CHAPTER-I

1. **INTRODUCTION**

Urinary tract infection (UTI) is defined as condition of multiplication of the organisms in the urinary tract and the presence of more than 10^5 organisms/ml of mid stream urine. Primarily, bacteria that colonize in the bowel and are capable of proliferating in urine cause UTI. Bacteriuria may indicate infection of urinary tract, contamination by exogenous bacteria or the removal of normal urethral flora with first flow of urine.

Urinary tract infection in adults can be categorized into five groups: young women with acute uncomplicated cystitis, young women with recurrent cystitis, young women with acute uncomplicated pyelonephritis, all adults with complicated urinary infection, and all adults with asymptomatic bacteriuria (Walter, 1993).

Urinary tract Infections are the second most common type of microbial infection. UTI occurs in all populations, from the neonate to the geriatric patient, but has a particular impact on females of all ages, kidney transplant patients, and anyone with functional or structural abnormalities of the urinary excretory system. Urinary tract infection (UTI) affects humans throughout their life span. UTI is among the most common reasons patients seek medical care. Urinary tract infection is a common disorder that occurs in approximately 25% of young women and 50% of all women during their lifetime (Fihn, 2003).

Women are especially prone to UTIs for reasons that are not yet well understood. UTIs in men are not as common as in women but can be very serious when they do occur. Pregnant women seem no more prone to UTIs than other women. However, when a UTI does occur in a pregnant woman, it is more likely to involve kidneys. The incidence ratio of UTIs in middle-aged women to men is 30:1; however, during later decades of

life, the ratio of infection in women to men with bacteriuria progressively decreases (Boscia and Kaye, 1987).

Discovering over 10^5 bacteria /ml of voided urine indicates significant bacteriuria, a presumably pathological condition (Forbes *et al.,* 2002). Asymptomatic bacteriuria (ASB), in which urine culture reveals a significant growth of pathogens, that is greater than 10^5 cfu/ml, but without the patient showing symptoms of UTI, can be found in both pregnant and non pregnant women. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric outcomes such as prematurity, low-birth weight, and higher fetal mortality rates. Although UTI may not always lead to complications in the mother, it is still a cause of significant morbidity (Turpin *et al*., 2007). Untreated ASB is a risk factor for acute cystitis (40%) and pyelonephritis (25-30%) in pregnancy. These cases account for 70% of all cases of symptomatic UTI among unscreened pregnant women (Jones, 2009).

Asymptomatic bacteriuria (ASB) in healthy, non pregnant women is common and is benign. However, a group of women who are at risk for symptomatic urinary tract infection is reported (Nicolle, 2000). The prevalence of asymptomatic bacteriuria in pregnancy varies from 4-7 % (range 2-11%) and is similar to that observed in non pregnant women (Patterson , 1987; Norden and Kass, 1986). More than a quarter of patients with bacteriuria later develop symptomatic UTI or acute pyelonephritis if left untreated. Continuing bacteriuria is associated with premature delivery and increased perinatal mortality (Martin, 1992).

Nicolle (1994) stated that the gold standard in screening for asymptomatic bacteriuria is urine culture in early pregnancy of 12 to 16 weeks gestation. Screening for bacteriuria is recommended among all pregnant women at the first prenatal visit and in the last trimester of pregnancy. The most effective method is urinalysis followed by culture. In

the presence of risk factors for UTI (previous history of UTI, pre-existing renal disease, diabetes), it is recommended to do urinalysis every month (Bahadi, 2010).

Gram negative bacilli and Enterococci are the primary enteric microorganisms capable of proliferating in the human urine. Among the microorganisms, *Escherichia coli* is the most common cause of UTI. Other pathogens include *Proteus mirabilis*, *Klebsiella* spp.,other *Enterobacteriaceae* and *Staphylococcus saprophyticus.* In more complicated UTIs, particularly in recurrent infections, the relative frequency of infection caused by *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Enterobacter* spp. increases. Hospitalized patients are most likely to be infected by *E. coli, Klebsiella* spp., *Proteus mirabilis,* staphylococci and other *Enterobacteriaceae*, *Pseudomonas aeruginosa* and enterococci. In addition, UTIs are leading cause of Gram negative sepsis in hospitalized patients and are the origin for about half of all nosocomial infections caused by urinary catheters (Forbes *et al.*, 2002).

Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory (Chakraborty, 2001).

UTI is also reported as a common nosocomial infection among Nepalese. According to the annual report published by Department of Health Services (2059/60) morbidity of UTI in Nepal was 1, 25,058. About 61.4% of people in our country are illiterate and do not have any proper concept of good hygiene and sanitation. So they are always vulnerable to infections. In many parts of Nepal, the facilities for urine culture and antimicrobial susceptibility testing are not available thus leading to incorrect diagnosis and management of UTI (Sharma, 1983). Most of the people in our country are not financially sound to have a routine check-up of their health status. People generally seek for medical services only when the symptoms of the disease begin to become evident. Asymptomatic diseases in patients are simply ignored and in many cases, this negligence ultimately leads to serious complications.

Shree Birendra Hospital is a tertiary care hospital. In this hospital military personnel and their relatives come for their haalth check up. In the gynecological ward of this hospital about 50 pregnant women are checked in a day. Therefore the present study was conducted with a broad objective to isolate the bacteria causing UTI in pregnant women who do not have any symptoms of UTI. The next objective of this study was to determine the trend of their antimicrobial resistance.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To isolate the bacteria causing asymptomatic bacteriuria in pregnant women and determine their antimicrobial resistance pattern at Shree Birendra Hospital, Chhauni.

2.2 SPECIFIC OBJECTIVES

1. To isolate pathogenic bacteria from urine sample of asymptomatic pregnant women.
2. To determine the occurence of urinary tract infection among pregnant women of different age group.
3. To compare the UTI in different trimesters and gravidae.
4. To study predisposing factors of UTI among pregnant women.
5. To determine antibiotic sensitivity pattern of bacterial isolates.

.

CHAPTER-III

3. LITERATURE REVIEW

3.1 URINARY TRACT

The urinary tract consists of two pairs of kidneys, ureters, bladder and urethra. The urinary system helps to maintain proper water and salt balance through out the body.. The female urethra is relatively short compared with the male urethra and also lies in close proximity to the warm, moist, perirectal region, which is teeming with microorganisms. Because of the short urethra, bacteria can reach the blader more easily in the female host (Forbes, 2002). The two kidneys located on each side of the vertebral column plays very important role like concentrating urine, regulating electrolytes, and maintaining acid-base homeostasis. Urine passes from each kidney to the bladder through ureters. The bladder store urine for sometime and then urine is excreted out of the body via urethra.

3.2 ESTABLISHMENT AND MULTIPLICATION OF BACTERIA IN URINE

Human urine can support the bacterial growth due to its favorable chemical composition (Asscher *et al.*, 1968; Chernew, 1962). Though urinary antibodies have been demonstrated there has been no specific study indicating a role for urinary antibodies as a defense mechanism against infection. Thus, infections of the urinary tract (UTI) are a common source of morbidity and mortality. The bladder and urinary tract are normally sterile. The urethra however may contain a few commensals and also the perineum can contaminate urine when it is collected (Cheesbrough, 2000). Although urethra has a resident bacterial flora, these organisms do not commonly cause bladder infection in normal person (Leigh, 1990).

UTI are caused primarily by bacteria that colonize the bowel and are capable of proliferating in urine because human urine contains no humoral and cellular defenses against bacterial growth (Fowler, 1990).

Normal urine does not contain significant quantities of lysozyme or immunoglobulin, and any complement present is inactivated. Phagocytosis of bacteria is impaired both by the absence of opsonins and the wide range of osmolality in urine (Chernew and Braude, 1962).

The ability of the urine to support bacterial growth is related to urinary pH, osmolality, and chemical constituents such as glucose, amino acids and organic acids. Optimal bacterial growth occurs within a pH range of 6.0-7.0. A lowered osmolality of the urine encourages bacteriuria (Kaye and sobel, 1986).

Normal urine usually contains sufficient glucose to support maximal growth rates of urinary pathogens and any lowering of the pH is prevented by its buffering capacity. The number of bacteria in the urine of diabetic patients was significantly higher than in that of nondiabetic controls (O'Sullivan *et al.,* 1961).

3.3 URINARY TRACT INFECTION

The presence of bacteria in urine is called bacteriuria (Cheesbrough, 2000). Infection of urinary tract is defined as bacteriuria, the multiplication of the organisms in urinary tract and the presence of more than a hundred thousand organisms per ml in the midstream sample of urine (Chakraborty, 2001).Urinary tract infection simply means the presence of bacteria undergoing multiplication in urine within urinary drainage system (Leigh, 1990).

