CHAPTER I

INTRODUCTION

Urinary tract infection could be defined as the persistent presence and proliferation of active microorganisms within the urinary tract. Urinary tract infection implies both microbial colonization of the urine and invasion of the lower or upper urinary tract by microorganisms. Urinary tract infection is the most prevalent disease of the urinary tract that has a high morbidity in both hospital and the community. (Foxman, 2002; Foxman *et al.,* 2000).

The urinary tract includes the kidneys, ureters, urinary bladder and urethra. Though in the distal part of the urethra there resides normal microflora such as *Staphylococcus epidermidis,* viridians and non-hemolytic *streptococci*, diptheroids, non pathogenic *Neisseria* spp. and the rest part of the urinary tract in the healthy human is sterile and do not contain any microorganisms. Urine secreted form kidney is also sterile unless the kidneys or other organs of the urinary tract are infected. But the sterile urine gets contaminated when it passes through urethra which contains a normal microbial flora, thus the voided urine may contain small numbers of bacteria in absence of UTI (Leigh, 1990; Forbes *et al*., 2002).

An infection of Urinary tract occurs when microorganisms usually from digestive tract move up to the urethra and multiply. This route which is now believed to be the most usual one is called as ascending route or haematogenous route. The spread through this route is increased by fecal incontinence in the infants and by sexual activity and possibly by poor hygienic habits in case of adults (Leigh, 1990; Forbes *et al*., 2002). Women have a higher risk of developing a UTI than men; approximately 50% to 70% of women will have UTI during their lifetimes, and 20% to 30% of women will have recurrent episodes. In general women are mostly in higher risk group due to the shortness of the female urethra in comparison to male as well as the proximity of the urethra to the anus from where the fecal bacteria can be easily transferred to the vagina

or the urethra. The prevalence of UTIs has been found to be age and sex dependent (Brooks *et al*., 2004).

Pregnant women are at increased risk for urinary tract infection due to the physiological and hormonal changes during pregnancy. Approximately 90% of pregnant women develop urethral dilatation, which will remain until delivery (Amiri *et al.*, 2009). Besides up to 70% of pregnant women develop glycosuria, which encourages bacterial growth in the urine. The prevalence of bacteriuria also rises with higher parity, older age and lower socio-economic status and in women with diabetes mellitus, sickle cell trait or a past history of UTI (Amiri *et al.*, 2009). Thus periodic testing of urine is recommended by physicians for the pregnant women. Primarily the pregnant women have asymptomatic bacteriuria that is presence of bacteria in urine without the symptoms of UTI and also the presence of leucocytes in urine which is called as pyuria. It is an important risk factor for pyelonephritis, hypertension, pre-eclampsia, fetal wastage, low birth weight and premature birth. Thus in case of pregnant women presence of causative organisms without any symptoms will be treated (Al-Haddad, 2005).

UTI is a serious health problem in the world affecting millions of people each year. It is one of the prime causes of morbidity and mortality in the world affecting all age groups in across the life spans. UTI is the one of the most common bacterial infection came across in clinical practice in Europe and North America. In the global basis, it is estimated that about 150 million cases of UTI occurs per year resulting in 6 billion dollars in direct health expenditure (Harding and Ronald, 1994). In Nepal also UTI is the common disease among Nepalese population. According to Annual Health Report (2006/2007) morbidity of UTI in Nepal is 144143. The geographical distribution of UTI has been found in mountain, hill and terai regions as follows 16175, 78052 and 49911 respectively. The figure may change in case of appropriate laboratory facilities are available (Annual Health Report, 2006).

Nepal which is one of the developing countries having 66% of people illiterate who are not aware of hygienic condition and its impact in their health (Sharma, 1983). These

people are always in the risk of infections caused by different infectious pathogens. Lack of knowledge and poor economic condition inaccessibility to well establish laboratory facilities, incomplete antibiotic therapy, lack of counseling with health professional have been the important reason for the increase in UTI incidence in Nepal. Other risk factor for UTI is the unhygienic and carelessness during the stay in hospitals which also increases the risk of UTI (Sharma, 1983).

Antimicrobial resistances have been the increasing global problem which was first seen in *Escherichia coli* in 1940. The primary factor for the development of and spread of antimicrobial resistance bacteria is due to the injudicious and carelessness in the use of antimicrobial agents (Urassa *et al*., 1997; Khan and Zaman, 2006).

Drug resistances that develop in pathogenic microorganism are confer by Plasmids. R plasmids have been found to be responsible for encoding the genes responsible for one and often many different antimicrobial agents (Brooks *et al*., 2004). These R plasmids are found to be transmitted from resistance strains to the sensitive one which has been found to be facilitated by the environment created by intensity of antimicrobial use together with highly susceptible population to the infection. The use of antibiotics is thus increasing the antimicrobial resistance in pathogenic bacteria. This antimicrobial resistance is now a growing problem in world. Culture and antibiogram shows the trend of bacterial sensitivity and resistance towards the antibiotics. (Collee *et al*., 2001; Sambrook and Russell, 2001).

According to Annual Health Report (2006/2007), yearly numbers of women and their neonates are in the risk of morbidity and mortality due to UTI in Nepal. The rationale of this study is to determine the prevalence of uropathogens in urinary tract infection and multidrug resistant uropathogens in patients visiting the hospital. This study also assessed the clinical symptoms, complaints as reported, age, parity, gravida etc in UTI positive patients. These risk factors associated with the UTI were collected by using semi-structured questionnaire and clinical history form of the patient visiting the Paropakar Maternity and Women's Hospital, Thapathali.

CHAPTER II.

2. OBJECTIVES

2.1. General objective

To conduct a microbiological analysis of UTI in patients visiting Paropakar Maternity and Women's Hospital, Thapathali.

2.2. Specific objectives

1. To isolate and identify the causative agents of UTI patients and perform its antibiogram.
2. To assess the prevalence of uropathogens in patients visiting the hospital.
3. To determine the multidrug resistant uropathogens.
4. To analyze clinical symptoms, occupation, literacy, parity, gravida etc associated with UTI positive cases in patients visiting the hospital.

CHAPTER III.

3. Literature review

3.1. Urinary Tract Infection

Urinary tract infection can be defined as the proliferation of active microorganisms in urine and within the urinary tract which are harmful to their environment. Generally growth of $>10^5$ organisms ml^{-1} from an appropriately and aseptically collected mid-stream clean catch urine sample indicates UTI and this presence of bacteria in urine is called as bacteriuria. The presence of *Chlamydia* spp. and Gonococci in urine is not included because of their unique characters and strict localization in the urethra and genital system (Pokharel, 2004).

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

The clinical signs and symptoms along with urinalysis which reveals bacteriuria and pyuria are considered clinically diagnostics of UTI. The sign and symptoms of UTI may involve fever, chills, dysuria, urinary urgency, frequency, and cloudy or malodorous urine. Bacteriuria refers to the presence of bacteria in the urine. These organisms multiply in the urine and present in a count, which is excessively high or unexplainable by urethral contamination. Pyuria refers to the presence of white blood cells in the urine. It is a marker of inflammation in response to bacterial infection (Griebling, 2009).

According to Kass, Marple and Sandford the criteria to interpret significant bacteriuria is if bacterial count is less than 10^4 CFU/ml indicates contamination, equal to or more

than 10^5 CFU /ml indicate significant bacteriuria and 10^4–10^5 CFU/ml bacterial count indicates low count significant bacteriuria.

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

3.2. Pathogenesis of UTI

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

According to Hooton (2000), the pathogenesis of UTI is complex and is influenced by many host biological and behavioural factors and by the properties of the infecting uropathogens. In any health women primarily most of the uropathogens originate in the rectal flora and enter the bladder through the urethra with a temporary phase of periurethral and distal urethral colonization. It has also been reported that vaginal colonization of uropathogens can also occur from a women's male sexual partner but it has been considered rarely the underlying cause of UTI. However, vaginal colonization has been taken as prerequisite for the UTI and other severe complication of UTI, different risk factors that play important role in causation of UTI primarily do so at least in part by facilitating vaginal colonization. In 6-26% of women vaginal colonization of *E. coli* have been found to occur. Symptomatic UTIs develop only after

the uropathogens present in bladder or kidney induce or stimulate cytokine release which results in inflammatory response and symptoms whereas in case of haematogenous spreading of the UTI by potential uropathogens such as *Staphylococcus aureus* is more likely to occur in the setting of persistent bloodstream infection or urinary tract obstruction (Hooton, 2000; Griebling, 2009).

3.3. Urinary tract and bacterial multiplication in Urine

Except urethra which contains normal microbial flora, all the other organs of the urinary tract are sterile including urine in case of healthy humans. However voided urine may contain small numbers of microbes in absence of UTIs which enters in urine from the urethra (Brooks *et al*., 2004; Simon and Shortlife, 2003).

Human urine have been found as the good culture medium as it contains sufficient glucose and it acts as buffer preventing the change in pH as well as the absent of significant quantities of lysomzyme or immunoglobulin and absence of other defense mechanism of immune system.(Simon and Shortlife, 2003)

3.4. Risk Factors of UTI

There are many different factors which increases the risk of urinary tract infection in humans such as

Vaginal microecology: Vaginal microflora play critical role in facilitating vaginal colonization with coliforms and other uropathogens and thus UTI. The normal flora of vagina, Lactobacilli maintains the pH to less than 4. Any alteration in the presence or concentration of these normal flora which may be due to repeated or prolonged antibiotics use that inhibits Lactobacillus, or use of soap that increase the pH more than 4 have been postulated to have an important role in vaginal colonization with uropathogens. These factors that predispose to vaginal colonization by uropathogens can lead to UTI. However vaginal colonization with uropathogens doesnot inevitably leads to UTI. It is likely that vaginal colonization is usually a necessary predeterminant to UTI, but that other factors, such as sexual intercourse, generally must occur to allow infection to occur (Hooton, 2000; Griebling, 2009).

Gender and sexual activity: The female urethra appears to be particularly prone to colonization with colonic Gram negative bacilli because of its proximity to the anus, its short length (about 4 cm) and its termination beneath the labia. Sexual intercourse causes the introduction of bacteria into the bladder and is temporarily associated with the onset of cystitis; it thus appears to be important in the pathogenesis of UTIs in younger women. An important factor predisposing to bacteriuria in men is urethral obstruction due to prostatic hypertrophy (Stamm, 2003; Griebling, 2009; Leibovici, 1991).

Pregnancy: This predisposition to upper tract infection during pregnancy results from decreased urethral tone decreased ureteral peristalsis and temporary incompetence of the vesicourethral valves (Stamm, 2003). UTIs during pregnancy pose particular risks for both mother and child. It increases the risk for premature birth, infant mortality and later chronic kidney disease. UTIs occurring in the first and third trimester of pregnancy increase the risk for mental retardation and developmental delay in the infant from 1.2% to 2.0%. Infants of women who harbor *Ureaplasma urealyticum* also have increased risk for respiratory infections (Todar, 2002). About 2.0-11.0% of pregnant women have asymptomatic bacteriuria in early pregnancy. The higher prevalence occurs in women of lower socioeconomic status and those with a past history of UTI. From 13.0% to 27.0% of women with asymptomatic bacteriuria in early pregnancy will experience acute pyelonephritis later in pregnancy (Nicolle, 1994; Griebling, 2009).

Bacterial Virulence Factors: Not all strains of *E. coli* are equally capable of infecting the intact urinary tract. Bacterial virulence factors markedly influence the likelihood that a given strain once introduced into the bladder, will cause UTI. Most *E. coli* strains that cause symptomatic UTIs in noncatheterized patients belong to a small number of specific O, K, and H serogroups (Stamm, 2003). Numerous investigations suggest that the strains of *E. coli* that cause UTIs possess certain virulence factors that enhance their ability to colonize and invade the urinary tract. Some of these virulence factors include increased adherence to vaginal and uroepithelial cells by bacterial surface structures (adhesions, in particular, pili), alpha-hemolysin production and resistance to serum-killing activity (Forbes *et al.,* 2002). Uropathogenic *E. coli* (UPEC) causes 90.0% of

UTI in anatomically-normal, unobstructed urinary tracts. The adhesin that has been most closely associated with uropathogenic *E. coli* is the P fimbria. UPEC usually produce siderophores that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce hemolysins which are cytotoxic due to formation of transmembranous pores in host cells. Another factor thought to be involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement-dependent bactericidal effect of serum (Todar, 2002).

The adherence property has also been demonstrated with other species of bacteria. *Proteus* spp. is able to facilitate their adherence to the mucosa of the kidneys. *Proteus* spp. are able to hydrolyze urea via urease production, which results in an increase in urine pH that is directly toxic to kidney cells and also stimulates the formation of kidney stones. Similar findings have been made with *Klebsiella* spp. and *S. saprophyticus*. Motility may be important for organisms to ascend to the upper urinary tract against the flow of urine and cause pyelonephritis (Forbes *et al.*, 2002; Griebling, 2009).

Genetic factors: Increasing evidence suggests that host genetic factors also influence susceptibility to UTI. The number and type of receptors on uroepithelial cells to which bacteria may attach are at least in part genetically determined. Many of these structures are components of blood group antigens and are present on both erythrocytes and uroepithelial cells. For example, P fimbriae mediate attachment of *E. coli* to p-positive erythrocytes and are found on nearly all strains causing acute uncomplicated pyelonephritis (Stamm, 2003; Zaffanello, 2010).

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

Kidney Stones: Kidney stones, in some cases, can cause obstruction followed by infection, particularly pyelonephritis. Symptoms of severe UTI in people with a history of kidney stones may indicate obstruction of the urinary tract, which is a serious condition. Formation of infectious urinary calculi is the most common complication accompanying UTI by members of the genus *Proteus* spp. supported by other studies (Li *et al.,* 2002; Torzewska *et al.,* 2003). Recent studies have shown that men have higher risk of forming renal stone than women (Curhan *et al.,* 1998; Yagisawa *et al.,* 1999). In a study on bacteriology of urinary calculi in relation to UTI, out of 52 patients, 37.0% patients had calculi associated UTI with *E. coli* and *P. mirabilis* being the most common causative microorganisms (Nass *et al.,* 2001). Kumar (2003) found that the prevalence of Renal Stone (RS) was higher without UTI (44.4%) than those with UTI (27.8%) in males. In case of females, the result showed 17.6% and 5.1% in cases with and without UTI.

