, develop more resistance and hence, are more difficult and takes longer time to treat (Magalit et al., 2004). The high rate of antimicrobial resistance in pathogens isolated in the hospital could possibly be explained by the selective effect of treatment with multiple antimicrobial for a single patient, which may result in the amplification of antimicrobial resistance in some organisms (Archibald et al., 1997; Eickhoff, 1992). The hospital pathogens developed more resistance and therefore, are more difficult and take longer time to treat (Magalit et al., 2004).

CHAPTER- VI
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study showed that *E. coli* was the most common cause of UTI in both outpatients and inpatients in Bir hospital, Nepal. Urinary tract infection is more common in outpatients than in inpatients. UTI is more common in females than males. The present study highlight the resistance of *E. coli* isolates recovered from urine samples obtained from suspected UTI patients to widely prescribed oral antibiotics. Most *E. coli* isolates are highly resistance to commonly prescribed antibiotics (ampicillin, cephalexin, quinolones and co-trimoxazole). This is may be due to injudicious use of antibiotics. It is therefore the prescription of these agents as empiric therapy for suspected UTI should be avoided. Although *E. coli* isolates maintained susceptibility to nitrofurantoin, which should be considered a preferred therapeutic agent once the organism is identified.

6.2 Recommendations

1. As resistance rate of all antibiotics (expect for nitorfurantion) is over 40.0%, polices on prescribing antibiotics must be considered.

2. More than ninety percent MDR strains of *E. coli* were isolated from suspected urinary tract infection patients. Therefore, suspected patients are only treated with antibiotics after obtaining result of antimicrobial susceptibility testing.

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