Urinary tract infection is defined as the detection of both bacteriuria 10^5 cfu/ml and pyuria i.e.10 leucocytes/HPF (Goya *et al.,* 1997). Bacteriuria which may lead to the infection of the prostate, epididymis or the testes are also included in the definition of UTI (Fowler and Mariano, 1990).

The term urinary tract infection (UTI) refers to the invasion of the urinary tract by a non-resident infectious organism. In order to confirm UTI with reasonable confidence, the criteria of clinical features, bacteriuria and pyuria must be met. Significant

bacteriuria is defined as the presence of bacteria in the urine. Organisms are actually multiplying in the urine and present in a count, which is excessively high or unexplainable by urethral contamination. UTI encompasses a wide variety of clinical entities whose common denominator is microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus. Infection of the prostate and epididymis is also included in the definition (Pokhrel, 2004).

Signs and symptoms of UTI include hematuria, dysuria, suprapubic discomfort, frequency, urgency, and nocturia. These symptoms are often difficult to distinguish from those due to pregnancy itself (Jones, 2009).

The criteria to interpret significant bacteriuria given by Kass, Marpal and Sandford;

- Less than 10^4cfu/ml indicate contamination,
- Equal to or more than 10^5cfu/ml indicate significant bacteriuria,
- 10^4-10^5cfu/ml indicates low count significant bacteriuria.

Low count significant bacteriuria subject to the following conditions

- Urine was collected before the organisms reached to log phase of growth after the entry of bacteria into the urinary tract.
- Patient under treatment.
- Obstruction in the ureter
- Some times in younger female, the count is low such as honeymoon cystitis.
- Patient with certain endocrine disorder e.g. diabetes.
- Chronic kidney infection where concentration power of kidney is low.
- Infection with relatively slow growing organisms e.g. *S. saprophyticus*, Streptococci other than Enterococci, *Haemophilus influenzae* etc.

In certain circumstances lower threshold should be considered as significant for example, where the organism is more difficult to grow (*Staphylococcus saprophyticus*,

Chlamydia, mycobacterium) or if the specimen has been collected from a urinary catheter (Wilkie ME *et al.*, 1992).

3.4 ETIOLOGICAL AGENTS OF UTI

Among the microorganisms causing UTI, *E. coli* is responsible for 74.6%, *Proteus* spp. responsible for 8.0%, *Klebsiella* spp. responsible for 2.0%, *Pseudomonas* spp. is responsible for 2.0% and other Gram negative organisms are responsible for 13.3% of the total cases of UTI (Herm *et al*., 2003).

In a study done by Astal *et al.* (2002) *E. coli* (25.9%), was found to be the major isolate followed by *Proteus* spp. (4.4%), *Enterobacter* spp. (3.3%), *Klebsiella* spp. (3.0%), *Pseudomonas* spp. (2.6%), *S. saprophyticus* (2.2%), *Enterococcus* spp. (1.5%), *Acinetobacter* spp. (1.1%), *Citrobacter* spp. (0.4%) and *S. aureus* (4%).

E. coli is the most common infecting organism in patients with uncomplicated UTI (Johnson, 1991). In the complicated UTI that occur in the abnormal or catheterized urinary tract, particularly in hospital patients, *E. coli* is still the commonest causative organism, but other members of *Enterobacteriaceae* such as *Klebsiella* spp., *Enterobacter* spp., Indole positive *Proteus* spp. and *Citrobacter* spp. are also frequent.

Other pathogens include *P. mirabilis*, which is a common cause of urinary tract infections in boys and men, and is associated with renal abnormalities. In hospital patients, *Proteus* spp. may cause chronic UTI in association with the use of instrument.

Gram positive pathogens such as *E. faecalis, S. saprophyticus* and group B streptococci can also infect the urinary tract. Urinary tract infections due to *E. faecalis* are usually associated with the use of instruments or catheterization (Collier *et al.*, 1998).

Candida infection may occur in diabetic and immunocompromised patients. Rarer infecting organisms include *S. agalactiae, S. milleri,* other Streptococci and *Gardnerella vaginalis* (Collins *et al.*, 1986).

Novobiocin resistant *S. saprophyticus* is a true primary pathogen of the urinary tract, which is responsible for 20.0% of urethritis and cystitis in sexually active but otherwise healthy young women.

The urethra has resident microflora that colonize its epithelium in the distal portion. The resident microflora of urethra includes coagulase negative staphylococci (excluding *S. saprophyticus*), viridans and non-hemolytic streptococci, diptheroids, anaerobic cocci and anaerobic Gram negative bacilli. Potential pathogens, including Gram negative aerobic bacilli primarily *Enterobacteriaceae* and occasional yeasts, are also present as transient flora, which contaminate urine in passage, so the voided urine may contain small number of bacteria in absence of urinary tract infection (Brooks *et al.*, 2004)

M. tuberculosis and other atypical mycobacteria may be found where cultures for acid fast bacteria are requested, they do not grow under routine aerobic conditions and may be found during the evaluation for sterile pyuria (Schaeffer *et al.*, 1998).

3.5 PATHOGENESIS

There are mainly two potential routes of infection: the ascending route, from the urethra to the bladder, then by the ureters to the kidneys; the hematogenous route, with seeding of the kidney during the course of bacteremia. (Forbes *et al.*, 2002). Other routes are: lymphatic route, intestine to kidney by way of lymphatics and direct infection.

Ascending Infection: There is considerable clinical evidence that most infections of the kidney result from ascension of fecally derived organisms from the urethra and periurethral tissues into the bladder and then by the ureter to the renal pelvis, with subsequent invasion of the renal medullae at this site (Forbes *et al.*, 2002)).

Hematogenous Infection: In humans, blood-borne infection of the kidneys and urinary tract accounts for less than 3% of the cases of UTI and pyelonephritis. Whereas *E. coli*, the other *Enterobacteriaceae*, *S. saprophyticus*, and *E. faecalis* account for

approximately 95% of UTIs, the major causes of hematogenous infection are *S. aureus*, *Salmonella* spp., *P aeruginosa*, and *Candida* spp. Because the kidneys receive 20% to 25% of the cardiac output, any microorganism that reaches the bloodstream can be delivered to the kidneys ((Forbes *et al.*, 2002)).

Most UTIs result from the ascending route, only a minority occurs after bacteraemia. The first step in the pathogenesis is the colonization of the periurethral tissue with uropathogens. Secondly, these uropathogens may gain access to the urethra. A symptomatic or asymptomatic infection of the bladder may result. A few organisms may finally ascend the ureters to the kidneys. These entire steps take place depends on the inoculum size, the virulence properties of the invading microorganism and the defense mechanisms of the host (Forbes *et al.*, 2002).

Upon entering the urinary tract, UPEC strains face a formidable array of host defenses, including the flow of urine and antimicrobial factors. Infection with type 1-piliated *E. coli* can trigger a number of host responses, including cytokine production, inflammation, and the exfoliation of infected bladder epithelial cells. Despite numerous host defenses and even antibiotic treatments that can effectively sterilize the urine, recent studies demonstrate that uropathogens can persist within the bladder tissue.

Strains of uropathogenic *Escherichia coli* (UPEC) are the causative agents in the vast majority of all urinary tract infections. To gain an initial foothold within the bladder, most UPEC strains encode filamentous surface adhesive organelles called type 1 pili that can mediate bacterial attachment to, and invasion of, bladder epithelial cells. Invasion provides UPEC with a protective environment in which bacteria can either replicate or persist in a quiescent state (Mulvey *et al*., 2000). Uropathogenic *E. coli* causes 90.0% of the urinary tract infections in anatomically normal, unobstructed urinary tracts. The bacteria colonize from feces or perineal region and ascend the urinary tract to the bladder. The fimbriae bind not only to red cells but to a specific

galactose disaccharide that is found on the surfaces uroepithelial cells in approximately 99.0% of the population

Uropathogenic strains of *E. coli* 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. These bacteria may serve as a reservoir for recurrent infections (Mulvey *et al*., 2000).

The K antigens of *E. coli* are capsular antigens. These may be able to promote bacterial virulence by decreasing the ability of antibodies and/or complement to bind to the bacterial surface, and the ability of phagocytes to recognize and engulf the bacterial cells.

Once introduced into the urinary tract, *Proteus* strains appear to be uniquely suited to cause significant disease in the urinary tract. These strains are able to facilitate their adherence to the mucosa of the kidneys. Also, *Proteus* spp. is 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* also adheres better to uroepthelial cells than do *S. aureus* and *S. epidermidis.*

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).