Diabetes: UTI is an important clinical problem for people with diabetes. UTI is 2-3 times more common in adult diabetic patients than in non-diabetics (Leigh, 1990). There is an increased prevalence of asymptomatic bacteriuria in diabetic women, but not in diabetic men (Zhanel *et al.,* 1991). On a population basis, diabetic women, depending on age, are 6-24 times more likely than non-diabetic women to be admitted for acute pyelonephritis; and diabetic men are 3.4-17 times more likely than their non-diabetic counterparts to be admitted for the same condition (Nicolle *et al.,* 1996). The risk for symptomatic complicated UTIs may also be higher in people with diabetes. In fact, certain UTI-related abscesses are reported only in patients with diabetes. These patients are also at higher risk for fungal-related UTIs. The suggested mechanisms of an increased susceptibility to UTI are decreased antibacterial activity due to 'sweet urine', defects in neutrophil function and increased adherence to uroepithelial cells (Todar, 2002).

Renal transplantation: UTIs are the most common infections following renal transplantation. Their importance is debated. Some reports suggest that UTIs are mostly benign, while other suggests that they may induce graft loss. About 80.0% of patients with cellular rejection had a UTI, suggesting that UTI might trigger a graft rejection

(Takai *et al.,* 1998). UTI is an important cause of morbidity in renal transplant recipients. Around 50.0% of patients suffer from at least one episode of the infection during the first 6 months post transplant (Part *et al.,* 1985). About 20.0% of UTIs occurs during the first year of transplantation. Female recipients have significantly more UTI than males (Russel *et al.,* 2000).

Different other factors.

These uropathogenic bacteria possess certain characteristics that enhance their virulence such as pyelonephrites associated pili (PAP) pili called as P. frimbria. These P. frimbria binds to the P blood group antigen of red blood cells and on a specific galactose disaccharide found on the surfaces of uroepithelial cells. As well as type I fimbria play a similar role in the attachment of the E. coli to adhere to uroendotheial cells. Siderophores produced by E. coli cause hemolysis of the red blood cells to release iron required for its survival. They also lyse lymphocytes and inhibit phagocytosis of neurophils (Todar, 2002). These factors enhance the risk of getting urinary tract infection in humans.

3.5. Causative agents of UTIs

Enteric bacteria (in particular, *E. coli*) have been and remain the most frequent cause of UTI that is the most common microorganism, contributing to 90% of UTIs, is *Escherichia coli, w*here as *Klebsiella, Proteus, Pseudomonas and Staphylococcus* has been encountered less frequently. *E. coli* is the facultative anaerobic Gram negative rods that are found as the normal flora in the intestine of humans and animals. This bacterium is ubiquitous in nature and can well adapt to the changes in the environment such as chemicals, pH, temperature and osmolarity. Uropathogenic *E. coli* first colonizes the feces; its presence in the region increases the risk for it to proceed up the urinary tract and into the bladder during sexual intercourse or by other means (Todar, 2002).

3.6. Categorization of UTI

There have been no definitive criteria for the categorization of UTIs. It has been categorized by different scientist on the basis of different criteria. The classification of UTIs as given by Norby (1990) is shown in table below:

Table 1. Classification of Urinary Tract Infection

Classification on the basis of	Groups	Definition
Symptoms	Asymptomatic	UTI symptoms during the preceding two weeks.
	Symptomatic	Symptoms during the preceding two weeks
Level	Lower (Cystitis)	Bacteriuria limited to the bladder
	Upper (pyelonephritis)	Bacteriuria involving kidneys
Complications	Uncomplicated	No identified anatomical defects, foreign bodies or tumors.
	Complicated	Identified anatomical defects, foreign bodies or tumors.
Recurrences	Sporadic	<2 episodes of UTI in the preceding six months and <3 episodes in the preceding year.
	Recurrent	>2 episodes of UTI in the preceding six months and >3 episodes in the preceding year.

3.6.1. On the basis of level and symptoms

UTIs can also occur without symptoms and with symptoms but very low bacterial levels.

Asymptomatic Urinary Tract Infection (Bacteriuria): When a person has no symptoms of infection but significant numbers of bacteria have colonized the urinary tract, the condition is called asymptomatic UTI (also called bacteriuria). The condition is harmless in most people and rarely persists, although it does increase the risk of developing symptomatic UTIs. Screening for asymptomatic bacteriuria is not necessary during most routine medical examinations except in pregnant women, immune-compromised patients and people undergoing urologic surgery, in which the condition can lead to serious infection (Forbes *et al.*, 2002).

Acute Urethral Syndrome: Patients with this syndrome are primarily young, sexually active women, who experience dysuria, frequency, and urgency but yields fewer

organisms than 10^5 CFU/ml urine on culture. This condition is usually caused by *E. coli or* other bacteria that cause cystitis, but in lower numbers, or by a sexually transmitted disease such as Chlamydia or Gonorrhoea (Forbes *et al.*, 2002).

3.6.2. On the basis of complication

They are also sometimes further defined as either being uncomplicated or complicated depending on the factors that trigger the infections.

Uncomplicated Urinary Tract Infections (UTIs): Uncomplicated infections are only associated with bacterial infection, most often *E. coli.* They occur primarily in otherwise healthy females and occasionally in male infants and adolescent and adult males. Cystitis, pyelonephritis and urethritis are the examples of uncomplicated UTIs (Forbes *et al.*, 2002).

Cystitis: Cystitis is the most common urinary tract infection and is sometimes referred to as *acute uncomplicated UTI.* It occurs in the lower urinary tract (the bladder and urethra) and nearly always in women. Typically, patients with cystitis complain of dysuria, frequency and urgency. These symptoms are due not only to inflammation of the bladder but also to multiplication of bacteria in the urine and urethra (Forbes *et al.*, 2002).

Pyelonephritis: Pyelonephritis usually refers to inflammation of the kidney parenchyma, calices and pelvis after bacterial infection. The typical clinical presentation of an upper urinary tract infection includes fever and flank pain and frequently, lower tract symptoms and sometimes systemic signs of infection such as vomiting, diarrhea, chills, increased heart rate and lower abdominal pain (Forbes *et al.*,2002).

Urethritis: When infection is limited only to the urethra, the infection is known as urethritis. Approximately 30% of women with acute dysuria, frequency and pyuria have mid stream urine cultures that show either no growth or insignificant bacterial growth. Because *Chlamydia trachomatis, Neisseria gonorrhoea* and *Trichomonas vaginalis* are

common causes of urethritis and are considered to be sexually transmitted, it is discussed as a sexually transmitted disease (Forbes *et al.,* 2002).

Complicated UTIs: Complicated UTIs, which occur nearly as often in men as women, are also caused by bacteria but they occur as a result of some anatomical or structural abnormality, such as catheter use in the hospital setting, bladder and kidney dysfunction or kidney transplant. The common feature in most complicated UTIs is the inability of the urinary tract to clear out bacteria because a physical obstruction to urine flow hinders treatment success (Forbes *et al.*, 2002).

Recurrence is common after both complicated and uncomplicated UTIs. After a single uncomplicated acute urinary tract infection, recurrence occurs in approximately 27% to 48% of women. Recurrence is often defined as either reinfection or relapse. About 80% of recurring UTIs are reinfections. A reinfection occurs several weeks after antibiotic treatment has cleared up the initial episode and is caused by a different organism from the one that caused the original episode. Relapse is the less common form of recurrent UTI. It is diagnosed when a UTI recurs within two weeks of treatment of the first episode and is caused by the same organism (Todar, 2002).

3.6.3. On the basis of recurrence

UTIs are classified as primary or recurrent, depending on whether they are the first acquired infection or whether they are repeated infections.

3.6.4. On the basis of anatomic sites

On the basis of anatomic structures UTI is subdivided into two general categories which include (Chakraborty, 2001):

1. Lower tract infection

a. Urethritis

b. Cystitis

2. Upper tract infection

a. Acute pyelitis that is infection of pelvis and kidney.

b. Acute pyelonephritis that is infection of parenchyma of kidney.

3.6.5. On the basis of sources

3.6.5.1. Community acquired UTI

This commonly occurs in patients who are not admitted to the hospital at the time when they are infected. Most commonly the community acquired UTI is caused by *E. coli* (Griebling, 2009)

3.6.5.2. Hospital acquired UTI

This type of UTI occurs due to instrumentation in urinary tract most commonly catheterization. This type of UTI primarily catheter associated bacteriuria is usually asymptomatic. It has been found that bacteriuria occurs in 10-20% of patients who are catheterized but UTI in only 2-6%. Bacteraemia mostly gram negative are the primary causative agents of UTI and it develops in 1- 4% of catheterized patients with UTI causing a significant morbidity (increasing hospital stay and costs). It has a mortality of 13-30%. The organisms originate from the patients perineal flora or the hands of the health care staffs during catheterization, or through the periurethral route along the external catheter surface, or the intraluminal route as a consequence of faulty catheter care. Patients who are catheterized for >30 days or for long term, the prevalence of bacteriuria is virtually 100%, with the change in infecting strains and polymicrobial infection may be present. Treatment of asymptomatic bacteriuria has not found to reduce complication in patients who are catheterized and is thought to be the source for the emergence of resistant strains (Griebling, 2009).

3.6. Urinary tract infection in pregnancy

Pregnancy increases the risk of UTIs. It is the most common medical complication in pregnancy. At around 6th week of pregnancy, due to the physiological changes of pregnancy the ureters begin to dilate. This is also known as "hydronephrosis of pregnancy", which peaks at 22-26 weeks and continues to persist until delivery. Both progesterone and estrogens levels increase during pregnancy and these will lead to decreased ureteral and bladder tone. Increased plasma volume during pregnancy leads to decrease urine concentration and increased bladder volume, interference of and minor injury to the vulvo-vaginal-urethral anatomy during labour. The combination of all these factors leads to urinary stasis and uretero-vesical reflux. Glycosuria in

pregnancy is also another well-known factor which predisposes mothers to UTI (Loh and Sivalingam, 2007).

3.7.1. Types of urinary tract infections in pregnancy

There are three major types of UTI in pregnancy. They are asymptomatic bacteriuria, acute cystitis and acute pyelonephritis. The clinical presentations of these conditions vary.

Asymptomatic bacteriuria: Asymptomatic bacteriuria is defined as a finding of more than 10^5 colony-forming units per ml of urine in a clinically asymptomatic person. This condition may be present even before the mother gets pregnant. There are reports that 1.2 to 5 % of young girls will demonstrate asymptomatic bacteriuria at some time before puberty. The prevalence of asymptomatic bacteriuria in pregnancy is about 10%. Lower serum interleukin-6 levels and serum antibody responses to *E. coli* antigens which occurs in pregnancy has been associated with increased incidence of asymptomatic bacteriuria in pregnancy. Neonatal complications which are associated with asymptomatic bacteriuria include intrauterine growth restriction, low birth weight and pre-term premature rupture of membrane. Maternal complications which are associated with asymptomatic bacteriuria are hypertension, pre-eclampsia and maternal anemia. Without treatment, this condition leads to symptomatic cystitis in about 30% of pregnant mothers of whom about 50% will eventually develop acute pyelonephritis (Loh and Sivalingam, 2007).

Table 2. Maternal and fetal complications of asymptomatic bacteriuria in pregnancy

Maternal complications	Fetal complications
Hypertension	Intrauterine growth retardation
Pre-eclampsia	Intrauterine death
Anaemia	Low birth weight
Chorioamnionitis	Prematurity
Symptomatic acute cystitis	
Acute pyelonephritis	

Acute cystitis: Acute cystitis relates to infection of the urinary bladder. Very often the urethra is also infected. The major distinguishing feature of acute cystitis from asymptomatic bacteriuria is the presence of dysuria, urgency and frequency. Usually the patient remains afebrile. Severe systemic symptoms such as nausea, vomiting, high grade fever and distress are usually absent. Most mothers may not be aware that they are having the infection because urgency and frequency are common symptoms in a normal pregnancy (Loh and Sivalingam, 2007).

Acute pyelonephritis: Acute pyelonephritis is infection of the kidney and the pelvic ureter. It is a serious systemic illness affecting 1-2% of all pregnancies and the most common non-obstetric cause of hospital admission during pregnancy. This complication is characterised by high-grade fever, chills and rigors, headache, nausea, vomiting, lumbar pain and in serious cases, reduced urine output. Without treatment it can cause preterm labour and maternal septicaemia. Recurrent pyelonephritis has been implicated as a cause of intra uterine growth restriction and foetal death. The overall incidence of recurrence is about 2-3% and it can recur during the same pregnancy (Loh and Sivalingam, 2007). Urinary tract infections during pregnancy pose particular risks for both mother and child. If asymptomatic bacteriuria is not detected and treated promptly in pregnant women, as many as 25% develop kidney infection (pyelonephritis), which in turn increases the risk for premature birth, infant mortality, and later chronic kidney disease. Even if kidney infection does not develop, untreated UTIs occurring in the first and third trimester of pregnancy increase the risk for mental retardation and developmental delay in the infant from 1.2% to 2%. Certain strains of *E. coli* can increase the risk for complications during pregnancy, including miscarriage or premature delivery, even if pyelonephritis does not develop. Infants of women who harbor *Ureaplasma urealyticum* also have an increased risk for respiratory infections (Loh and Sivalingam, 2007).

3.7.2. Risk factors of UTI in pregnant women

Although pregnancy does not increase the rates of asymptomatic bacteriuria, it does increase the risk that it will progress to a full−blown infection. About 2% to 11% of pregnant women have asymptomatic bacteriuria and, of those, 13% to 27% will

develop a kidney infection late in their term. (It should be noted, however, that in early pregnancy, frequent urination, a common symptom of UTI, is most likely due to pressure on the bladder.) Although all pregnant women should be tested for UTIs, those at particularly high risk are those with diabetes, sickle cell trait, members of low-income groups, women who have had many children, history of childhood UTIs, women who have undergone a cesarean section with catheterization of the bladder, women who have received epidural anesthesia (Zeighami *et al*., 2008; Amiri *et al*., 2009).