3.6 HOST DEFENCE MECHANISM

The presence of bacteria in the urinary tract does not necessarily result in infection. The size of the inoculum, virulence of the organism, and defense mechanisms inherent in the urinary tract will determine whether infection is established. Urine itself is inhibitory to some of the urethral flora such as anaerobes. In addition, a low pH, high or low

osmolality, high urea concentration, or high organic acid content of urine may inhibit even those organisms that can grow in urine.

Protection of the urinary tract against infection is strongly related to the constant flow of urine and regular emptying of the bladder. Reduction in regular flow of urine by, for e.g. bladder-neck obstruction, prostatic hypertrophy or neurological disorders of the bladder, favors an increase in numbers of bacteria and the development of infection (Leigh, 1990).

The impermeability of the urothelium to bacterial invasion is another most critical component of defense mechanism. A number of factors appear to inhibit the adherence of bacteria to the urothelium. The glycosaminoglycans component of the luminal transitional cell surface is extremely hydrophilic and may prevent bacterial attachment (Parsons and Mulholland, 1978).

3.7 CLINICAL MANIFESTATION

Irritability, fever and alteration of established voiding patterns may be the only manifestations of urinary tract in neonates and young children. In older children and adults, bacteriuria is manifested primarily by frequent urination, precipitous voiding, and a sensation of incomplete bladder emptying after micturition.

This symptom complex results from bladder inflammation and is commonly referred to as irritative voiding. Approximately 30.0% to 50.0% of women and as many as 95.0% of men with these symptoms have sterile urine. Painful urination or dysuria, is also a nonspecific complaint that usually results from urethral inflammation.

The common symptoms are frequency of micturition, haematuria, suprapubic pain and tenderness, smelly urine and dysuria (Collee *et al.,* 1999).

3.8 SITE OF INFECTION

The urinary tract is a complex drainage system consisting of distinct anatomical and physiological areas. The great majority bacterial infections occur in the bladder (cystitis) after the ascending migration of bacteria from the urethra or perineum. Infection of kidney may follow the haematogenous spread of bacteria, but more often the organism ascend from the bladder via the ureter and the renal pelvis calyces. Infection of the renal substances may thus be either a renal abscess or acute pyelonephritis. Infection limited to the pelvicalyceal system and the ureter without renal involvement (pyelitis) is very rare, but may occur when there is ureteric dilatation and an increased residual volume of urine. The urethra has a normal bacterial flora but acquisition of other organisms may lead to inflammation called urethritis. Infection may also occur in the prostrate gland (prostatitis) and seminal vesicles in men and the paraurethral gland in women (Leigh, 1990).

3.9 CATEGORIZATION OF UTI

It is clinically important to classify UTIs by type of infection, presence or absence of symptoms, tendency to recur, and presence or absence of complicating factors. Recurrent infections can be subdivided into reinfections caused by new bacterial strains and relapses caused by the same strains that caused the preceding infections. Complicating factors are host factors facilitating establishment and maintenance of bacteriuria or worsening the prognosis of UTIs engaging the kidneys. Different classifications have been devised to help physicians choose treatments and determine the causes of UTIs.

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

3.9.2 Uncomplicated and Complicated UTIs: 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 urethra (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).

Acute uncomplicated cystitis is a common cause of morbidity in women. Those most at risk are sexually active young women. It is estimated that between 20.0% and 50.0% suffer from UTI at sometime (Smith and Easmon, 1990).Recurrent cystitis occurs in more than three episodes per year. About 20.0% of young women with an episode of cystitis have recurrent infection (Stamm, 1993). Occasionally, such recurrence are due to persistent focus of infection, but well over 90.0% of recurrence in young women are episodes of exogenous re-infection (Stamm and Hooton, 1993). About 95.0% of all recurrent infections in female are re-infection of urinary tract (Schaeffer, 1998).

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).

The clinical spectrum of pyelonephritis in young women ranges from Gram-negative septicemia to cystitis like illness. Acute pyelonephritis will develop in about 20.0% to 40.0% of pregnant women with asymptomatic bacteriuria detected in the first trimester, if left untreated (Fihn, 1992).

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.

Complicated UTIs: Complicated UTI occurs in a patient who have functionally, metabolically or anatomically abnormal urinary tract or those caused by pathogens that are resistant to antibiotics (Schaffer, 1998). A broad range of bacteria can cause complicated infections and many are resistant to multiple antimicrobial agents (Stamm, 1998). 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.

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).

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

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

Hospital acquired UTI (catheter associated or nosocomial UTI): Hospital acquired UTIs are those developing in patients after admission to the hospital, which were neither present nor in incubation at the time of hospitalization. As many as 20.0% of all hospitalized patients, who receive short-term catheterization develop UTI. Catheter-associated UTIs account for 40.0% of nosocomial infection and are the most common source of Gram negative bacterimia in hospitalized patients (Warren, 1997).

Hospitalized patients are most likely to be infected by *E. coli, Klebsiella* spp.*s, Proteus mirabilis,* staphylococci, other Enterobacteriaceace, *Pseudomonas aeruginosa* and enterococci (Forbes *et al.,* 2002). Studies have demonstrated the importance of the attachment and growth of bacteria on the surfaces of the catheter in the pathogenesis of catheter-associated UTI. The encrustations formed on the catheter surface provide a refuge for bacteria and may protect them from antimicrobial agents and phagocytes (Stamm, 2003).

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

Asymptomatic urinary tract infection (Bacteriuria): The term asymptomatic bacteriuria refers to the presence of a positive urine culture in an asymptomatic person (Hooton *et al., 1997*). When a person has no symptoms of infection but significant

numbers of bacteria have colonized the urinary tract, the condition is called asymptomatic UTI.

Screening and treatment of asymptomatic bacteriuria should be done only for those patients who are immunosuppresed, pregnant women and patients before genitourinary procedures or pre-prosthetics, in whom the condition can lead to serious infection.

There is no indication for screening and no benefit in treating elderly, patients with chronic catheters or spinal cord injuries (Regier, 2007).

50% of elderly women and 30% of elderly men have asymptomatic bacteriuria (Chilton, 2008).Treating the patients who are asymptomatic does not provide benefit and may contribute to resistance. Therefore, asymptomatic residents should not be treated (Fitzgerald, 2002).The condition is harmless in most people and rarely persists, although it does increase the risk of developing symptomatic UTIs.

It is defined as the presence of more than 10^5 cfu/ml of bacteria (i.e. significant numbers of bacteria) in urine of patients with no symptoms of urinary tract infection. Many UTI are asymptomatic but may lead to serious infections and it is not known whether symptomatic UTIs are preceded by asymptomatic bacteriuria (Hansen, 1964; Vejlsgaard, 1966). Moreover, even in the absence of any detectable urinary tract malformations making way for infections, many patients have repeated episodes of UTI, which are often asymptomatic.

Asymptomatic bacteriuria is a common infection during pregnancy and it increases the risk of symptomatic UTI and preterm birth (Fatima, 2006). A cost-analysis study found that screening is cost-effective when the prevalence of bacteriuria is >2% (Wadland *et al*., 1989). The condition is detectable and treatable; its consequences are preventable so that screening for asymptomatic bacteriuria is justifiable and ultimately cost- effective (Gratacos *et al*., 1994)

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).

3.10 URINARY TRACT INFECTON IN PREGNANCY

There is no doubt that certain anatomic abnormalities, systemic diseases, and manipulative procedures carry an increased risk of UTI. A common abnormality is obstruction of urine flow. Anatomical abnormalities of the urethral orifice, changes in bladder-neck function and weakness of the pelvic floor and uterine prolapse during old age increase the risk of UTI. Obstructions to the flow of urine by anatomical or pathological abnormalities are major predisposing factors at any age.

Pregnancy causes numerous changes in the body of a woman. Hormonal and mechanical changes increase the risk of urinary stasis and vesicoureteral reflux. These changes, along with an already short urethra (approximately 3-4 cm in females), which allows uropathogens access from the vagina to the lower urinary tract, and difficulty with hygiene due to a distended pregnant belly, increase the frequency of urinary tract infections (UTIs) in pregnant women (Jones, 2009; Conolly and Thorp, 1999). The kidneys enlarge during pregnancy because of an increase in renal parenchyma as well as an increase in intra renal fluid. By the third trimester 97% of women have evidence of hydronephrosis (Cardozo, 1996).

Major anatomical and physiological changes affecting the entire urinary tract occur during pregnancy. Renal function dramatically alters with a 50% increase in renal blood flow and glomerular filtration rate. Increased circulation hormone levels and the ressure effects of the pregnant uterus on the collection system result in dilation of the ureter renal pelvis and calyx. Several factors predispose pregnant females to developing UTI. They include the following:

- Mechanical factors such as compression and stretching of the ureters by the uterus.
- Hormonal factors which cause inhibition of peristalsis of the urinary tract, reduction of the uretero-bladder sphincter tone and promoting the adhesion of germs to the urothelium
- Chemical factors such as alkalinization of urine and physiological glycosuria.