Women who have had a UTI before or during pregnancy also have a higher risk of developing recurrent urinary tract infections after delivery. Approximately 25% to 33% of women who experience bacteriuria during pregnancy will have another urinary tract infection, sometimes as long as 10 to 14 years later (Zeighami *et al*., 2008; Amiri *et al*., 2009; Bookallil *et al*.,2005).

3.7. Laboratory diagnosis of UTI

For the diagnosis of UTI the most common sample is midstream clean catch urine in a suitable transport medium (Gill *et al.*, 2000, Collee *et al.*, 2001; Leigh 1990).

3.8.1. Collection of sample and transport

For the accurate diagnosis of UTI urine specimen free form contamination is required and the sample must be examined as quickly as possible. Generally Mid-Stream Urine is taken as the most satisfactory sample for diagnostic purposes (Gill *et al.*, 2000, Collee *et al.*, 2001; Leigh 1990).

Bacteria grow rapidly in urine in room temperature so in case of delay that is if specimen cannot be examined within 2 hrs it must be stored at 4^{o}C. If refrigeration is not available then boric acid can be used as preservatives (Gill *et al.*, 2000, Collee *et al.*, 2001; Leigh 1990).

3.8.1. Urinalysis

A urinalysis involves a physical and chemical examination of urine. In addition, the urine is spun in a centrifuge to allow sediments containing blood cells, bacteria, and other particles to collect. This sediment is then examined under a microscope. A urinalysis, then, offers a number of valuable clues for an accurate diagnosis such as observing the color and cloudiness in urine, measurement of acidity and and counting the White blood cells (leukocytes). A high count in the urine is referred to as *pyuria*. (A leukocyte count over 10 per microliter is considered to indicate pyuria.) This is very accurate in identifying the disease when it's present, but it also tests positive in many people who do not have a UTI. Pyuria is usually sufficient for a diagnosis of UTI in nonhospitalized patients if other standard symptoms (or just fever in small children) are also present (Vandepitte, 2003). Treatment can be started without the need for further tests if the white cell count is high with cloudy urine in patients with symptoms and signs of UTIs (Vandepitte, 2003).

3.8.2. Chemical and enzymatic analysis of urine

There are now numbers of chemical and enzyme based test available for the examination of urine such as Triphenltetrazolium chloride reduction test, detection of low glucose concentration, detection of nitrite, bacterial endotoxin and ATP, leucocyte esterase activity (Vandepitte, 2003).

3.8.2. Urine Culture

For urine culture, urine specimen is required which is inoculated on agar plate medium and then incubated in the laboratory for 24 to 48 hours. It is then examined for the presence of bacterial growth. Urinary tract infection is nearly always caused by a single species of bacteria, notably *E. coli*. A urine culture is usually performed if the dipstick results are positive, but even if the results are negative, a culture may still be helpful under certain circumstances (Vandepitte, 2003).

The presence of at least 100,000 bacteria per milliliter of any single type of bacterium in urine culture usually provides conclusive evidence of infection in women with symptoms. A count of 100,000 bacterial per milliliter in a woman without symptoms

indicates asymptomatic bacteriuria. In young women with symptoms of cystitis, a diagnosis of infection can reasonably be made with counts as low as 1000 bacteria per milliliter. Men are considered to have an infection with a bacterial count of only 1,000 (Vandepitte, 2003).

3.8.3. Quantitative culture

3.8.5.1. Standard loop method

According to this method, urine is shaken gently to maintain homogeneity. A sterile calibrated metal loop having known dimension is dipped into the urine. The loop is allowed to touch the surface so that the urine is sucked up in the loop. The agar plate, MacConkey agar and Blood Agar, is then inoculated and spread. It is then incubated and the bacterial count is then calculated from the number of colony forming units per ml of urine sample (Vandepitte, 2003).

3.8.5.2. Interpretation of quantitative urine culture results

Previously only the presence of at least 10^5 colony forming units (CFU) per ml in clean-catch midstream urine specimen was considered clinically diagnostic for UTI. But now some experts have challenge that the presence of 10^4 CFU or even fewer may indicate infection. World Health Organization (Vandepitte, 2003) have recommended for reporting the following category:

Catergory 1: fewer than 10^4 CFU ml^{-1} report as probable absence of UTI. (Exceptions: if fewer than 10^4 CFU per ml are present in urine taken directly from the bladder by suprapubic puncture or cystoscopy, in; symptomatic women, or in the presence of leukocytura, report the identification and the result of the susceptibility test).

Category 2: 10^4–10^5 CFU ml^{-1} if the patient is asymptomatic, request a second urine specimen and repeat the count. If the patient has symptoms of UTI, proceed with both identification and susceptibility tests if one or two different colony types of bacteria are present. Bacterial counts in this range strongly suggest UTI in symptomatic patients, or in the presence of leukocyturia. If the count, the quality of the urine specimen, or the

significance of the patient's symptoms is in doubt, a second urine specimen should be obtained for retesting. Report the number of CFU.

Category 3: More than 10^5 CFU ml^{-1} report the count to the physician and proceed with identification and susceptibility tests if one or two different colony types of bacteria are present. These bacterial counts are strongly suggestive of UTI in all patients, including asymptomatic females. If more than two species of bacteria are present in urine samples in categories 2 and 3, report as "Probably contaminated; please submit a fresh, clean-catch specimen".

3.8.4. Antimicrobial Susceptibility Test or Antibiogram

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

World health organization has recommended modified Kirby-Bauer disk diffusion method as standard for laboratories to test routinely for antimicrobial susceptibility test. In this test, antimicrobial resistance is detected by allowing the antibiotics to diffuse from a point source, commonly in the form of an impregnated filter paper disc, into an agar medium that has been seeded with the test organism. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level for the test strain. Following incubation, the diameter of the zone of inhibition around each antibiotic disc is measured in millimeters (Collee *et al*., 2001).

1.8.7. Bacterial resistance to antibiotics

Careless and injudicious use of antibiotics as well as empirical antimicrobial therapy has been the major contributing factor in the development of Multiple Drug Resistant bacteria. These antibiotic resistances have been the emerging problem in world (Collee *et al*., 2001; Khan and Zaman, 2006).

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

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

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

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

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

Bacteria often contain extra-chromosomal genetic elements called plasmids. Genetic material and plasmids can be transferred by transduction, transformation and conjugation. By the process of conjugation, resistance plasmids may be transferred between and within different species and genera; and can code for multiple antibiotic resistance. Plasmid-mediated resistance has been increasingly recognized among Gram negative enteric pathogens. Some plasmids carry genes for resistance to one and often several antimicrobial drugs. Plasmid genes for antimicrobial resistance often control the formation of enzymes capable of destroying the antimicrobial drugs. Thus, plasmids determine resistance to Penicillins and Cephalosporins by carrying genes for the formation of beta-lactamases. Plasmids code for enzymes that acetylate, adenylate, or phosphorylate various aminoglycosides; for enzymes that determine the active transport of Tetracyclines across the cell membrane and for others (Smith, 2004).

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

Mechanism of Antimicrobial Resistance

According to Brooks *et al*., (2004) and Smith, (2004), there are many different mechanisms by which microorganisms might exhibit resistance to drugs.

1. There are many different mechanisms by which microorganisms might exhibit resistance to drugs.
2. Microorganisms produce enzymes that destroy the active drug. Examples: *Staphylococci* resistant to penicillin G produce a Beta-lactamase that destroys the drug. Other Beta-lactamases are produced by Gram negative rods.
3. Microorganisms change their permeability to the drug. Examples: Tetracyclines accumulate in susceptible bacteria but not in resistant bacteria. Streptococci have a natural permeability barrier to Aminoglycosides.
4. Microorganisms develop an altered structural target for the drug. Examples: Erythromycin-resistant organisms have an altered receptor on the 50S subunit of the ribosome, resulting from methylation of a 23S ribosomal RNA. Resistance to some Penicillins and Cephalosporins may be a function of the loss or alteration of Penicillin binding proteins (PBPs).
5. Microorganisms develop an altered metabolic pathway that bypasses the reaction inhibited by the drug. Example: Some Sulphonamide-resistant bacteria do not require extracellular para-amino benzoic acid (PABA) but, like mammalian cells, can utilize preformed folic acid.
6. Microorganisms develop an altered enzyme that can still perform its metabolic function but is much less affected by the drug. Example: In Trimethoprim-resistant bacteria, the dihydrofolic acid reductase is inhibited far less efficiently than in Trimethoprim-susceptible bacteria.

Multiple Drug Resistance

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

R-factors: One of the earliest examples was in Japan in 1959. Previously sensitive *E. coli* became resistant to multiple antibiotics through acquisition of a conjugative plasmid (R-factor) from resistant *Salmonella* and *Shigella* isolates. A number of R-factors have now been characterized including RP4, encoding resistance to Ampicillin, Kanamycin, Tetracycline and Neomycin, found in *P. aeruginosa* and other Gram negative bacteria; R1, encoding resistance to Ampicillin, Kanamycin, Sulphonamides, Chloramphenicol and Streptomycin, found in Gram negative bacteria and pSH6, encoding resistance to Gentamicin, Trimethoprim and Kanamycin, found in *S. aureus* (Brooks, 2004; Smith, 2004).

Mobile gene cassettes and integrons: Many Gram negative resistance genes are located in gene cassettes. One or more of these cassettes can be integrated into a specific position on the chromosome termed as integron. Thus, integrons are genetic elements that recognize and capture multiple mobile gene cassettes (Smith, 2004). Although integrons by themselves are not mobile, due to their presence in plasmids and transposons, they can be transferred horizontally. Integrons for these reasons a major mechanism for the spread and maintenance of MDR (Brooks, 2004; Smith, 2004).

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

3.8.8. Multiple Antibiotics Resistance (MAR) Index

Antimicrobial resistance of pathogenic bacteria has been an emerging issue of critical importance. Now the interest has been focused on the potential for treatment failure and the selection of bacteria that no longer respond to currently available and prescribed antimicrobial agents. In this context, the research has been primarily targeted on to provide a better understanding of the emergence, dissemination and maintenance of resistant bacterial isolates in human populations and the environment. There have been several index developed to access the pattern of antibiotics resistance of an antibiotics as well as of the pathogens such as Minimum Inhibitory Concentration (MIC) level, MAR index also called as Antibiotic Resistance Index (ARI), Formula for Rational Antimicrobial Therapy (FRAT) etc. These indices can be used to summarize the proportion of resistance among several isolates and or antibiotics (Wagner *et al*., 2003).

The MAR index has been derived from ecological science. The MAR index has been used to identify sources of fecal contamination in food and water and for tracking changes in a pattern of antimicrobial resistance in calves over time (Wagner *et al*., 2003).

MAR index helps us to delineate the pattern of antibiotics resistance of an isolates as well as of the antibiotics against the tested isolates. MAR index helps to distinguish the sources of the causative agents. By using MAR index, one can separates whether the pathogenic bacteria have originated from any natural sources or from humans or animal hosts. It helps us to know whether the bacteria have got any previous contact with the antibiotics. A MAR Index of an isolates more than 0.20 implies that the strains of such bacteria originate from an environment where several antibiotics are used (Wagner *et al*., 2003).

MAR index for isolates

According to Wagner *et al*., (2003)

$$\text{MAR index} = \frac{\text{Number of antibiotics to which the isolate is resistance}}{\text{Number of antibiotics tested}}$$

MAR index of antibiotics

$$\text{MAR index} = \frac{\text{Number of antibiotics resistance to the isolates}}{\text{Number of antibiotics X Number of isolates}}$$

3.9. Epidemiology of UTI

In world context

Foxman *et al*., (2000) found in his study that at least 11.0% of women in US were estimated to suffer from physician-diagnosed UTI per year, and there exist a lifetime probability that a woman will have a UTI is 60%.

The clinical treatment of UTI have been complicated due to the increasing incidence of infection caused by strains of *E. coli* that are resistant to commonly used antibiotics therapy. It has been found in a recently conducted study that in US, the resistance to Trimethprim-Sulfamethoxazole in an Uropathogenic *E. coli* is increasing at the rates of 15.0-18.0 % (Kahlmeter, 2000; Talan *et al.,* 2000).

There have been reports of community-wide outbreaks of UTI by certain strain of multidrug-resistant, uropathogenic lineage of *E. coli* from South London in 1987 and 1988 (Phillips *et al.,* 1988) and from Barcelona, Spain (Prats *et al.,* 2000) though UTI is considered as an epidemic infection.

With the exception of Nitrofurantoin, there have been reports of increasing resistance to different antimicrobial class introduced for the treatment of UTI over the decade. Within this last 30 years there have been report of resistance to TMP/SMX up to 18% across US, similarly trend have been observed for Ciprofloxacin. These increases in resistance have made a great imapact on antibiotics prescribing (Stamm, 2003). Outside US, high rates of resistance to Cotrimoxazole have been reported in Israel (31.0%), Spain (32.0%) and Bangladesh (60.0%). Although the prevalence of resistance to Ciprofloxacin and other Fluoroquinolones has generally remained low, it has reached 18.0% in Bangladesh and 4.0% in Israel. Resistance to Norfloxacin is 13.0% in Spain (Gales *et al.,* 2000).

The ECO.SENS Project was the first international survey to investigate the prevalence and susceptibility of pathogens causing community acquired uncomplicated UTIs in women at 240 centres in 17 countries. *E. coli* accounted for the majority (80.0%) of uropathogens isolated in all 17 countries. The rates of resistance among *E. coli* strains were: Ampicillin and Sulphamethoxazole (30.0%), Trimethoprim alone or with Sulphamethoxazole (15.0%), Nalidixic acid (6.0%), Ciprofloxacin (3.0%), Amoxicillin-Clavulanic acid, Mecillinam, Cefadroxil, Nitrofurantoin and Fosfomycin (≤2.0%) (Kahlmeter, 2000).

In a continuous surveillance report performed to study resistant patterns to the most commonly prescribed antibiotics, namely Norfloxacin, Amoxicillin, Trimethoprim and Nitrofurantoin from 1989 to 1998 in >90,000 *E. coli* isolates; it was found that resistance to Norfloxacin increased from 1.3% in 1989 to 5.8% in 1998. Multiresistant, defined as resistance to Norfloxacin and at least two of the other three antibiotics, increased from 0.5% in 1989 to 4.0% in 1998 (Goetsch *et al.,* 2000).