Others: increase in bacteria in the vulvo perineal region during pregnancy (Bahadi , 2010).

The main clinical presentations include the following:

Asymptomatic bacteriuria*:* This is the most common form found in 5 to 10% of pregnant women and leads to pyelonephritis in about 10% of affected patients; hence the importance of screening.

Symptomatic UTI*:* This is represented by acute cystitis and acute pyelonephritis (APN); both can result in complications such as the threat of premature birth and uncontrolled diabetes. *E. coli is* isolated in more than 80% of patients with bacteriuria and 95% of those with APN (Bahadi, 2010).

Pregnancy appears to be associated with an increased incidence of bacteriuria. About 2.0% to 11.0% of pregnant women have asymptomatic bacteriuria and of those, 13.0% to 27.0% will develop a kidney infection late in their term. It is possible that increased incidence is related in part to the urethral dilatation that occurs during pregnancy. Approximately 90 percent of pregnant women develop ureteral dilatation Beginning in week 6 and peaking during weeks 22 to 24, which will remain until delivery (hydronephrosis of pregnancy). Increased bladder volume and decreased bladder tone, along with decreased ureteral tone, contribute to increased urinary stasis and ureterovesical reflux (Patterson, 1987).

The physiologic increase in plasma volume during pregnancy decreases urine concentration. Up to 70 percent of pregnant women develop glycosuria, which encourages bacterial growth in the urine. Increases in urinary progestins and estrogens may lead to a decreased ability of the lower urinary tract to resist invading bacteria. This decreased ability may be caused by decreased ureteral tone or possibly by allowing some strains of bacteria to selectively grow (Patterson, 1987; Lucas, 1993).

There are some researches in which the significant relationship between BMI (body mass index) and frequency of UTI is found. In the study conducted by Giuliani (2002), anemia and readmission were significantly more common in lean women than in women with normal BMI. Obese women have significantly more urinary tract infection than women with normal BMI. Some women with more BMI may be more prone to UTI (Griffin, 2011).

The physiological change that occurs during pregnancy has direct impact on the causation of UTI. The glomerular filtration rate and effective renal plasma flow increase by about 50% in pregnancy. These changes start to occur soon after conception and are well established by the end of the first trimester. Towards the end of first trimester, there is a moderate fall in both the glomerular filtration rate and the effective renal plasma flow.

During pregnancy glucose excretion increases on after conception and may be as high as 10 times as that in non-pregnant woman. About 2/3 of healthy pregnant women develop glysosuria detectable on urinalyses at sometime during pregnancy.The changes in glomerular filtration rate are also responsible for the increased renal clearance of other urinary constituents, in particular urea and uric acid, both of which decrease considerably during the first trimester of pregnancy. During late pregnancy, however the circulating concentrates of uric acid tend to increase. This may reflect a decrease in glomerular filtration or altered renal handling (Cardozo, 1996).

Special attention to the pregnant women is one of the most important points in health care. One of the problems in pregnancy is UTI (Mittal, 2005; Saidi, 2005). There is an increased incidence of UTI with asymptomatic and symptomatic bacteriuria. The incidence of lower Urinary tract symptoms rises during pregnancy and incontinence often occurs for the first time. Neglecting the treatment of UTI in pregnant women may result in some health and economic problems. Due to the increase in sex hormones and the anatomic and physiologic changes during pregnancy, bladder and kidney infection is more likely and may result in hypertension, preeclampsia, low birth weight, prematurity, septicemia, and maternal death (Saidi, 2005; Abyad, 1991; Deljell,2000).

UTI in pregnancy may be associated with an increased neonatal mortality (Whalley, 1967) and it can also be a source of gram negative septicaemia which so frequently proves fatal. Recently, it was found that about 20% of patients had pyelonephritis as the cause of primary renal disease (Wing, 1978).

Bacteriuria often develops in the first month of pregnancy and is frequently associated with a reduction in concentrating ability, suggesting involvement of the kidney (Kaitz, 1961). The smooth muscle relaxation and subsequent ureteral dilatation that accompany pregnancy are thought to facilitate the ascent of bacteria from the bladder to the kidney. As a result, bacteriuria during pregnancy has a greater propensity to progress to pyelonephritis (up to 40 percent) than in non pregnant women (Sweet, 1977; Kass, 1960).

The prevalence of asymptomatic bacteriuria in pregnancy varies form 4-7 % (range 2-11%) and is similar to that observed in non pregnant women (Patterson, 1987; Norden and Kass, 1986). More than a quarter of patients with bacteriuria later develop symptomatic UTI or acute pyelonephritis if left untreated. Continuing bacteriuria is associated with premature delivery and increased perinatal mortality. (Wilkie ME *et al.*, 1992). Untreated ASB is a risk factor for acute cystitis (40%) and pyelonephritis (25-

30%) in pregnancy. These cases account for 70% of all cases of symptomatic UTI among unscreened pregnant women (Jones, 2009).

A specimen obtained at 12-16 weeks will identify 80% of women who will ultimately have asymptomatic bacteriuria during pregnancy (Stenqvist *et al.*, 1989). A single urine specimen obtained from 12-16 weeks of pregnancy will identify most women with asymptomatic bacteriuria (Nicolle, 2003).

3.11 MICROBIOLOGY OF UTI IN PREGNANT PATIENTS

Bacteriuria occurs in 2 to 7 percent of pregnancies, particularly in multiparous women, a similar prevalence as in non pregnant women. The organisms are also similar in species and virulence factors in pregnant and non pregnant women. Thus the basic mechanism of entry of bacteria into the urinary tract is likely to be the same for both groups (Stenqvist *et al.*, 1987).

The most common bacterial isolates from MSU samples of asymptomatic pregnant women were *E. coli* in 66.7%, followed by coagulase-negative staphylococci in 12.5 % (Abdullah, 2005). Similar findings have been reported by other researchers (Mohammad, 2002; Chongsomchai, 1999). *E. coli* is the most common microorganism in the vaginal and rectal area, and because of the anatomical and the functional changes that occur during pregnancy, the risk of acquiring UTI from *E. coli* is high (Mohammad, 2002).

Escherichia coli accounts for 80 to 90 percent of infections. Other gram-negative rods such as *Proteus mirabilis* and *Klebsiella pneumoniae* are also common. Gram-positive organisms such as group B streptococcus and *Staphylococcus saprophyticus* are less common causes of UTI. Group B streptococcus has important implications in the management of pregnancy (Delzell, 2000).

3.12 DIAGNOSIS OF URINARY TRACT INFECTION

A sample of urine from a patient with a suspected UTI is the most common type of specimen received by most clinical microbiological laboratories. The schedule for routine examination should be carefully determined with a view to obtaining the necessary diagnostic information with the greatest possible economy of labor and resources.

3.12.1 Methods of specimen collection

Procurement of a specimen that parallels the status of urine within the bladder is required for meaning interpretation of virtually all investigations.

Prevention of contamination by normal vaginal, perineal and anterior urethral flora is very vital. Invasive techniques for the procuring urine directly from the bladder may be necessary if the patient is unable to micturate, contamination of the urine is unavoidable or in some research settings (Jackson and Fowler, 1990).

Cheesbrough (2000) suggests that whenever possible, the first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most concentrated and therefore the most suitable for analysis.

Clean-catch, midstream urine (CC-MSU)

The least invasive procedure, the clean-catch, midstream urine specimen collection must be performed carefully for optimal results. The detail procedure for the collection of sample is mentioned in Appendix IV.

Straight catheterized urine specimen

Although slightly more invasive, urinary catheterization may allow collection of bladder urine with less urethral contamination. Risk exists, however, that urethral organisms will be introduced into the bladder with the catheter.

Suprapubic bladder aspiration

Urine is withdrawn directly into a syringe through a percutaneously inserted needle, thereby ensuring a contamination-free specimen. The bladder must be full before performing the procedure. If good aseptic techniques are used, this procedure can be performed with little risk in premature infants, infants, small children, and pregnant women and other adults with full bladders.

Indwelling catheter

Specimen collection from patients with indwelling catheters requires scrupulous aseptic technique. The catheter tubing should be clamped off above the port to allow collection of freshly voided urine. In catheterized patients, urine should be collected directly from the catheter and not from the collection bag.

3.12.2 Screening procedures

Up to 80.0% of the urine specimen received in laboratory for culture may contain no etiological agent of infection or may contain only contaminants. There are various procedures tried to screen out such samples so that time, reagents and money of the laboratory is saved. Of these, a simple Gram stained smear of the urine has been found to be least expensive and probably the most sensitive and reliable screening method.