In one of the study conducted in 14 medical centers of Asia-Pacific region between the time period of 1998 and 1999 found that over 50.0% of the 958 pathogens were *E. coli and Klebsiella* spp. followed by *P. aeruginosa, Enterococcus* spp. and *Enterobacter* spp. Susceptibility for the three enteric bacilli was high for Carbapenems (100.0%), 'fourth generation' Cephalosporins (Cefepime 94.9-98.6%) and Amikacin (≥93.0%) (Turnidge *et al.*, 2002).

Tabiban *et al*., (2008) conducted study to assess the association between host characteristics and uropathogens in USA found that *Ps. aeruginosa* can cause UTI to those patients that have undergone urinary tract procedures (43% versus 15% overall), have a neurogenic bladder (29% versus 12% overall), have received recent antibiotic therapy (52% versus 24% overall), and a male (76% versus 28% overall). *P. mirabilis* can cause UTI primarily to those patients who have a foreign body in the lower urinary tract (48% versus 30% overall). According to the author these data may be useful for planning future targeted prophylaxis studies.

The prevalence of UTI is influenced by factors such as age, sex, population sample, urine collection method, testing methodologies, diagnostic criteria, and culture. Age and sex are important factors in causation of UTI (Raszka and Khan, 2005).

Fatima *et al*., (2007) reported in her study conducted in India including 580 women that *E. coli* to be the causative organism in 78.6% while 21.4% cases were found to be by other uropathogens. 35.7 % bacteriuric women had positive past history of UTI compared to only 9.7% non-bacteriuric women which indicates significant result regarding recurrent infection. Anaemia was not found significant, bacteriuria was found as a causative factor for preterm labour as 21.4% bacteriuric women compared with 4.9% non-bacteriuric women went into preterm labour ($p<0.05$).

Amiri *et al*., (2009) performed a study in Iran to assess an association between hygiene practices and sexual intercourse to UTI and found that sexual intercourse ≥ 3 times per week (OR-5.62), recent UTI (OR=3.27), not washing genitals precoitus (OR=2.89), not voiding urine postcoitus (OR=8.62) and washing genitals from back to front (OR=2.96) were found to be associated with UTI.

Hooton *et al*., (1996) found that the prevalence of bacteriuria rises with parity, older age and lower socioeconomic status and in women with diabetes mellitus sickle cell trait or a past history of UTI. In low economic population the prevalence of bacteriuri is about 2% in primiparas younger than 21 years compared with 8%-10% in grandmultiparas older than 35 years.

UTI in Nepal

In a study done by Ghimire *et al*., (1994) at Maternity Hospital, Thapathali, the significant bacteriuria was found 15.9% in the urine samples among pregnant women whereas it was only 5.0% among non-pregnant women. The prevalence uropathogenic *E. coli* was found higher (52.5%), followed by *Klebsiella* spp. (40.7%) and *Proteus* spp. (6.8%). Among the isolated *E. coli*, 100.0%, 50.0%, 30.0%, 25.0% and 5.0% of the organisms were found to be resistant to Ampicillin/Amoxicillin, Cephalexin,

Tetracycline, Cotrimoxazole and Ciprofloxacin respectively. And 94.5%, 60.0%, 38.0%, 44.0% and 0% of the isolated *Klebsiella* spp. were found to be resistant to same antibiotics respectively.

In the study performed by Gautam *et al*., (1997), *E. coli* was found as the most predominant pathogen (57.0%) followed by *K. pneumoniae* (24.0%), *Proteus* spp. (10.0%), *Ps. aeruginosa* (1.7%), *Sal. typhimurium* (1.7%), *Sh. boydii (*1.7%), *S. faecalis* (1.7%) and *S. aureus* (1.7%). In antibiogram profiling Nitrofurantoin (88.0%) was found susceptible to all isolated uropathogens followed by Ciprofloxacin (81.0%), Nalidixic acid (69.0%) and Chloramphenicol (60.0%) whereas Cotrimoxazole and Amoxicillin were least effective antibiotics against these bacterial isolates.

Dhakal *et al*., (1997) found *E. coli* (47.4%) as the most predominant bacteria followed by *Klebsiella* spp. (13.2%), *S. aureus* (10.5%) and *Ps. aeruginosa* (7.9%). Antimicrobial sensitivity test showed that Nitrofurantoin (84.2%) was only the effective drug followed by Norfloxacin (28.9%) and Ampicillin (10.5%) against the bacterial isolates.

Dhital *et al*., (2000) in his study also found *E. coli* (78.0%) as the most prevalent pathogen followed by *K. pneumoniae* (9.0%), *P.* mirabilis (2.0%), *Ps. aeruginosa* (2.0%), *Citrobacter* spp. (2.0%) and *Enterobacter* spp (1.0%). In this study, 80.0% of the Gram negative bacteria were resistant to Ampicillin, 72.0% to Nalidixic acid, 70.0% to Cotrimoxazole and 54.0% to Chloramphenicol. The most susceptible antibiotic was Norfloxacin (73.0%). Amikacin showed 29.0% resistant and Ciprofloxacin, Nitrofurantoin and Gentamicin was 32.0% resistant. Rai *et al*., (2000) also found *E. coli* (61.8%) as the most prevalent pathogen followed by *K. pneumoniae* (12.2%) and *S. aureus* (12.2%). In this study Cephalexin (100%) was most effective against Gram negative isolates, followed by Nitrofurantoin (93.8%), Ciprofloxacin (85.7%), Cotrimoxazole (50.0%) and Norfloxacin (50.0%).

Tuladhar (2000) reported that in 1947 urine specimens, culture positive were found in 517 (26.6%) of which MDR bacterial strains were detected in 122 (23.6%) cases in which *E. coli 72* (13.1%), *Klebsiella* spp. 20 (3.9%) and *S. aureus* 13 (2.1%) were the

predominants. Out of 1479 urine specimens of hospitalized patients, there were 230 culture positive cases of which MDR bacterial strains were detected in 81 (35.2%) cases in which the most predominants were *E. coli* 51 (22.2%), *Klebsiella* spp. 14 (6.1%) and *S. aureus* 5 (2.2%).

E .coli was the most common isolate accounting for 77.5% of all bacterial isolates and was followed by *Proteus* spp., *Klebsiella* spp. *and Staphylococcus* spp. Ciprofloxacin was found to be most effective antibiotic against *E. coli* followed by Nalidixic acid. *Proteus* spp. was 100.0% susceptible to Nalidixic acid and Gentamicin. *Saphylococcus* spp. was susceptible to Nitrofurantoin (100.0%), Cotrimoxazole (100.0%) and Norfloxacin (60.0%) (Chhetri *et al.,* 2004).

In a retrospective study conducted in five hospitals of Kathmandu, the most common organisms causing UTI was found to be *E. coli* (49.0%), followed by *S. aureus* (23.0%), *Klebsiella* spp. (9.7%), *Proteus* spp. (3.6%), *Pseudomonas* spp. (0.8%) and *Citrobacter* spp. (2.8%). All the organisms causing UTI were found to be susceptible to Nitrofurantoin and Amoxycillin whereas Ciprofloxacin was found to be most effective (Jha and Bapat., 2005).

CHAPTER IV.

4. MATERIALS AND METHODS

4.1. Materials

Various materials used in this study is enlisted in the Appendix I.

4.2. Methods

This study was conducted at Maternity Hospital, Thapathali, Kathmandu. In this study approximately 2000 mid-urine samples were collected from the suspected patients of UTI and processed following the standard laboratory techniques in Maternity Hospital.

4.2.1. Study design

A Prospective hospital based study design was used.

Sampling methodology

Random sampling method was used for the collection of data and urine sample.

Population and sample size

Populations for the study were the patients visiting Maternity Hospital. Sample size was determined according to the existing prevalence as determined by the previous studies.

a). Sample size determination

The sample size for the study was according to the previous study. All the samples according to the criteria of the study were included in the study.

b). Inclusion criteria

The clinically suspected UTI patients visiting the Hospital were included in the study.

c). Exclusion criteria

The urine sample showing the multiple growths were again requested for urine sample.

d). Site of study

Site for the collection of data was the Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu and the sampling was done in the entire patient visiting the hospital.

Ethical consent

Ethical approval from the hospital was taken. After briefly informing the participant about the objectives of the study, the verbal and written consent was also taken from the participant.

Time frame

The collection of the data and laboratory work was conducted for six months from Jestha to Paush, 2066 BS.

4.2.2. Collection of the data

The data regarding the patient visiting the hospital was collected directly by interview method by using semi-structured questionnaire, clinical history of the patients involved in the study.

4.2.3. Urine sample collection

5-10 ml mid-stream urine sample was collected in aseptic condition in clean dry, sterile and leak proof container. The sample was taken to the laboratory for further analysis without any delay. In case of delay the sample was refrigerated at 4^{o}C. The guidelines for the collection of clean catch midstream urine sample are given in Appendix III (Vandepitte *et al*., 2003, Collee *et al*., 2004).

4.2.4. Urine sample evaluation

The sample collected was evaluated in terms of its acceptability, proper labeling (full name, age, sex, serial number of the patient, date and time of collection), visible signs of contamination any delay in getting the urine samples to the laboratory was also considered (Vandepitte *et al*., 2003, Collee *et al*., 2004).

4.2.5. Sample processing

a). Macroscopic examination

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

b). Microscopic examination

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

Wet mount preparation of urinary sediments was observed through microscope for the presence of WBC, pus cells and RBC. Number of WBC and RBC was estimated as number per HPF that is 40X objective of microscope (Vandepitte *et al*., 2003, Collee *et al*., 2004)..

c). Chemical examination

Chemical examination was conducted. Only albumin was done by using dipstick method.

4.2.6. Urine culture

Semi-quantitative culture technique was used to culture urine specimen and to detect the presence of significant bacteriuria by standard methods (Collee *et al*., 2004 and Leigh, 1990). A standard loop of standard dimension was used to take up approximately fixed ($\pm$10% error) and known volume (0.001 ml) of mixed uncentrifuged urine. It was then inoculated on the surface MacConkey Agar (MA) and Nutrient Agar (NA). Urine specimen was thoroughly mixed to ensure uniform suspension of bacteria before inoculating in the agar plates. The inoculated MA and NA plates were aerobically incubated overnight at 37^{o}C. The bacterial count was reported as described by Vandepitte (2003).

a). Semi-quantitative culture

This was carried out by using calibrated inoculating loop have diameter of 0.001 mm. The protocol was followed as recommended by WHO (Vandepitte *et al*., 2003).

b). Identification of isolates

The isolated colony from the plates showing significant growth was further preceded for identification. Plate showing no growth, mixed growth and bacterial growth of insignificant number was excluded from the study. The identification of significant isolate was done by standard microbiological methods as described in the Bergey's Manual. Identification of the bacteria isolated from the urine sample was conducted according to the protocol provided by the clinical hand book of medical microbiology and Collee *et al*., (2001). The single distinct colony was gram stained. A single distinct colony from MA for both the gram negative and gram positive bacteria was picked up by using sterile straight wire loop and inoculated on the NA. It was incubated at 37^0C for 24 hrs. After the overnight incubation, the culture was used to perform biochemical test and antibiotic sensitivity test.

Gram Negative bacteria

The pure culture of Gram negative bacteria were obtained on NA. Its colonial and morphological characteristics were recorded. Catalase test and oxidase test were conducted. It was then incubated in different biochemical media such as Triple Sugar Iron agar, SIM media, MR/VP media, Citrate media and Urease broth.

Gram positive bacteria

Similarly for gram positive bacteria pure culture was initially obtained. Its colonial and morphological characteristics were noted. Catalase test, Oxidase test and slide coagulase test were conducted. It was then inoculated in Hugh Leifson's media.

4.2.7. Antibiotics selection criteria

For Gram Negatives isolates: Since the study conducted was hospital based, primarily the antibiotics were chosen on the basis of the use in the hospital (Vandepitte *et al.*, 2003).

For Gram positive isolates: Similarly as for Gram negative isolates, antibiotics generally used in Hospital were tested (Vandepitte *et al.,* 2003). However, some antibiotics were used for the research purposes on the basis of available literature (Vandepitte *et al.,* 2003).

4.2.8. Antibiotics susceptibility test

The antibiogram susceptibility testing or antibiogram profiling of the urine isolates to different antimicrobial disks was done by modified Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory and Standard Institute (CLS, 2007) using Mueller Hinton Agar (MHA). For *Staphylococcus* MHA incorporated with 0.2 % NaCl was used.

For the sensitivity test, a single isolated colony was picked up by using sterile wire loop which was then inoculated in Nutrient broth tube and incubated at 37^{o}C for 2-4 hrs. After incubation period, the turbidity of the inoculated broth was maintained with McFarland number 0.5.

A plate of Mueller Hinton Agar was swabbed with the bacterial suspension by sterile cotton swab using carpet culture technique. The plate was left for about 5 minutes to let the inoculum to dry. Using sterile forceps, appropriate antimicrobial discs were placed on the inoculated MHA plate. It was then incubated at 37^{o}C for 24 hrs. After the incubation period, the diameter of zone of inhibition of antimicrobial discs was measured. The zone of inhibitions were compared with the standard interpretative chart provide by the company.

The organisms' showing resistant to more than three different class of antibiotics was taken as Multi-drug resistant isolates. MAR Index was calculated as recommended by Wagner *et al*., (2003)

4.2.9. Quality control

Strict quality control was maintained to obtain reliable microbiological results. The quality of each agar plate prepared was maintained by incubating one plate of each

batch in the incubator. Control strains of ATCC were used for the identification test and for the standardization of Kirby-Bauer test and also for correct interpretation of inhibition zones of diameter. Quality of sensitivity test was maintained by maintaining the thickness of MHA at 4mm and the pH of 7.2-7.4. Similarly antibiotics disks having correct amount as indicated was used. Strict aseptic condition was maintained while carrying out all the procedures.

4.2.10. Purity plate

Purity plate for each biochemical test was maintained to ensure the pure culture inoculums used as well as to assess that the biochemical tests were undertaken in an aseptic condition.