3.12.3 Macroscopic examination of urine

Color and turbidity of urine is noted in the very initial step (Cheesbrough, 2000).

3.12.4 Microscopic examination of urine

Microscopic examination of the urine is an indispensable tool in the diagnosis of genitourinary disorder (Fowler, 1990).

Erythrocytes: Erythrocytes are found in small numbers in normal urine. In normal male and female, occasional red cells (0-2/HPF or 3-12/µL) may be seen on microscopic examination of the sediment. Under high power, unstained RBC appears as

pale discs, usually 7 µm in diameter. They may become created in hypertonic urine and appear as small, rough cells with crinkly edges. Increased number of erythrocytes in the urine may be present in renal diseases like glomerulonephritis, extra renal disease like acute appendicitis, urinary schistosomiasis, leptospirosis, infective endocarditis, malignancy of urinary tract and hemorrhagic conditions. The finding of RBC count greater than 3/HPF is considered as abnormal (Froom *et al.,* 1986; Steward *et al.,* 1985; Wargotz *et al.,* 1987). The study done by Froom *et al.* (1986) concluded that the examination of the urine sediment by HPF method is not sufficiently sensitive to be used as a screening test for the detection of UTI in asymptomatic subjects. Microscopic haematuria may be present in 40.0% to 60.0% of patients with UTI (Faro and Fenner, 1998).

Leucocytes: These are round 10 to 15 µm in diameter cells that contain granules. In urinary infection they are often found in clumps (Cheesbrough, 1984). Normal urine contains 2-3 pus cells/HPF. Pyuria is usually regarded as significant when moderate or many pus cells are present i.e. > 10WBC/ml (Cheesbrough, 2000).

The visualization of leucocytes is suggestive of bacteriuria but may result from any inflammatory disorder of the urinary tract such as acute glomerulonephritis, renal tubular acidosis, and non-infectious irritation to ureter, bladder or urethra or may be due to dehydration, stress and fever (Godkar, 2001).

Pyuria is significant if more than or equal to 5 white blood cells or pus cells are seen per high power field in the sediment (Abyad, 1991; Chakraborty, 2001; Merila *et al.,* 1987; Steward *et al.,* 1985). Three or more fresh leucocytes per HPF suggest infection and are rarely found in normal non-bacteriuric patients (Stamm *et al.,* 1981). Pyuria with sterile routine culture may be found with renal tuberculosis, gonococcal urethritis, *C. trachomatis* infection and leptospirosis or when a patient with urinary infection has been treated with antimicrobials (Cheesbrough, 1984).

Epithelial Cells: Normally, few epithelial cells are found in urine. These cells are cuboidal in shape having small nuclei and granular cytoplasm (Fowler, 1990). When seen in large number, however, they usually indicate inflammation of the urinary tract or vaginal contamination of the specimen (Cheesbrough, 2000). Normally few cells (3-5/HPF) from genitourinary tract can be found in urine due to sloughing off of old cells (Godkar, 2001). Increased number of tubular epithelial cells suggests tubular damage. It can occur in pyelonephritis, acute tubular necrosis, salicylate intoxication and kidney transplants rejection (Godkar, 2001). Wargotz *et al.* (1987) reported that greater than or equal to five squamous epithelial cells per high power field is considered as abnormal.

3.12.5 Chemical examination of urine

The chemical examination of urine for protein and glucose plays a little part in the diagnosis of bacterial infection. Proteinuria may be increased by inflammatory exudates and vaginal secretions. Whilst it is an indicator of renal disease, detection of the presence of increased glucose in the urine is of some value because bacteriuria occurs frequently in diabetics but the routine testing of urine specimens for glucose in the laboratory is not indicated (Cheesbrough, 2000).

3.12.6 Bacteriological examination of urine

The diagnosis of UTI cannot be made without bacteriological examination of the urine because many patients with the frequency, dysuria syndrome have sterile urine and, conversely, asymptomatic bacteriuria is common condition.

Some microbes, like *Chlamydia* and *Mycoplasma*, can be detected only with special bacterial cultures. Infections with these organisms are suspected when a person has symptoms of a UTI and pus in the urine, but a standard culture fails to grow any bacteria.

Bacteriological culture of the urine is the only accurate way of diagnosing bacteriuria. Quantitative or semi-quantitative techniques are to be preferred but the particular one

chosen will depend upon the resources of the laboratory. The accurate methods of counting bacteria, e.g. the pour-plate technique or the surface-viable count, are time-consuming and expensive in use of materials. Most of the laboratories use a semi-quantitative technique. The standard loop, filter-paper strip (Leigh and Williams, 1964) dip-spoon and dip-slide are all useful means of examining large numbers of urine specimens, but they differ considerably in the amount of medium used and in performance time.

Standard Loop Method

An inoculating loop of standard dimensions is used to take up a small, approximately fixed and known volume of mixed uncentrifuged urine and inoculate on to an agar culture medium. The plate is incubated, the number of colonies is counted and this number is used to calculate the number of viable bacteria per ml of urine. Thus, if a 0.002ml loopful of urine yields 25 colonies, then the approximate number of cfu per ml of urine will be 25 x 500 = 12,500. Such a count should be reported as 10^4-10^5 colonies/ml (Collee *et al.*, 1999).

3.13 ANTIMICROBIAL TREATMENT OF ANTENATAL BACTERIURIA

A pregnant woman who develops a UTI should be treated promptly to avoid premature delivery of her baby and other risks such as high blood pressure. Some antibiotics are not safe to take during pregnancy. In selecting the best treatments, doctors consider various factors such as the drug's effectiveness, the stage of pregnancy, the mother's health, and potential effects on the fetus.

These are drugs with antibacterial effects limited to the urine. They fail to produce significant levels in tissues and thus have no effect on systemic infections. However, they effectively lower bacterial counts in urine and thus greatly diminish the symptoms of lower UTI. They are used only in the management of UTI.

Sulphonamides: Sulphonamides in combination of trimethoprim presents good level of renal excretion. It is extremely useful for the treatment of uncomplicated urinary tract infection caused by *E. coli* in domiciliary practice. Pregnant women should avoid TMP-SMX because of fetal hepatotoxicity (Simon *et al.*, 2000). It should not be used in the first trimester of pregnancy because there is possibility of tetratogenicity. During the last month of pregnancy, it moves bilirubin from its receptors causisng kernicterus syndrome in the newborn. Enterococcus species are resistant to SMX/TMP (Regier, 2005)

Nitrofuran compounds: Nitrofurantoin is active against most members of *Enterobacteriaceae*, but not against *Pseudomonas* spp. It may cause nausea and gastrointestinal distress. It is most active at acid pH. Nitrofurantoin presents good absorption and fast renal excretion as well as good action against gram negative bacteria. It does not induce resistance of fecal flora. This drug is contraindicated in patients with deficiency of glucose 6-phosphate dehydrogenase (G6 PD) because it can cause hemolytic anemia. It should be avoided at the end of pregnancy because of the possibility of neonatal hemolysis but can be used during breast feeding period.

4-quinolone antibacterials: Fluoroquinolones have become popular treatments for patients with uncomplicated UTI because of *E. coli*'s emerging resistance to other common medications. It should be avoided in pregnancy because of possible effect on fetus.

Nalidixic acid is avoided in pregnancy because of various side effects such as nausea, vomiting, visual disturbance and photosensitivity it produces. This drug is present at variable concentration in plasma level and has low toxicity against gram positive bacteria. Also bacteria can get resitance quickly. Nalidixic acid is active against several different types of Gram negative bacteria, whereas Gram-positive organisms are resistant. The Infectious Diseases Society of America (IDSA) guidelines recommend the use of Fluoroquinolones (e.g. Ciprofloxacin, Norfloxacin and Ofloxacin) as first-

line agents in communities with greater than 10.0% to 20.0% resistance rates to TMP-SMX (Naber, 2000).

Cephalosporins: Cephalosporins including Cephalexin (Keflex), Cefuroxime (Ceftin), and Cefixime (Suprax), can also manage UTIs. Increasing resistance, however, has limited their effectiveness (Nicolle, 2003). First generation Cephalosporins (Cephalexin) are very active against Gram positive cocci (except enterococci and Nafcillin-resistant staphylococci) and moderately active against some Gram negative rods (primarily *E. coli*, *Proteus* spp. and *Klebsiella* spp.s.) The second generations Cephalosporins (Cefuroxime) are active against organisms covered by first generation drugs including *Klebsiella* spp.and *Proteus* spp., but not *P. aeruginosa.* Third generation Cephalosporins have their enhanced activity against Gram negative rods, especially that of Ceftazidime and Cefoperazone against *P. aeruginosa.* Fourth generation Cephalosporins include Cefepime and Cefpirome. Cefepime has enhanced activity against *Enterobacte*r spp. and *Citrobacter* spp. (Correa, 2002).

Aminopenicillin: Among the most important Penicillins are Ampicillin and Amoxicillin, which are active against some enterobacteria. These are systemically absorbed oral drugs that are excreted in high concentrations in urine (Brooks *et al.*, 2004). They have been used frequently in the past for the treatment of UTI, but emergence of resistance in up to 30.0% of common urinary isolates has lessened the utility of these drugs (Hooton and Stamm, 1991).

Aminoglycoside: The aminoglycosides inhibit protein synthesis by interfering with the genetic transcription and finally causes cell membrane disruption. They are bactericidal in action. Gentamicin is active against many strains of Gram positive and Gram negative bacteria, including some strains of *P. aeruginosa*. Amikacin inhibit many Gram negative enteric bacteria (Hugo and Russell, 1993).

3.13.1 Bacterial resistance to antimicrobial agents

An antibiotic resistance is defined as the microbe, which is sensitive to certain antibiotic start gaining resistance against it. Infections caused by MDR strains often lead to death (Tuladhar *et al.,* 2001).

Antibiotics today are the front-line therapeutic means for the medical intervention in an infection, which plays a central role in the control and management of infectious diseases. However, due to misuse and overuse of antibiotics, most clinically relevant bacterial pathogens have acquired a selection process to adapt to the pressures of antimicrobial attack, so that certain strains are now no longer susceptible to one or more of these antimicrobial agents (Hugo and Russell, 1993).

In recent years resistance to first-line antibiotics such as Ampicillin, Tetracyclines, Chloramphenicol and Sulphonamides has been increasing. Most drug resistance in enteric bacteria is attributable to the widespread transmission of resistance plasmids. The abundant use of antimicrobial drugs favors the persistence and growth of drug-resistant bacteria, including *Enterobacter* spp.*, Klebsiella* spp.*s., Proteus* spp.*, Pseudomonas* spp. *and Serratia* spp. (Brooks *et al.,* 2004). Resistance may spread to a wide variety of bacteria from their genes. Any single organism can acquire multiple genes and become resistant to the full spectrum of available antimicrobial agents (Hugo and Russell, 1993).

3.13.2 Types of drug resistance

Drug resistance may be of two types- **natural and acquired**.

Natural drug resistance is an innate property of the bacterium and is unrelated to the previous exposure to the drug. An entire bacterial species may be resistant to an antibiotic even before the introduction of the drug. For e.g. *S. pyogenes* is resistant to Polymyxins and *P. mirabilis* is resistant to Colistin because of lack of penetration of the drug through the cell wall, lack of suitable cell wall or other target receptors that may

have existed before the introduction of the drug which may be lethal to the drug (Hugo and Russell, 1993).

Acquired drug resistance may be attained by bacteria either by mutation or by gene transfer. Resistance of *M. tuberculosis* to Streptomycin develops by mutation (Hugo and Russell, 1993). Most of the acquired drug resistance of *S. aureus* and Gram negative bacilli are R factor or plasmid mediated and achieved by conjugation. Genetic transfer of antimicrobial resistance can also take place through transposons (Hugo and Russell, 1993).

3.13.3 Mechanisms of antimicrobial resistance

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.

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.

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).

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.

Microorganisms develop an altered enzyme that can still perform its metabolic function but is much less affected by the drug. Example: In trimethoprim-resistant bacteria, the dihydrofolic acid reductase is inhibited far less efficiently than in trimethoprim-susceptible bacteria (Brooks *et al.*, 2004).

3.14 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing is an *in vitro* method for estimating the activity of drugs which will assist clinician in selecting an antimicrobial effective in inhibiting the growth of an infecting microorganism in vivo. The primary goal of antimicrobial susceptibility testing is to determine whether the bacterial etiology of concern is capable of expressing resistance to the antimicrobial agents that are potential choices as therapeutic agents for managing the infection. According to Greenwood (2000), since therapy of infection begins before laboratory results are available, antibiotic susceptibility testing primarily plays a supplementary role in confirming that the organism is susceptible to the agent that is being used. As antibiotics are concentrated in urine to higher levels than are found in the tissues, high-content test discs should be used.

WHO recommended modified Kirby-Bauer disc diffusion technique is used by most laboratories to test routinely for antimicrobial susceptibility. The antibiotics, commonly in the form of an impregnated filter paper disc, are allowed to diffuse from a point source into an agar medium that has been seeded with the test organism. After incubation, the diameter of the zone of inhibition around each disc is measured in millimeters (Collee *et al.*, 1999).

CHAPTER-IV

4. MATERIALS AND METHODS

The cross sectional study was conducted in “Shree Birendra Hospital,Chhauni” , Kathmandu from Nov 2009 to Feb 2010. About 20 ml of clean catch mid-stream urine samples were collected from five hundred pregnant women attending obstetric outpatient of the hospital and examined by routine examination, culture and antibiotic susceptibility tests.

4.1 MATERIALS

The materials required for this work are listed in Appendix VII.

4.2 METHODS .

Urine samples were collected randomly from pregnant female patients of different age groups in different trimesters of gestation visiting SHREE BIRENDRA HOSPITAL and processed for rapid test. The patients who were clinically suspected of UTI and those patients who are taking antibiotic were excluded in the study. The data regarding the patient visiting the hospital was collected directly by interview method. Then culture was done for isolation and various biochemical tests were performed for the identification of probable organisms causing UTI. Finally antibiotic sensitivity test was performed. The samples were collected only after complete filling questionnaire.

4.2.1 Data Collection

Each patient requested for urine culture was directly interviewed for his or her clinical history during sample collection. The gathered history of patients includes name, age, age at marriage, age at first pregnancy, past history, personal habit, previous UTI present or not, gravida, parity and trimester.

4.2.2 Specimen Collection

The patient was given a sterile, dry, wide-necked leak-proof container, which was labeled with date, name and number of the patient and the time of collection, for the collection of first morning clean-catch mid-stream urine. The patient was given instructions for the collection of CC-MSU. The detailed procedure is mentioned in Appendix IV. The container was delivered to the laboratory along with the request form as soon as possible. In case of delay, the samples were refrigerated at 4°C.

4.3 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.

4.3.1 Macroscopic examination

The specimen obtained in laboratory was observed for its color and turbidity.

4.3.2 Microscopic examination

The urine specimen was examined microscopically as a wet preparation primarily for detecting pus cells. WBCs in excess of 10^4 cells/ml (>10 cells/ ml) of urine will indicate significant pyuria. 1 WBC / LPF correspond to 3 cells/µL (Cheesbrough, 2000).

4.3.3 Chemical examination

The detection of protein, pH and sugar in urine was performed by using reagent strip for urinalysis. The strip was dipped into the urine specimen for few seconds and the change in color in test area was noted after 30 seconds for glucose and after 60 seconds for protein and pH. The results were interpreted according to the color change of the test area, comparing with that of the given standard color for detection of protein, pH and sugar.

4.3.4 Culture of specimen

A semi-quantitative calibrated loop technique was adopted for the primary isolation of the organism. A loopful of well-mixed uncentrifuged urine was streaked on to the surface of Blood agar and CLED agar. The method used for loop standardization is given in the Appendix III.

- A loopful of sample was touched to the centre of the plate, from which the inoculum was spread in a line across the diameter of the plate.
- Without flaming or re-entering urine, the loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies.
- The CLED agar and Blood agar plates were incubated aerobically at 35-37^{0}C overnight.
- The approximate numbers of colonies were counted and the number of bacteria, i.e. cfu/ml of urine was estimated in accordance to the volume of urine inoculated previously. For example, 100 colonies on inoculating 0.001 ml of urine would correspond to 10^5 cfu/ml.

The bacterial count was reported as:

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

However if the culture indicated the appearance of $\geq$3 organism types with no predominating organism, this was interpreted as due to possible contamination of the specimen and asked for another specimen. In addition to the previously described guidelines a pure culture of *S. aureus* was considered significant regardless of the number of colony forming unit (Forbes *et al.*, 2002).

4.3.5 Identification of the isolates

Identification of significant isolates was done by using microbiological techniques as described in the Bergy's manual which involves morphological appearance of the colonies, staining reactions and biochemical properties (Bailey & Scotts, 1990; Cheesbrough, 1984; Mackie and McCartney, 1998).