4.2.11. Statistical analysis

All the data obtained was statistically analyzed by using Statistical Package for Social Science (SPSS) version 16 software packages. The chi-square, one way ANOVA and ODDs Ratio test was used as per need to determine significant association between different factors for the causation of UTI.

CHAPTER V.

5. RESULTS

A total of 1246 patients visiting maternity hospital (out-patients and in-patients) suspected of UTI were included in this study. The prevalence of UTI was found 29.61%. A total of 722 out-patients and 474 in-patients were included in this study. In the total positive cases, UTI was found higher in out-patients (33.74%) than in in-patients (22.57%).

5.1. Distribution Patterns of Uropathogens in patients

Table 3. Distribution of patients according to the culture pattern

Distribution of culture positive patients				
Origin	**Total**	**UTI positive**	**MDR**	**Median age of UTI positive patients**
OPD	772 (62%)	246 (33.74%)	133 (54.07%)	27
In-patient	474(38%)	123 (22.57%)	66 (53.66%)	26
Total	**1246**	**369 (29.61%)**	**199 (53.93%)**	**27**

In the total isolates 199 (53.93% of the total positive cases) of the uropathogens were found to be Multiple Drug Resistant (MDR). The median age group of the patients who were found UTI positive was 20-30 years.

5.2. Distribution patterns of uropathogens according to growth positivity

Table 4. Result of Growth pattern of Microorganisms

S.N.	**Growth**	**No. of samples**	**Percentage of samples**
1.	Culture positive	369	29.61
2.	No Growth	877	70.38
	Total	**1246**	**100**

Out of the total 369 positive cases 219 samples were found susceptibility for

bacterial isolates that is >10^5 CFU /ml where as remaining samples of the UTI positive samples had less <10^5 CFU/ml (between 10^3CFU/ml – 10^4CFU/ml)

5.3. Distribution patterns of different uropathogens

Table 5. Distribution of uropathogens in patients visiting the hospital

ORGANISMS			
Uropathogens	**Number**	**Percent**	**MDR (%)**
Gram negative	**305**	**82.66**	**162 (81.41)**
Escherichia coli	230	62.3	128 (55.65)
Klebsiella pneumoniae	24	6.5	14 (58.33)
Proteus mirabilis	18	4.9	9 (50)
Klebsiella oxytoca	11	3	2 (27.27)
Proteus vulgaris	10	2.7	5 (50)
Pseudomonas aeruginosa	8	2.2	3 (37.5)
Citrobacter freundii	2	0.5	-
Enterobacter cloacae	2	0.5	-
GRAM POSITIVE	**64**	**17.34**	**37 (18.59)**
Staphylococcus aureus	28	7.6	23 (82.14)
CoNS	28	7.6	13 (46.43)
Streptococcus spp.	5	1.4	-
Streptococcus faecalis	3	0.8	1(33.33)
Total	**369**	**100**	**199 (53.93)**

A total of 369 bacterial isolates belonging to 12 different species were isolated. Among them 8 isolates were from Gram negative and other 4 belong to Gram positive. In Gram negative *E. coli* was the most predominant isolates (63.2%) followed by *K. pneumoniae* (6.5%), *P. mirabilis* (4.9), *K. oxytoca* (3.0%), *P. vulgaris* (2.7%), *Ps. aeruginosa* (2.2), *E. cloacae* (0.5) and *C. freundii* (0.5). *S. aureus* (7.6%) and CoNS (7.6%) were the major isolates in Gram positive followed by Streptococcus spp. (1.4%) and *S. faecalis* (0.8%)

5.4. Patterns of Uropathogens in Out-patients and in-patients

Table 6. Distribution of bacterial isolates in urine according to the origin of patients

ORGANISMS	**OPD**	**In-patient**
Escherichia coli	156	74
Staphylococcus aureus	18	10
Proteus mirabilis	12	6
CoNS	15	13
Klebsiella pneumoniae	18	6
Proteus vulgaris	8	2
Pseudomonas aeruginosa	4	4
Klebsiella oxytoca	6	5
Streptococcus spp.	5	0
Streptococcus faecalis	2	1
Cirobacter freundii	2	0
Enterobacter cloacae	0	2
Total (369)	**246**	**123**

The isolated uropathogens were found in high proportion in outpatients (246) in compare to inpatients (123). Among Gram negative, *E. coli* was the most frequent isolates in both groups. Among Gram positive isolates, *Staphylococcus aureus* and CoNS were highest in out-patients and in-patients respectively.

5.5. Age wise distribution of uropathogens

Table 7. Distribution of uropathogens according to Age groups of the patients

Age group	**<10**		**10-20**		**20-30**		**30-40**		**40-50**		**50-60**		**60-70**		**>70**	
Organisms	**I**	**O**	**I**	**O**	**I**	**O**	**I**	**O**	**I**	**O**	**I**	**O**	**I**	**O**	**I**	**O**
C. freundii	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
CoNS	0	0	2	3	7	9	1	0	0	3	1	0	0	0	2	0
E. cloacae	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
E. coli	0	1	8	24	50	84	11	21	3	16	1	4	1	4	0	2
K. oxytoca	0	0	2	0	2	5	1	1	0	0	0	0	0	0	0	0
K. pneumoniae	0	0	1	0	3	9	2	3	0	4	0	2	0	0	0	0
P. aeruginosa	0	0	2	0	2	2	0	2	0	0	0	0	0	0	0	0
P. mirabilis	0	0	0	0	1	6	3	4	0	0	0	0	2	2	0	0
P. vulgaris	0	0	0	0	1	4	0	2	1	1	0	1	0	0	0	0
S. aureus	0	0	0	3	6	8	2	2	1	2	1	3	0	0	0	0
Streptococcus spp.	0	0	0	2	0	3	0	0	0	0	0	0	0	0	0	0
S. faecalis	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0
Total	0	1	15	32	73	133	20	36	6	26	3	10	4	6	2	2

Note: I – inpatients, O – outpatients.

The isolated pathogenic bacteria were found highest in 20-30 years age group.

5.6. Antibiotics susceptibility pattern of uropathogens

Table 8. Antibiotics susceptibility pattern of Gram negative urine isolates

Antibiotic	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Nalidixic Acid	209	68.52	5	1.64	91	29.84
Ofloxacin	82	26.88	9	2.95	214	70.16
Ciprofloxacin	123	40.33	4	1.31	178	58.36
Norfloxacin	70	22.95	0	0	235	77.05
Tobramycin	28	9.18	10	3.28	267	87.54
Gentamycin	49	16.07	3	0.98	253	82.95
Cotrimoxazole	82	26.89	76	24.92	147	48.20
Cephalexin	56	18.36	80	26.23	169	55.41
Ampicillin	121	39.67	76	24.92	108	35.41

For Gram negative isolates 9 different antibiotics were tested. Among them, Tobramycin (87.54%) was found most susceptible followed by Gentamycin (82.95%), Norfloxacin (77.05%), Ofloxacin (70.16%), Ciprofloxacin (58.36%), Cephalexin (55.41%), Cotrimoxazole (48.20%), Ampicillin (35.41%) and Nalidixic Acid (29.84%). The difference in sensitivity pattern was found significant (p=0.000).

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

E. coli (N=230)							
Antibiotics group	Antibiotics	Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Quinolone\Fluoro quinolones	Nalidixic Acid	161	70	2	0.87	67	29.13
	Ofloxacin	64	27.83	3	1.30	163	70.87
	Ciprofloxacin	49	21.30	44	19.13	137	59.57
	Norfloxacin	55	23.91	0	0	175	76.09
Aminoglycosides	Tobramycin	17	7.39	6	2.61	207	90
	Gentamycin	42	18.26	3	1.30	185	80.43
Sulfamazole	Cotrimoxazole	71	30.87	42	18.26	117	50.87
Beta-lactam	Ampicillin	97	42.17	40	17.39	93	40.43
Cephalosporin	Cephalexin	90	39.13	1	0.43	139	60.43

As shown in Table 9., *E. coli* was found highly susceptible to Tobramycin (90%) in Aminoglycosides group, followed by Norfloxacin (76.09%) in Quinolone/Fluoroquinolones group, Cephalexin (60.43%) from Cephalosporin group, Cotrimoxazole (50.87%) in Sulfamazole group and Ampicillin (40.43%) from Beta-lactam group.

Table 10. Antibiotic susceptibility pattern of *Proteus vulgaris*

P. vulgaris (N=10)							
Antibiotics group	Antibiotics	Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Quinolone\Fluoro quinolones	Nalidixic Acid	7	70	0	0	3	30
	Ofloxacin	3	30	1	10	6	60
	Ciprofloxacin	0	0	6	60	4	40
	Norfloxacin	3	30	0	0	7	70
Aminoglycosides	Tobramycin	2	20	1	10	7	70
	Gentamycin	4	40	0	0	6	60
Sulfamazole	Cotrimoxazole	0	0	6	60	4	40
Beta-lactam	Ampicillin	1	10	6	60	3	30
Cephalosporin	Cephalexin	4	40	0	0	6	60

As shown in Table 10., In Quinolone/Fluoroquinolones group, Norfloxacin (70%) was the highly susceptible followed by Ofloxacin (60%), Ciprofloxacin (40%) and Nalidixic Acid (30%) for *Proteus vulgaris*. Similarly, in Aminoglycosides group, more susceptible was found Tobramycin (70%) followed by Gentamycin (60%), Cephalexin (60%) of Cephalosporin class, Cotrimoxazole (40%) of Sulfamazole group and Ampicillin (30%) of Beta-lactam group.

Table 11. Antibiotic susceptibility pattern of *Proteus mirabils*

P. mirabils (N=18)							
Antibiotics group	Antibiotics	Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Quinolone\Fluoro quinolones	Nalidixic Acid	13	72.22	2	11.11	3	16.67
	Ofloxacin	4	22.22	2	11.11	12	66.67
	Ciprofloxacin	4	22.22	2	11.11	12	66.67
	Norfloxacin	2	11.11	0	0	16	88.89
Aminoglycosides	Tobramycin	4	22.22	1	5.56	13	72.22
	Gentamycin	2	11.11	0	0	16	88.89
Sulfamazole	Cotrimoxazole	3	16.67	10	55.56	5	27.78
Beta-lactam	Ampicillin	4	22.22	11	61.11	3	16.67
Cephalosporin	Cephalexin	1	5.56	11	61.11	6	33.33

In Quinolone\Fluoroquinolones group, Norfloxacin (88.89) was highly susceptible and least was found Nalidixic Acid (16.67%).

In Aminoglycosides group, Gentamycin (88.89) was found most susceptible followed by Cephalexin (33.33%) of Cephalosporin class, Cotrimoxazole (27.78%) of Sulfamazole group and Ampicillin (16.67%) of Beta-lactam group.

Table 12. Antibiotic susceptibility pattern of *Klebsiella oxytoca*

K. oxytoca (N=11)							
Antibiotics group	Antibiotics	Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Quinolone\Fluoro quinolones	Nalidixic Acid	4	36.36	0	0	7	63.64
	Ofloxacin	2	18.18	0	0	9	81.82
	Ciprofloxacin	3	27.27	0	0	8	72.73
	Norfloxacin	2	18.18	0	0	9	81.82
Aminoglycosides	Tobramycin	2	18.18	0	0	9	81.82
	Gentamycin	0	0	0	0	11	100
Sulfamazole	Cotrimoxazole	1	9.09	3	27.27	7	63.64
Beta-lactam	Ampicillin	5	45.45	3	27.27	3	27.27
Cephalosporin	Cephalexin	1	9.09	3	27.27	7	63.64

In *K. oxytoca*, Gentamycin (100%) showed maximal susceptibility followed by Tobramycin (81.82%) of Aminoglycosides group, Norfloxacin (81.82%) and Ofloxacin (81.82%) of Quinolone/Fluoroquinolones, Cotrimoxazole (63.64%), Cephalexin (63.64%) and Ampicillin (27.27%).

Table 13. Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

K. pneumoniae (N=24)							
Antibiotics group	Antibiotics	Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Quinolone\Fluoro quinolones	Nalidixic Acid	17	70.83	1	4.17	6	25
	Ofloxacin	6	25	2	8.33	16	66.67
	Ciprofloxacin	18	75	1	4.17	5	20.83
	Norfloxacin	7	29.17	0	0	17	70.83
Aminoglycosides	Tobramycin	3	12.5	0	0	21	87.5
	Gentamycin	1	4.17	0	0	23	95.83
Sulfamazole	Cotrimoxazole	7	29.17	6	25	11	45.83
Beta-lactam	Ampicillin	12	50	7	29.17	5	20.83
Cephalosporin	Cephalexin	5	20.83	7	29.17	12	50

As shown in Table 13., for *K. pneumoniae* Norfloxacin (70.83%) was found most susceptible followed by Ofloxacin (66.67%), Nalidixic Acid (25%) and Cephalexin (20.83%), in Aminoglycosides group Gentamycin (95%) was found more susceptible than Tobramycin (70.83%) followed by Ciprofloxacin (50%), Cotrimoxazole (45.83%) and Ampicillin (20.83%).

Table 14. Antibiotic susceptibility pattern of *Citrobacter freundii*

C. freundii (N=2)							
Antibiotics group	Antibiotics	Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Quinolone\Fluoroquinolones	Nalidixic Acid	1	50	0	0	1	50
	Ofloxacin	1	50	0	0	1	50
	Ciprofloxacin	1	50	0	0	1	50
	Norfloxacin	0	0	0	0	2	100
Aminoglycosides	Tobramycin	0	0	1	50	1	50
	Gentamycin	0	0	0	0	2	100
Sulfamazole	Cotrimoxazole	0	0	2	100	0	0
Beta-lactam	Ampicillin	0	0	2	100	0	0
Cephalosporin	Cephalexin	0	0	2	100	0	0

As shown in Table 14., *C. freundii*, was found 100% susceptible to Norfloxacin and Gentamycin whereas Cotrimoxazole, Ampicillin and Cephalexin were 0%.