Each of the organisms was isolated in pure form before performing biochemical and other tests. Gram staining of an isolated colony was done from primary culture. For gram negative organism a speck of single isolated colony from CLED agar and for gram positive the same from BA was transferred into the nutrient broth and incubated at 37ºC for 4 hours. It was then subcultured on dried nutrient agar plate and incubated at 37ºC for 24 hours. Thus obtained overnight incubated culture of organism on nutrient agar was used to perform catalase test, oxidase test, other biochemical tests and antibiotic susceptibility test. The Gram- staining procedure is mentioned in the appendix III.

Appropriate biochemical tests were performed for the confident identification of the bacterial isolates. For that, the pure colonies on the media plates were inoculated onto different biochemical media.

Gram-positive organisms were identified primarily on the basis of their response to gram's staining, catalase test, oxidase test and coagulase tests.

The biochemical tests used for the identification of gram-negative bacterial isolates include Catalase test, Oxidase test, Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Oxidation Fermentation test, Triple Sugar Iron (TSI) test, Motility test and Gas production tests.

The composition and preparation of biochemical media and reagents used in the biochemical test are mentioned in the appendix II. The procedure for performing biochemical tests are mentioned in appendix VI.

4.4 Antibiotic susceptibility testing

The antibiotic sensitivity testing was performed according to the recommended Kirby-Bauer sensitivity testing method (NCCLS, 1999). Antibiotics were selected which were used in the hospital for the urine isolates.

Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer.

The pH of the medium 7.2-7.4 and the depth of the medium at 4 mm (about 25 ml per plate) were maintained in petridish.

Using a sterile wire loop, a single isolated colony of which the sensitivity pattern was to be determined was touched and inoculated into a nutrient broth tube and was incubated for 2-4 hrs.

After incubation in a good light source, the turbidity of the suspension was matched with the turbidity standard of Mac Farland 0.5.

Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture technique.

Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) was placed, evenly distributed on the inoculated plates, not more than 12 discs were placed on a 150 mm diameter Petri plate.

Within 30 minutes of applying the discs, the plates were taken for incubation at 35^0C for 16-18 hrs.

After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and results interpreted.

4.5 Purity plate

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

4.6 Quality control for test

Quality of each test was maintained by using standard procedures. Strict aseptic conditions were maintained while carrying out all the procedures. The quality of each agar plates prepared was tested by incubating one plate of each lot on the incubator. During identification of organism, for each test ATCC control positives and control negatives was taken simultaneously. Quality of sensitivity tests was maintained by maintaining the thickness of Mueller-Hinton agar at 4mm and the pH at 7.2-7.4. Similarly antibiotic discs containing the correct amount as indicated were used.

4.7 Statistical analysis

All the data obtained was statistically analyzed by chi-square test using Statistical Package for Social Science (SPSS) version 16 software packages.

CHAPTER -V

5. RESULTS

5.1. MICROBIOLOGICAL PATTERN OF RESULTS

5.1.1 Growth pattern of bacteria in urine sample.

Out of 500 samples cultured, only 33 samples showed significant growth where as 100 showed insignificant growth. However, many samples were culture negative or shown mixed growth (Table 1)

Table 1: Growth pattern of bacteria in urine sample.

Growth pattern	No of isolates	Percent of isolates
[1]Significant growth	33	6.6
[2]Insignificant growth	100	20
[3]No growth	277	55.4
[4]Mixed growth	90	18

[1] more than 10^5 CFU/ml of MSU

[2]less than 10^5 CFU/ml of MSU

[3] no colony isolated

[4]More than 2 types of Colonies

5.1.2 Bacterial genera isolated from infected urine samples

A total of 33 organisms were isolated from growth positive urine samples. Among all the isolates, *E. coli* was predominant organism followed by *Staphylococcus aureus* and other species (Table 2).

Table 2: **Bacterial genera isolated from infected urine samples**

Organism isolated	**Number**	**Percentage (%)**
Gram negative bacteria		
Escherichia coli	14	42.4
Citrobacter fruendii	3	9.1
Klebsiella oxytoca	2	6.1
Enterobacter species	1	3
Pseudomonas aeruginosa	1	3
Total	**21**	**63.6**
Gram positive bacteria		
Staphylococcus aureus	8	24.2
Staphylococcus saprophyticus	4	12.1
Total	**12**	**36.3**

5.2 CHEMICAL OBSERVATION OF URINE

5.2.1 Relation of albumin test with culture result.

Out of total samples, 92.2% (461/500) of samples were negative for albumin test; however 5.6% (26/461) showed significant bacteriuria on culture. Similarly, 7.8% of samples showed positive albumin test out of which 17.9% (7/39) showed significant bacteriuria (Table 3). The sensitivity, specificity, positive predictive value and negative predictive value of albumin test with positive culture result was 21.12%, 93.15%, 17.95% and 94.36% respectively (Appendix IX).

Table 3: Relation of albumin test with culture result.

Albumin test	Culture positive (%)	Culture negative (%)	Total (%)
Positive (≥1+)	7 (17.9)	32 (82)	**39 (7.8)**
Negative (<1+)	26 (5.6)	435 (94.4)	**461 (92.2)**
Total	**33(6.6)**	**467(93.4)**	**500(100)**

5.3 MICROSCOPIC OBSERVATION OF URINE

5.3.1 Pus cells count and culture positivity.

Among the 500 samples, 9% (479/500) of samples showed insignificant pyuria, however among these, 6.1% (29/479) of samples gave positive culture results. Similarly, 21 (4.2%) of total samples showed significant pyuria, and among these 19% (4/21) samples gave positive culture results (Table 4). The sensitivity, specificity, positive predictive value and negative predictive value of pyuria with positive culture result was 12.12%, 96.35%, 19.05% and 93.95% respectively (Appendix IX).

Table 4: Pus cells count and culture positivity.

Pyuria	Culture positive (%)	Culture negative (%)	Total (%)
Significant (≥5WBC/HPF)	4 (19)	17 (80.9)	**21(4.2)**
Insignificant (<5WBC/HPF)	29(6.1)	450 (93.9)	**479(9)**
Total	**33(6.6)**	**467(93.4)**	**500(100)**

5.3.2 RBC count and culture positivity.

Among the 500 samples, 99.4% (497/500) of samples showed insignificant haematuria, however among these, 6.6% (33/497) of samples showed positive culture results. Similarly, 3 (0.6%) of total samples showed significant haematuria, and none of these samples showed positive culture result.

Table 5: RBC count and culture positivity.

Haematuria	Culture positive (%)	Culture negative (%)	Total (%)
Significant (≥3RBC/HPF)	0 (0)	3(100)	**3 (0.6)**
Insignificant (<3RBC/HPF)	33 (6.6)	464(96.9)	**497 (99.4)**
Total	**33(6.6)**	**467(93.4)**	**500(100)**

5.3.3: Epithelial cell count and culture positivity

Out of the total 500 samples, 95.2% (476/500) showed significant epithelial cell count, however among these, 28 (5.9%) of samples showed positive culture results. Similarly, 24 (4.8%) of total samples showed significant epithelial cell count, and 20.8% (5/24) of these samples showed positive culture result (Table 6). The sensitivity, specificity, positive predictive value and negative predictive value of epithelial cell count with positive culture result was 5.15%, 95.93%, 20.83% and 94.12% respectively (Appendix IX).

Table 6: Epithelial cell count and culture positivity.

Epithelial cells	Culture positive (%)	Culture negative (%)	Total (%)
Significant (≥5WBC/HPF)	5 (20.8)	19(79.2)	**24 (4.8)**
Insignificant (<5WBC/HPF)	28 (5.9)	448 (94.1)	**476 (95.2)**
Total	**33(6.6)**	**467(93.4)**	**500(100)**

5.4 PATTERN OF PATIENTS REQUESTED FOR URINE CULTURE

5.4.1 Age wise distribution of cases requested for urine culture.

In this study, the age of the patient ranged from 17 years to 41 years. The highest number of patients 423 (84.6%) belonged to the age group 20-30 followed by 42 (8.4%) who belonged to age group below 20, 35 (7%) who belonged to age group above 30.The highest percentage of infection (7.1%) was found in the age group below 20. statistically, there was no association between age group of patients and culture positive result (Table 7).

Table 7: Age wise distribution of patients.

Age group	Total suspected cases (N=500)		Total culture positive case..s N=(33)		p value (χ^2 test)
	No.	Percent	No.	Percent	
<20	42	8.4	3	7.1	0.96 (insignificant)
20-30	423	84.6	28	6.6	
>30	35	7	2	5.7	

5.4.2 Geographical wise distribution of the patients

Out of 500 pregnant woman, 315 (63 %) were from rural area and 37% (185) were from urban area. The higher number 7.6% (14/185) of positive cases were from urban area which was slightly different from that of rural area 6% (19/315). No statistically significant association between geographical distribution of patients and presence of bacteriuria (Table 8).