Table 15. Antibiotic susceptibility pattern of *Enterobacter cloacae*

E. cloacae (N=2)							
Antibiotics group	Antibiotics	Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Quinolone\Fluoro quinolones	Nalidixic Acid	0	0	0	0	2	100
	Ofloxacin	0	0	1	50	1	50
	Ciprofloxacin	0	0	0	0	2	100
	Norfloxacin	0	0	0	0	2	100
Aminoglycosides	Tobramycin	0	0	0	0	2	100
	Gentamycin	0	0	0	0	2	100
Sulfamazole	Cotrimoxazole	0	0	2	100	0	0
Beta-lactam	Ampicillin	0	0	2	100	0	0
Cephalosporin	Cephalexin	0	0	2	100	0	0

In *E. cloacae*, Nalidixic Acid (100%), Cephalexin (100%), Norfloxacin(100%), Tobramycin (100%) and Gentamycin (100%) showed maximum susceptibility followed by Ofloxacin (50%), whereas Cotrimoxazole, Ampicillin, Cephalexin didn't showed any zone of susceptibility.

Table 16. Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

P. aeruginosa (N=8)							
Antibiotics group	Antibiotics	Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Quinolone\Fluoro quinolones	Nalidixic Acid	6	75	0	0	2	25
	Ofloxacin	2	25	0	0	6	75
	Ciprofloxacin	3	37.5	0	0	5	62.5
	Norfloxacin	1	12.5	0	0	7	87.5
Aminoglycosides	Tobramycin	0	0	1	12.5	7	87.5
	Gentamycin	0	0	0	0	8	100
Sulfamazole	Cotrimoxazole	0	0	5	62.5	3	37.5
Beta lactam	Ampicillin	2	25	5	62.5	1	12.5
Cephalosporin	Cephalexin	0	0	5	62.5	3	37.5

As shown in Table 16., In *Pseudomonas aeruginosa*, Gentamycin (100%) showed the highest susceptibility whereas least was found in Ampicillin (12.5%).

Antibiotics susceptibility pattern of Gram positive isolates from urine samples

Table 17. Antibiotic susceptibility pattern of *Staphylococcus aureus*

Staphylococcus aureus (N=28)							
		Resitance		Intermediate		Sensitive	
Antibiotics class	Antibiotics	N	%	N	%	N	%
Quinolone/Fluoro quilones	Gatifloxacin	19	67.86	2	7.14	7	25
	Ciprofloxacin	17	60.71	4	14.26	7	25
	Moxifloxacin	24	85.71	0	0	4	14.26
Aminoglycosides	Gentamycin	1	3.57	2	7.14	25	89.26
	Amikacin	0	0	1	3.57	27	96.43
Beta-lactam	Oxacillin	16	57.14	0	0	12	42.86
	Ampicillin	15	53.57	0	0	13	46.43
	Penicillin G	21	75	0	0	7	25
	Methicillin	14	50	0	0	14	50
Glycopeptide	Vancomycin*	0	0	0	0	14	100
Macrolide	Erythromycin	12	42.86	9	32.14	7	25
Sulphonomide	Cotrimoxazole	14	50	2	7.14	12	42.86
Chloramphenicol	Chloramphenicol	2	7.14	1	3.57	25	89.26
Lincosamide	Clindamycin	7	25	0	0	21	75

In *S. aureus*, Gatifloxacin (25%) and Ciprofloxacin (25%) were found more efficient where as least was found Moxifloxacin (14.26) in Quinolone/Fluoroquinolone group. In Aminoglycosides group, Amikacin (96.43%) was found more susceptible followed by Gentamycin (89.46%). All MRSA isolates were found Vancomycin susceptible.

5.7. Age wise Distribution pattern of *S. aureus*

Table 18. Age wise distribution of *Staphylococcus aureus*

Age group	**Isolates**	**Percent**	**MRSA**	**MRSA %**
10-20	2	7.14	2	100
20-30	10	35.71	3	30
30-40	10	35.71	5	50
40-50	2	7.14	2	100
50-60	4	14.26	2	50
Total	28	100	14	50

S. aureus was found high in 20-30 years and 30-40 years age group. A total of 14 Methicillin Resistant *S. aureus* were isolated. Highest isolates of MRSA were found in the age group 30-40 years of patients.

Table 19. Antibiotics sensitivity pattern of CoNS **and** *Streptococcus* spp.

(N=36)	Resistant		Intermediate		Sensitive	
Antibiotics	No.	%	No.	%	No.	%
Cotrimoxazole	16	44.44	4	11.11	16	44.44
Penicillin G	19	52.78	1	2.78	16	44.44
Oxacillin	10	27.78	2	5.56	24	66.67
Erythromycin	15	41.67	1	2.78	20	55.56
Cloxacillin	11	30.56	1	2.78	24	66.67
Ciprofloxacin	15	41.67	1	2.78	20	55.56
Ampicillin	10	27.78	1	2.78	25	69.44
Nitrofurantoin	7	19.44	2	5.56	27	75

As shown in Table 19., in CoNS, *Streptococcus faecalis* and other *Streptococcus* spp., Nitrofurantoin (75%) and least was found Cotrimoxazole (44.44%) and Penicillin G (44.44%)

Table 20. Antibiotic susceptibility pattern of CoNS

CoNS (N=28)						
	Resistance		Intermediate		Sensitive	
Name of Antibiotics	n	%	n	%	n	%
Cotrimoxazole	16	53.14	4	14.28	8	28.57
Penicillin G	15	53.57	1	3.57	11	39.28
Oxacillin	8	28.57	2	7.14	18	64.29
Erythromycin	13	46.43	1	3.57	14	50
Cloxacillin	10	35.71	1	3.57	17	60.71
Ciprofloxacin	14	50	1	3.57	13	46.43
Ampicillin	10	35.71	1	3.57	17	60.71
Nitrofurantoin	7	25	2	7.14	19	67.86

As shown in Table 20., for CoNS, most susceptible was Nitrofurantoin (67.86%) and least was found Cotrimoxazole (28.57%).

Table 21. Antibiotic susceptibility pattern of *Streptococcus* spp.

Streptococcus spp. (N=5)						
	Resistance		Intermediate		Sensitive	
Antibiotics	n	%	n	%	n	%
Cotrimoxazole	0	0	0	0	5	100
Penicillin G	3	60	0	0	2	40
Oxacillin	2	40	0	0	3	60
Erythromycin	2	40	0	0	3	60
Cloxacillin	1	20	0	0	4	80
Ciprofloxacin	0	0	0	0	5	100
Ampicillin	0	0	0	0	5	100
Nitrofurantoin	0	0	0	0	5	100

As shown in Table 21., *Streptococcus* spp., was found susceptible to Cotrimoxazole

(100%), Ciprofloxacin (100%), Ampicillin (100%), Nitrofurantoin (100%) followed by Cloxacillin (80%), Oxacillin (60%), Erythromycin (60%) and Penicillin G (40%).

Table 22. Antibiotic susceptibility pattern of *Streptococcus faecalis*

S. faecalis (N=3)						
	Resistance		Intermediate		Sensitive	
Antibiotics	n	%	n	%	n	%
Cotrimoxazole	0	0	0	0	3	100
Penicillin G	0	0	0	0	3	100
Oxacillin	0	0	0	0	3	100
Erythromycin	0	0	0	0	3	100
Cloxacillin	0	0	0	0	3	100
Ciprofloxacin	1	33.33	0	0	2	66.67
Ampicillin	0	0	0	0	3	100
Nitrofurantoin	0	0	0	0	3	100

As shown in Table 22., in *Streptococcus faecalis*, Cotrimoxazole (100%), Penicillin G (100%), Oxacillin (100%), Erythromycin (100%), Cloxacillin (100%), Ampicillin (100%), Nitrofurantoin (100%) showed susceptibility followed by Ciprofloxacin (100%).

5.8. Age wise distribution of Gram positive uropathogens

Table 23. Age wise distribution of CoNS, *Streptococcus* spp. **and** *Streptococcus faecalis*

	Microorganisms		
Age group	CoNS	*Streptococcus* spp.	*Streptococcus faecalis*
10-20	3	1	0
20-30	11	3	0
30-40	9	1	2
40-50	3	0	0
50-60	2	0	0
>60	0	0	1
Total isolates	28	5	3

The CoNS, Streptococcus spp. were found highest in 20-30 years age group, where as

S. faecalis was found high in 30-40 years age group.

5.9. Multiple Antibiotics Resistance (MAR) Index of Uropathogens

Table 24. MAR index of isolates

MAR index	*E. coli*	*K. oxytoca*	*K. pneumoniae*	*P. mirabilis*	*P. vulgaris*	*P. aeruginosa*	*C. freundii*	*E. cloacae*	*S. aureus*	CoNS	*Streptococcus spp.*	*S. faecalis*	Total
<0.2	74	7	7	10	4	3	1	1	5	11	1	0	124
>= 0.2	155	4	17	8	6	5	1	1	23	17	4	2	245
Total	**230**	**11**	**24**	**18**	**10**	**8**	**2**	**2**	**28**	**28**	**5**	**3**	**369**

The MAR index above 0.2 was found highest in *E. coli*, followed by *S. aureus*, CoNS, *K. pneumonia*e, *K. oxytoca, Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa, Streptococcus* spp., *S. faecalis* and *Citrobacter freundii* and *Enterobacter cloacae* respectively of the total isolates.

5.10. Multiple Antibiotics Resistance (MAR) index of antibiotics

Table 25. MAR Index of antibiotics for Gram negative uropathogens

Gram negatives uropathogens (N=305)		
Antibiotic	**Resistant**	**MAR index**
Nalidixic Acid	209	0.086
Ofloxacin	82	0.034
Ciprofloxacin	123	0.050
Norfloxacin	70	0.029
Tobramycin	28	0.011
Gentamycin	49	0.020
Cotrimoxazole	82	0.034
Cephalexin	56	0.023
Ampicillin	121	0.050

A total 9 different antibiotics were tested for Gram negative uropathogens. The MAR

index was found highest in Nalidixic acid (MARI=0.086) followed by Ciprofloxacin (MARI=0.050), Ampicillin (MARI=0.050), Ofloxacin (MARI=0.034), Cotrimoxazole (MARI=0.034), Norfloxacin (MARI=0.029) and Cephalexin (MARI=0.023) and least was found in Tobramycin (MARI=0.011) for Gram negative uropathogens.

Table 26. MAR Index for Gram positive uropathognes (*Staphylococcus aureus*)

Gram positive *Staphylococcus aureus* **(N=28)**		
Antibiotics	**Resistant**	**MAR Index**
Moxifloxacin	24	0.066
Penicillin G	21	0.058
Gatifloxacin	19	0.052
Ciprofloxacin	17	0.047
Oxacillin	16	0.044
Ampicillin	15	0.041
Cotrimoxazole	14	0.038
Methicillin	14	0.038
Erythromycin	12	0.033
Clindamycin	7	0.019
Chloramphenicol	2	0.005
Gentamycin	1	0.003

A total 14 different antibiotics was tested for *S. aureus* isolates in which no resistant isolates was found against Amikacin and Vancomycin.

The MAR index of antibiotics in case of *S. aureus* was found highest in Moxifloxacin (MARI=0.066) followed by Penicillin (MARI=0.058), Gatifloxacin (MARI=0.052), Ciprofloxacin (MARI=0.047), Methicillin (MARI=0.038), Erythromycin (MARI=0.033), Clindamycin (MARI=0.019), Chloramphenicol (MARI=0.005) and least was found in Gentamycin (MARI=0.003).

Table 27. MAR Index of antibiotics for Gram positive uropathognes (CoNS, *Streptococcus faecalis* and *Streptococcus* spp.)

Gram positive CoNS, *Streptococcus faecalis* and *Streptococcus* spp. **N=36**		
Antibiotics	**Resistant**	**MAR Index**
Cotrimoxazole	16	0.056
Penicillin G	19	0.066
Oxacillin	10	0.035
Erythromycin	15	0.052
Cloxacillin	11	0.038
Ciprofloxacin	15	0.052
Ampicillin	10	0.035
Nitrofurantoin	7	0.024

A total of 8 different antibiotics were assessed against CoNS, *Streptococcus* spp. and *Streptococcus faecalis*. The MAR index of antibiotics was found highest in Penicillin G (0.066) and minimal was found in Nitrofurantoin (0.024).

5.11. Host factors in UTI positive patients

Table 28. Different host factors in UTI positive patients visiting Materity hospital

a). Patients personal profile

FACTORS	ORIGIN		TOTAL (N=369)
	IN PATIENT (n=123)	OPD (n=246)	
Age			
<10	0	1	1(0.27%)
10-20	15	32	47 (12.74%)
20-30	73	133	206 (55.83%)
30-40	20	36	56 (15.18%)
40-50	6	26	32 (8.67%)
50-60	3	10	13 (3.52%)
60-70	4	6	10 (2.71%)
>70	2	2	4 (1.08%)
Problems or complaints for visit			
Urinary Tract Infection	26	36	62 (16.80%)
Routine checkup	12	12	24 (6.50%)
Pregnancy	69	91	160 (43.36%)
Complications	16	107	123 (33.33%)
Marital status			
Married	111	236	347 (90.04%)
Unmarried	12	10	22 (5.96%)
Education			
Illiterate	65	108	173 (46.88%)
Only read and write	13	34	47 (12.74%)
Below 10	20	48	68 (18.43%)
SLC	7	25	32 (8.67%)
Above SLC	18	31	49 (13.28%)
Occupation			
Housewife	70	217	287 (77.78%)

Employee	20	10	30 (8.13%)
Business	20	13	33 (8.96%)
Other (Students)	13	6	19 (5.15%)
Previous complication			
Yes	39	88	127 (34.42%)
No	84	158	242 (65.58%)
Kidney problem			
Yes	4	7	11 (2.98%)
No	119	239	358 (97.02%)
Previous antibiotics used			
Yes	59	145	204 (55.28%)
No	44	121	165 (44.72%)
Parity			
No children	47	97	144 (39.02%)
1 child	32	52	84 (22.76%)
2 children	27	51	78 (21.14%)
3 children	7	20	27 (7.32%)
4 children	2	9	11 (2.98%)
5 children	3	8	11 (2.98%)
6 children	0	4	4 (1.08%)
7 children	1	2	4 (1.08%)
8 children	4	3	7 (1.90%)

b) According to symptoms

Pain in urination			
Yes	77	157	234 (63.41%)
No	46	89	155 (42.01%)
Color of urine (Yellow)			
Yes	86	141	227 (61.52%)
No	37	105	142 (38.48%)

Presence of blood in urine according to patients			
Yes	7	31	38 (10.30%)
No	116	215	331 (89.70%)
Midnight urination			
Yes	109	207	316 (85.64%)
No	14	39	53 (14.36%)
Frequency per day			
Infrequent	14	40	54 (14.63%)
1 times	33	40	73 (19.78%)
2 times	27	42	69 (18.70%)
3 times	37	81	118 (31.98%)
4 times	6	35	41 (11.11%)
5 times	3	4	7 (1.90%)
6 times or more	3	4	7 (1.90%)
Back pain			
Yes	104	203	307 (83.18%)
No	19	43	62 (16.80%)
Fever			
Present	19	39	58 (15.72%)
Absent	104	207	311 (84.28%)
Duration of UTI			
less than one month	18	40	58 (15.72%)
1 month-6 months	37	60	97 (26.29%)
> 6 months – <than 1 year	1	7	8 (2.17%)
1 year	66	125	191 (51.76%)
more than one year	4	22	24 (6.50%)

c). According to microscopic analysis

Pyuria			
>=5+	48	110	158(42.82%)
<5+	75	136	211 (57.18)

Haematouria			
<3+	95	186	281 (76.15%)
>=3+	28	60	88 (23.84%)
Albumin			
<1+	93	185	278 (75.34%)
>=1+	30	81	111 (30.08%)

Among the patients visiting the Paropakar Women's and Maternity Hospital Nepal who were diagnosed positive for UTI 43.36 % were found to pregnant, followed by complication of pregnancy and other severe conditions of urinary tract infection (33.33%), suspected UTI (16.80%) and for routine checkups (6.50%).

Most of the patients diagnosed with UTI were found to be married (90.04%). Regarding educational status, most of the patients were found to be illiterate (46.88%), followed by, educational level below 10 (18.43%), above SLC (13.28%), only read and write (12.74%) and up to SLC (8.67%). In terms of occupation most UTI positive patients were found to be housewife (77.78%). In case of symptoms, 234 (63.41%) UTI positive patients have complaint about pain during urination, 141 (61.52%) have yellow urine, 31 (10.30%) have blood in urine and 207 (85.64%) have a micturition in midnight. 173 (46.88%) of UTI positive patients have to urinate three times and more than three times. Among the total UTI positive cases 307(83.18%) have got the back pain and 58 (15.72%) have a fever.

Most of the UTI positive patients have got the problem from 1 year or more than 1 year which represents 58.27% of total UTI cases. Recurrent UTI was found in 127 (34.42%) of positive cases of UTI.

Only 11 (2.98%) UTI positive patients have got the problem of Kidney. Of the UTI positive patient 204 (55.28%) have the history of previous antibiotics used. Among the total positive cases of UTI 144 (39.02%) patients have no children.

Among the total uropathogens positive patients Haematouria, Pyuria and albumin was found positive in 23.84%, 42.82% and 30.08% respectively.

5.12. Association between previous antibiotics and MDR

Table 29. Chi-square test of previous antibiotics and MDR

		previous antibiotics use		Total	p-value
		Yes	No		
Multiple Drug Resistant	Yes	137	62	199	p=0.039
	No	99	71	170	
Total		236	133	369	

5.13. Association between previous complication and MDR

Table 30. Chi-square test of previous complication and MDR

		Previous Complication		Total	p-value
		Yes	No		
MDR	Yes	71	128	199	0.660
	No	56	114	170	
Total		127	242	369	

Between previous antibiotics use and MDR, strong association was found (p=0.039), and the ODDs ratio was found 1.585 (CI: 1.033 to 2.430). But no association was found between previous complication and MDR isolates.

CHAPTER VI.

6. Discussion and Conclusion

6. 1. Discussion

The present study was conducted to find the prevalence of UTI in the patients visiting Maternity hospital and to determine the MDR bacterial isolates and also to assess the different factors associated with the UTI in the patients visiting maternity hospital, Thapathali. All together 1246 mid-stream urine samples were collected from the patients visiting maternity hospital and were subjected to standard microbiological procedure.

Among 1246 urine samples, 1057 (70.39%) urine samples didn't showed any growth and 369 (29.61%) samples were found to be culture positive. Similar previous studies have also reported the low growth rate (Chhetri *et al*., 2001), this might be due to the inclusion of patients for routine checkups only. Besides, the low growth positivity might be due the inclusion of kidney transplant patients, samples from patients under treatment, infection by the slow growing organisms or due to those organisms that are not able to grow in routine culture media (Manandhar, 1996). As low as 4.6% growth positivity have been reported in one study (Talukdar, 1987).

In the 369 UTI positive cases, the uropathogens were found higher in Outpatients (66.67%) than in inpatients (33.33%) which were found statistically insignificant (p=0.280).

In this study only females visiting the maternity hospital were included. Pregnant women have increase risk of developing UTI due to different anatomical and physiological changes that takes place during the period of pregnancy. In the present study also 43.36 % of UTI positive patients were pregnant women. In the study conducted by Kahlmeter (2000), and Warren (2000) demonstrated that if untreated, asymptomatic bacteriuria increased the frequency of premature delivery and neonates

with low birth weight and also it was likely to cause severe infections such as pyelonephritis at a rate of 15-30%.

In this study, age group 20-30 years had got the high prevalence of UTI. A total of 134 (36.31%) patients of the total UTI positive cases were found in this age group. Previous study done by Shrestha *et al.,* (2005) at Kathmandu Model Hospital, Steenberg *et al*., (1985) Rajbhandari and Shrestha (2002) also found the similar result. This result suggests that sexually active and women of childbearing age are more susceptible to UTI. Leigh (1990) had found that Nuns and unmarried women have lower prevalence of UTI in compared to married women. In the present study also, among the total positive cases only 22 (5.96%) were unmarried women whereas 347 (94.03%) were married. Many previous studies have also found the similar results and suggested that sexual intercourse is an important determinant factor for the pathogenesis of UTI. But in some other cases, the high growth positivity has been found in higher ages (Leigh, 1990).

Among the 369 uropathogens, 305 (82.66%) were Gram negative rods and remaining 64 (17.34%) were Gram positive cocci. In the similar previous study done by Shrestha (2004), Levett (1993) in India, Karki *et al*., (2004) in Kathmandu had found higher percentage of Gram negative rods.

Altogether, there were 12 different bacterial species were isolated, in which 8 species were Gram negative and 4 species were Gram positive. Among the Gram negative, *E. coli* (62.3%) was the most prevalent uropathogens followed by *K. pneumoniae* (6.5%), *P. mirabilis* (4.9%), *K. oxytoca* (3%), *P. vulgaris* (2.7%), *Ps. aeruginosa* (2.2%), *C. freundii* (0.5%) and *E. cloacae* (0.5%). In this study, the higher number of *E. coli* was observed which is similar to the result obtained by Manandhar *et al*., (2005), Dhakal *et al*., (2002), Farrell *et al*., (2003), Mohammed *et al*., (2006).

In Gram positive cocci, equal number of *S. aureus* (7.6%) and CoNS (7.6%) were isolated followed by *Streptococcus* spp. (1.4%) and *S. faecalis* (0.8%) from the total positive cases. In the similar previous study conducted by Dhakal and Pokhrel (2001),

among the total isolates, 79% were Gram negative rods and 21% were Gram positive cocci which revealed that UTI is primarily caused by Gram negative bacteria compare to Gram positive cocci. This finding agrees with the study done in the other parts of the world (Nordenstam *et al*., 1986; Tsunoda *et al*., 1979). Okada *et al*., (1994) also found 70.2% Gram negative rods and 29.8% Gram positive isolates. Similarly in a study done by Obi *et al.,* (1996) in Africa among 10 species of bacteria, the distribution of gram negative and gram positive bacteria were 88.5% and 9.7% respectively in UTI positive sample.

In total 230 *E. coli* isolates, 156 were from Outpatients and 74 were from inpatient. The most prevalent uropathogens from out-patients were *E. coli*, *S. aureus*, *P. mirabilis*, CoNS, *K. pneumoniae*, *K. oxytoca*, *Streptococcus* spp., *S. faecalis*, *C. freundii*. *Ps. aeruginosa* was found equal in both in patients and out patients. Only *E. cloacae* was isolated from in-patients. Similar result was obtained in the study conducted by Shrestha (2004) at Kathmandu Model Hospital.

E. coli is the major Gram negative isolate in this study. It is suggested that *E. coli* is the most predominant organism to colonize the urethral meatus (Schaeffer and Chmiel, 1983) and perineum (Leigh, 1990) before ascending to the bladder. Pathogenic *E. coli* expresses specific adhesions such as P *fimbriae* and produce alpha and beta hemolysins. In a study performed by Petrof *et al.,* (1999) in USA and Leonid *et al.,* (2006) in Russia concluded that these factors are thought to play a role in attachment, ascent and colonization of different tissue surface during progression of steps involved in urinary tract.

In a study done by Hooton *et al.,* (1996) in England found that the current prevailing theory regarding a reservoir for *E. coli* strains causing UTI is that they originate from the gastorointestinal tract flora. In USA, most of the urinary tract infections were caused by cystenie- requiring *E. coli* (Borderon *et al.,* 1978, Melver *et al*., 1995). During cystitis, uropathogenic *E. coli* (UPEC) subvert innate defenses by invading superficial umbrella cells and rapidly increasing in numbers to form intracellular bacterial communities (IBCs) (Justice *et al.,* 2006) in USA.

In the study performed by Ana *et al*., (1998) in USA found that *E. coli* was the most common urinary pathogen and found that most of the *E. coli* strains possessed urovirulence determinants which include mannone-resistant hemagglutination, F fimbriae, *P fimbriae*, hemolysins and aerbactin.

In this study, *K. pneumoniae* (6.5%) was the second predominant organisms. Similar result was obtained in the study conducted by Astal and Sharif, (2002) in Eastern region, Shrestha (2005) in Kathmandu, Zhanal *et al*., (2005) in Canada, Kumari *et al*., (2005), Das *et al*., (2006), Lautenbach *et al*., (2005) in Philadelphia, USA, Manadhar *et al*., (2005). *K. oxytoca* was also isolated in the present study. According to the Fowler (1990), *K. pneumoniae* is the primary pathogen in the genus although *K. oxytoca* may also cause bacteriuria.

The third and fifth most common isolates in this study was *P. mirabilis* and *P. vulgaris*. *Proteus* spp. is able to produce urease enzyme that catalyzes the hydrolysis of urea with the liberation of ammonia. Thus, if the *Proteus* causes the UTI it makes the urine alkaline leading to the stone formation and makes the acidification of the urine virtually impossible. It can also invade the urinary tract due to its rapid motility (Brooks *et al*., 2004). Besides, it can also enhance the formation of urinary calculi which have been accounted as the most common complication accompanying the UTI by the members of the genus *Proteus* as found in previous studies (Li *et al*., 2002 and Torzewska *et al*., 2003). *Proteus* spp. is primarily the causative agents of UTI in males and in hospitalized patients. In the latter group it may cause UTI in association with obstruction or use of instrument (Leigh, 1990).

Ps. aeroginusa was isolated as the sixth predominant isolates. *Ps. aeroginusa* is opportunistic pathogens. It is one of the primary causes of nosocomial infection. Mohammed *et al*., (2007) in his study found *Ps. aeruginosa* more prevalent in the middle age females in India. In this, study also, *Ps. aeruginosa* was found more prevalent in female of the age group 10-20 years, 20-30 years and 30-40 years female patients. In many other studies it have been found that *Ps. aeruginosa* plays an important role in the bladder infection and is considered as primary pathogens in

compromised host (Dolan *et al*., 1989; Jones *et al*., 1999) and also in uncomplicated urinary tract infection (Kosakai *et al*., 1990).

Two *E. cloacae* and *C. freundii* were isolated in this study. *Enterobacter* spp. and *Citrobacter* spp. are common fecal coliform bacteria. These bacteria ascend through the anus to urethra and cause UTI. And are commonly found in patients who have community acquired UTI (Manges, 2005; Raszka and Khan, 2005).

In the Gram positive isolates, *S. aureus* and CoNS were most prevalent followed by *Streptococcus* spp., this result is strongly supported by the study conducted by Latham *et al*., (1983) in USA. They found that most of the CoNS that cause UTI belong to two species, *S. epidermidis* and *S. saprophyticus* where as in the study conducted by Kosakai *et al*., (1990), *S. hemolytic*, *S. hominis*, *S. simulans* and *S. cohanii* were isolated from urine in addition to the commonly isolated strains. CoNS is the most predominant species colonizing the urethra and the perineum in both the male and female genital organs. CoNS are opportunistc pathogens and can cause infection when the immune system is weak (Forbes *et al*., 2002). Presence of *S. aureus* in urine is an indication of pyelonephritis primarily acquired through haematogenous route. So a pure culture of *S. aureus* is considered significant regardless of number of CFUs (Forbes *et al*., 2002). In the study conducted by Karki *et al*., (2004) also found *S. aureus* as the primary Gram positive isolates. According to Raszka and Khan (2005) *Streptococcus* spp. may cause up to 5% of UTI and often associated with more complex genitourinary tract abnormalities. In contrast, Shigemura *et al*., (2005) found large numbers of *Enterobacter faecalis*.

Antimicrobial resistance is a global problem. It is now generally accepted as major public health issue and has significant implication on health and patient care. Resistance to antimicrobial drugs is associated with high morbidity and mortality, high health-care cost and prolonged hospitalization. The problem antimicrobial resistance is more troublesome to developing countries. WHO and the European Commission (EC) have recognized the importance of studying the emergence and determinants of resistance and the need for strategies for its control.

In this study, Cefalexin (93.17%) of the Cephalosporin antibiotics was found most efficient, followed by Tobramycin and Gentamycin of the Aminoglycosides group (90.61%), Cotrimozaxole (90%) of Sulfamethaxazole group, Ampicillin (85.24%) of Beta lactam group and the least susceptible was found Quinolone/Fluoroquinolones group (40.38%). In the similar study performed by Jha and Bapat (2005) at Sukhraraj Tropical Hospital, 92.5% of urinary isolates were susceptible to Gentamycin.

In Gram positive *S. aureus* isolates, Aminoglycosides group (99%) of antibiotics were found more susceptible followed by Chloramphenicol (98%), Lincosamide (93%), Macrolide (88%), Sulphonamide (86%), Quinolone/Fluoroquinolones group (40%) of antibiotics and the least susceptible was found Beta-lactam group of antibiotics (34%).

In the urine isolate *E. coli*, Nalidixic acid (29.13%) was found the least susceptible. In other antibiotics of Fluoroquilones group Norfloxacin was found most efficient antibiotics followed by Ofloxacin (70.87%) and Ciprofloxacin (59.57%). Similarly Ampicillin (40.43%) from Beta lactam group was found least susceptible. The results found in this study is strongly supported by different other researches. In a study conducted by Karki *et al*., (2004) among out-patient and in-patient of Kathmandu Medical College Teaching Hospital, the *E. coli* isolates were found least susceptible to Nalidixic acid and the most potent antibiotic was found Nitrofuratoin and Norfloxacin followed by Ofloxacin. In the study conducted by Arosio *et al*., (1978) and Obi *et al*., (1996) resistant to Ampicillin was observed. Sharma (1983), in his study found 93.0% *E. coli* resistant to Ampicillin. Similar type of result was found by Modi and Erch (2006).

K. pneumoniae was found most susceptible to Gentamycin (95.83%) and least susceptible to Ciprofloxacin (20.83%) and Ampicillin (20.83%). In *K. oxytoca*, Gentamycin (100%) was found most susceptible and least was found Ampicillin (27.27%). In the similar study done by Karki *et al*., (2004) also found the similar results in which Ciprofloxacin was found least susceptible in *Klebsiella* spp.

P. mirabilis was found maximally inhibited by Norfloxacin (88.89%) in

Fluoroquinolones group and Gentamycin (88.89%) and minimal susceptibility was found against Ampicillin (16.67%) and Nalidixic acid (16.67%). In *P. vulgaris*, most efficient antibiotics were found Norfloxacin (70%) of Fluoroquinolones group, and Tobramycin (70%) of Aminoglycosides group and the low susceptibility were shown by Nalidixic acid (30%) and Ampicillin (30%). Karki *et al*., (2004) also found Norfloxacin highly susceptible to *Proteus* spp.

In Uropathogenic *Ps. aeruginosa*, Gentamycin was found 100% efficient. The least efficient antibiotic was found Ampicillin (12.5%). In contract to this study, Karki *et al*., (2004) found, all used antibiotics resistance against *Ps. aeruginosa*. Similarly, in the study conducted by Abubakar (2009), *Ps. Aeruginosa* was found most resistant to Ampicillin.

E. cloacae isolates was found 100% susceptible against Nalidixic acid, Ciprofloxacin, Norfloxacin, Tobramycin and Gentamycin

In *C. freundii*, most susceptible was found Norfloxacin (100%), Gentamycin (100%) and least susceptible were found Cotrmozaxole (0%), Ampicillin (0%) and Cefalexin (0%). In the stludy conducted by Abubakar (2009) least susceptible was found Ampicillin (15.4%) and Gentamycin (15.4%) and most susceptible was found Agumentin (61.2%), Nalidixic acid (53.9%).

In antibiotics susceptibility test of Gram positive isolates, *S. aureus*, most susceptible was found Amikacin (96.43%) and least was found Moxifloxacin (14.26%).

In CoNS, most susceptible was found Nitrofurantoin (67.86%) and least was found Cotrimoxazole (28.57%).

Streptococcus spp. was found highly susceptible to Cotrimoxazole (100%), Ciprofloxacin (100%) and Ampicillin (100%) and least susceptible to Penicillin G (40%).

S. faecalis was found susceptible to all antibiotics tested except Ciprofloxacin (66.67%).

Multiple Drug Resistance (MDR) is defined as resistance to three or more of the antimicrobial agents as evaluated in the study (Kurtepe *et al*., 2005). Of the total 369 uropathogens, 199 isolates (53.93%) were found to be MDR out of which 35.18% (70) were isolated from in-patients and 64.82% (129) were from out-patients among the total isolates. In Gram negative isolates evaluated for 9 different antibiotics, 53.11% (162/305) were MDR. In Gram positive isolates 57.81% (37/64) were found MDR. The lower prevalence of MDR, 13.9 % was found in the study done by Oteo *et al*., (2001) when MDR criterion was resistance to 3 or more antibiotics.

In the present study, 55.65% (128/230) of *E. coli* isolates were found MDR, *K. pneumoniae* showed 58.33% (14/24) MDR, similarly 50% (9/18) of *P. mirabilis*, 27.27% (2/11) of *K. oxytoca*, 50% (5/10) of *P. vulgaris*, 37.5% of *Ps. aeruginosa*, 50% (1/2) in *C. freundii* were found MDR. No MDR isolates were found in *E. cloacae*.

In Gram positive isolates *S. aureus*, evaluated for 14 different antibiotics showed 82.12% (23/28) MDR and 50% (14/28) were MRSA. All MRSA were found to be Vancomycin susceptible. Two case of invasive infection caused by penton-valentine Leukocidin positive, community associated Methicillin resistant *S. aureus* after kidney transplantation have been reported (Adeyemi *et al*., 2008).

Community associated Methicillin resistant *S. aureus* (CA-MRSA) continue to emerge as a cause of serious infection, chiefly of the skin and soft tissues. (Calvano *et al*., 2009).

In order to determine the prevalence of Methicillin resistant *S. aureus* (MRSA) colonization among adult in community setting in Taiwan and identifying its risk factors. MRSA colonization in adult in community setting in Taiwan was 3.8%. They found smoking appear to be protective against MRSA colonization, ($p<0.0001$) (Wong *et al*., 2009).

CoNS, *Streptococcus* spp. and *S. faecalis* tested for 8 antibiotics showed 46.43% (13/28), 0% (0/5) and 33.33% (1/3) MDR isolates respectively.

Multiple Drug Resistance (MAR) Index of isolates and antibiotics against bacterial isolates were calculated. Among the total 369 isolated uropathogens, 243 (66.4%) of the uropathogens showed the MAR index of bacterial pathogens higher than 0.2 which implies that strains of such bacteria originate from an environment where several antibiotics are used. The result found was strongly supported by the study conducted by Krumpermann (1983), Ehinmidu (2005) and Tambedkar *et al*., (2006).

MAR index of antibiotics against Gram negative isolates was found highest in Nalidixic acid (MARI 0.086) and least was found in Tobramycin (MARI 0.011). In *S. aureus* MAR Index was found highest in Moxifloxacin (MARI 0.066) and least in Amikacin (MARI 0) and Vancomycin (MARI 0). This results is supported by the study conducted by Krumpermann (1983), Ehinmidu (2005) and Tambedkar *et al*., (2006)

Thus from the present study indicate large portion of bacteria were exposed to the antibiotics. Besides, large number of the bacterial isolate in this study showed multiple antibiotics resistance. The present study data gives idea about the common trend increased antibiotics resistance of uropathogens in UTI in this region, which may be due to geographic variation or indiscrimate or sublethal use of antibiotic. According to Ehinmidu (2005) and Tambekar *et al*., (2006), the MAR index of isolates not only help in proper treatment of UTI patients but also discourage the indiscrimate use of antibiotics and prevent further development of bacterial drug resistance and also help the clinicians to give proper treatment and prescription of most sensitive antibiotic to the patient and avoid use of resistant antibiotics. According to Krumpermann (1983), Ehinmidu (2005) and Tambedkar *et al*., (2006) multiple antibiotics resistance (MAR) index is a tool that reveals the spread of bacteria resistance in a given population

In this study, high number of patients who were diagnosed UTI positive belongs to the age group 20-30 years (55.83%). In the present study, maximal numbers of culture positive patients visiting the hospital were married (90.04%) pregnant women

(46.36%). This result is supported by the study conducted by Steenberg *et al* (1985), Manandhar *et al* (1996), Rajbhandari and Shrestha (2002), Regmi *et al* (2003), Shrestha *et al* (2005) and Jha and Bapat (2005). Risk of developing UTI is high in females who are sexually active and are of childbearing age. Sexual activity has been considered as the major factor in the causation of UTI in women.

Primarily high numbers of urine culture positive patients were illiterate (46.88%) women and high proportion were found to be housewife (77.78%). In the study conducted by Haider *et al*., (2010) educational status has been found as an important factor in prevalence of UTI.

Among the urine culture positive patients most of them have the symptoms of back pain (83.18%), pain during urination (63.41%), yellow urine (61.52%), dysuria, urination for 3 times and more (46.88%). Of the total case positive women, few of them have the symptoms of blood in urine (10.30%), fever (15.72%), and kidney problem (2.98%) and have the history of previous complication (34.42%). Symptoms such as increased frequency of micturition, dysuria and urgency indicate lower UTI where as high temperature, dysuria and nausea indicates upper UTI (Car, 2006).

In the total 369 uropathogens positive patients, 125 (55.28%) had a history of previous antibiotics used. Strong association was found in previous antibiotics use and MDR isolates (p=0.039). The odd ratio of previous antibiotics use and MDR isolates was found 1.585 (CI: 1.033 to 2.430).

Among the total case positive women most of them were found to have one or more children (61.25%) during the visit to the hospital.

6.2. CONCLUSION

The Primary objective of this study was to assess the prevalence of UTI in patients visiting Paropakar Maternity and Women's Hospital as well as to assess different factors responsible in the cause of UTI in the patients.

From this study, the prevalence of UTI in the patient visiting Maternity hospital was found 29.61%. The prevalence of UTI was comparatively found higher in out-patients than in in-patients. From the urine sample of 1246, a total of 369 uropathogens belonging to 12 different species were isolated. Gram negative uropathogens were found predominant. Among Gram negative, *E. coli* was the major isolates. In Gram positive *S. aureus* and CoNS were found maximal.

Previous antibiotics used were found significantly associated with the MDR in the UTI positive cases.

CHAPTER VII.

7. SUMMARY AND RECOMNENDATIONS

7. 1 SUMMARY

1. The study was conducted in the microbiological laboratory of Paropakar Maternity and Women's Hospital Nepal from Jestha to Paush, 2067 BS in the UTI suspected patients. A total of 1246 samples were collected, in which 369 were found to harbor uropathogens.

2. The prevalence of UTI was found to be 29.61%, and comparatively higher proportions of out-patients were found to have UTI than in-patients.

3. Among the isolates 12 different uropathogens were isolated. In which 82.65% (305/369) belongs to Gram negative and 17.34% (64/369) were from Gram positive. *E. coli* (63.2%) was found most predominant in Gram negative isolates followed by followed by *K. pneumonia* (6.5%), *P. mirabilis* (4.9), *K. oxytoca* (3.0%), *P. vulgaris* (2.7%), *Ps. aeruginosa* (2.2), *E. cloacae* (0.5) and *C. freundii* (0.5). In Gram positive, *S. aureus* (7.6%) and CoNS (7.6%) were the major isolates followed by *Streptococcus* spp. (1.4%) and *S. faecalis* (0.8%).

4. The most efficient antibiotics for Gram negative was found Tobramycin (57.54%) followed by Gentamycin (82.95%), Norfloxacin (77.05%), Ofloxacin (70.16%), Ciprofloxacin (58.36%), Cefalexin (55.41%) and Ampicillin (35.41%).

5. For *S. aureus* the most susceptible antibiotics was found Amikacin (96.43%) and least was found Penicillin G and Erythromycin (25%), for CoNS, *Streptococcous* spp. the most susceptible antibiotics was found Netilimycin and least susceptible was found Penicillin G.

6. In the total uropathogens, 199 (53.93%) isolates were found to be MDR, in Gram negative *K. pneumoniae* showed highest MDR and in Gram positive *S. aureus* showed highest MDR.

7. In *S. aureus*, 50% (14/28) of isolates was found to be MRSA. All MRSA isolates were found to be susceptible to Vancomycin. Highest MRSA isolates were found in the age group 30-40 years.

8. MARI above 0.20 was found in 66.94% of the total isolates.

9. For the 9 antibiotics used for Gram negative the highest MARI was found in Nalidixic acid (MARI 0.086) and least was found in Tobramycin (MARI 0.011). For the 14 antibiotics used for S. aureus highest MARI was found for Mefixcillin (MARI 0.066) and least was in antibiotics Gentamycin (MARI 0.003) where as for CoNS and Streptococcus spp. MARI was found highest in antibiotics Penicillin G (MARI 0.066) and least was found for Nitrofurantoin (MARI 0.024).

10. For the different factors assessed for the UTI culture positive patients, in age group 20-30 years was found to have high uropathogens positive patients. Similarly, of the total uropathogens positive patients pregnant women accounts for 43.36% whereas other factors such as in martial status married women (90.04%), in Literarcy illiterate patients (46.88%), in occupation housewife (77.78%), in Previous complication with no such complaints (65.58%) and in Previous antibiotics used affirmative patients (55.28%) were found to have higher proportion of culture positive patients.

11. Previous antibiotics used and MDR isolates were found to have strong association (p=0.039) and odd ratio was found 1.585 (CI: 1.033 to 2.430).

7. 2 RECOMMENDATION

1. More UTI positive patients were found in 20-30 years age group which is the sexually active age and childbearing age. Thus women in this age group should be regularly monitored for urinary tract infection.

2. Among the total UTI positive cases, 43 % were pregnant women, thus they should be regularly checked for urinary tract infection.

3. Previous antibiotics used were found to be associated with the multiple drug resistant isolates thus women who have used the antibiotics before should be regularly checked.

4. Women who have complains with pain in urination, colored urine, high frequency of midnight urination, and more than 3 times urination per day should be tested for urinary tract infection.

5. Empirical antibiotics therapy should be discouraged. Antibiotics should be strictly used only on the basis of Laboratory results (Antibiotics susceptibility test) and strict rules and regulation should be made in this matter and sophisticated antibiotics treatment policy should be made.

6. Nearly more than the half of the urine isolates were found to be MDR with MAR index higher than 0.20, so regular surveillance and monitoring of such multiple drug resistant bacteria should be made by the authoritative bodies.

7. Different host factors such as hygiene, literacy, parity, gravida etc. and its association with UTI should be frequently examined.

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