Table 8: Geographical wise distribution of the patients.

Locality	**Total suspected cases (N=500)**		**Total culture positive case..s N=(33)**		**p value (χ^2 test)**
	No.	**Percent**	**No.**	**Percent**	
Rural	315	63	19	6	0.504
Urban	185	37	14	7.6	

5.4.3 Occupation wise distribution of the patients.

Out of 500 pregnant woman, the highest number 461(92 percent) were housewives. Of them 6.5% was culture positive. While 16 sample (3.2 %) were from women involved in service out of which 3 samples (18.8%) were culture positive. None of culture positive sample was from women of other occupations i.e agriculture, business and student (Table 9).

Table 9: Occupation wise distribution of the patients.

occupation	Total suspected cases (N=500)		Total culture positive case..s N=(33)		p value (χ^2 test)
	No.	Percent	No.	Percent	
Agriculture	13	2.6	0	0	0.243 (insignificant)
Service	16	3.2	3	18.8	
Housewife	461	92.2	30	6.5	
Business	3	0.6	0	0	
Student	7	1.4	0	0	

5.4.4 Education wise distribution of the patients

Among total suspected cases, 254 (50.8%) had secondary level of education and 9.1% (23/254) of culture positive cases were in this group. Stasistically, there is no association between education and bacteriuria (Table 10).

Table 10: Education wise distribution of the patients

Education	Total suspected cases. (N=500)		Total culture positive cases. (N=33)		p value (χ^2 test)
	No.	Percent	No.	Percent	
Illiterate	23	4.6	0	0	0.082
Primary	58	11.6	1	1.7	
Secondary	254	50.8	23	9.1	
More	165	33	9	5.5	

5.4.5 Distribution of patients according to their age at marriage

The highest numbers (52.4 %) of the cases married between age 20-30 years followed by age group below 20 and above 30. The highest percentage (8.4%) was found in those cases who married at the age group 20-30 years in comparison to other age groups but no statistically significant difference was found in the incidence of bacteriuria and age at marriage (Table 11).

Table 11: Distribution of patients according to their age at marriage.

Age at marriage	**Total suspected cases. (N=500)**		**Total culture positive cases. (N=33)**		**p value (χ^2 test)**
	No.	**Percent**	**No.**	**Percent**	
<20	211	42.2	11	5.2	0.14
20-30	262	52.4	22	8.4	
>30	27	27	0	0	

5.4.6: Distribution of patients according to their age at first pregnancy.

Among total 500 urine samples from asymptomatic pregnant woman, 319 (63.8 percent) belonged to age group 20-25. The highest culture positive cases 50 % (1/2) was found in the age group above 30. This result was also proved to be statistically significant i.e there is association between age at first pregnancy and culture positive result (Table 12).

Table 12: Distribution of patients according to their age at first pregnancy.

Age at first pregnancy	Total suspected cases. (N=500)		Total culture positive cases. (N=33)		p value (χ^2 test)
	No.	Percent	No.	Percent	
<20	179	35.8	9	5	0.03
20-30	319	63.8	23	7.2	
>30	2	0.4	1	50	

5.4.7 Distribution of patients according to gravida.

Among total suspected cases the 276 (55.2 %) were multigravidae and 224 (44.8%) were primigravidae. The higher culture positive were also found in multigravidae as compared to primigravidae. Statistically, there is no significant association between gravidae and culture positive result (Table 13).

Table13: Distribution of patients according to gravida.

Gravidae	Total suspected cases. (N=500)		Total culture positive cases. (N=33)		p value (χ^2 test)
	No.	Percent	No.	Percent	
Primigravidae	224	44.8	13	5.8	0.518
Multigravidae	276	55.2	20	7.2	

5.4.8 Distribution of patients according to parity.

As shown in the table out of 500 samples, 232 (46.4 %) were nulliparous, 182 (36.4%) were of first para, 62(12.4 %) were of second para and 24 (4.8 %) had more than two children. The highest percent of culture positive urine samples (8.8%) belonged to those

having one child. Statistically, there is no association between parity and culture positive result (Table 14).

Table 14: Distribution of patients according to parity.

Parity	Total suspected cases. (N=500)		Total culture positive cases. (N=33)		p value (χ^2 test)
	No.	Percent	No.	Percent	
0	232	46.4	14	6.03	0.315
1	182	36.4	16	8.8	
2	62	12.4	3	4.8	
>2	24	4.8	0	0	

5.4.9 Distribution of patients according to presence of previous UTI.

Among 500 cases, 16 (3.2%) had previous UTI whereas 484 (96.8%) did not had previous UTI. Among them, higher percent of 25 %(4/16)) of culture positive result was found in the patients who already had UTI. Statistically significant result was drawn regarding presence of previous UTI in patients and asymptomatic bacteriuria (Table 15).

Table 15: Distribution of patients according to presence of previous UTI.

Previous UTI	Total suspected cases (N=500)		Total culture positive cases N=(33)		p value (χ^2 test)
	No.	Percent	No.	Percent	
No	484	96.8	29	6	0.003
Yes	16	3.2	4	25	

5.4.10. Trimester-wise distribution of patients.

Among total 500 MSU samples 206(41.2 %) were from patients in third trimester while highest (7.7%) of growth positive culture was found in second trimester followed by first trimester (6.3%) and third trimester (5.3%).(Table 16).

Table 16: Trimester-wise distribution of patients.

Trimester	**Total suspected cases. (N=500)**		**Total culture positive cases. (N=33)**		**p value (χ^2 test)**
	No.	**Percent**	**No.**	**Percent**	
First	112	22.4	8	6.3	0.937
Second	182	36.4	14	7.7	
Third	206	41.2	11	5.3	

5.5 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE ISOLATES

5.5.1 Antibiotic Susceptibility Pattern of bacterial isolates

Among the antibiotics evaluated, *E. coli* was found to be highly susceptible towards nitrofurantoin (100%) followed by gentamycin (92.8%) and cotrimoxaxole (71.4%). 42.8 % of E. coli were resistant to nalidixic acid and amoxicillin respectively.

Staphylococcus was found 100% susceptible to nitrofurantoin and 87.5% susceptible to gentamycin and ofloxaxin. It was found to be 37.5% resistant to both amoxicillin and norfloxaxin (Table 17).

Table 17: Antibiotic Susceptibility Pattern of bacterial isolates

S.N.	Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
			Resistant		Moderate		Susceptible	
			No.	%	No.	%	No.	%
1.	*Escherichia coli* N=14	Amoxycillin	6	42.9	0	0	8	57.1
		Cephalexin	5	35.7	2	14.3	7	50
		Cotrimoxaxole	3	21.4	1	7.1	10	71.4
		Gentamycin	1	7.1	0	0	13	92.9
		Nitrofurantoin	0	0	0	0	14	100
		Norfloxaxin	3	21.4	2	14.3	9	64.3
		Nalidixic acid	6	42.8	2	14.3	6	42.9
		Ofloxacin	5	35.7	2	14.3	7	50
2.	*Citrobacter fruendii* N=3	Amoxycillin	2	66.7	0	0	1	33.3
		Cephalexin	0	0	1	33.3	2	66.7
		Cotrimoxaxole	0	0	0	0	3	100
		Gentamycin	0	0	0	0	3	100
		Nitrofurantoin	0	0	1	33.3	2	66.7
		Norfloxaxin	0	0	0	0	3	100
		Nalidixic acid	1	33.3	0	0	2	66.7
		Ofloxacin	2	66.7	0	0	1	33.3
3.	*Klebsiella oxytoca* N=2	Amoxycillin	2	100	0	0	0	0
		Cephalexin	0	0	0	0	2	100
		Cotrimoxaxole	0	0	0	0	2	100
		Gentamycin	0	0	1	50	1	50
		Nitrofurantoin	0	0	0	0	2	100
		Norfloxaxin	1	50	0	0	1	50
		Nalidixic acid	1	50	0	0	1	50
		Ofloxacin	1	50	0	0	1	50
4.	*Enterobacter species* N=1	Amoxycillin	1	100	0	0	0	0
		Cephalexin	0	0	0	0	1	100
		Cotrimoxaxole	1	100	0	0	0	0
		Gentamycin	0	0	0	0	1	100
		Nitrofurantoin	0	0	0	0	1	100
		Norfloxaxin	0	0	0	0	1	100
		Nalidixic acid	1	100	0	0	0	0
		Ofloxacin	1	100	0	0	0	0

5.	*Pseudomonas* species N=1	Amoxycillin	1	100	0	0	0	0
		Cephalexin	0	0	0	0	1	100
		Cotrimoxaxole	0	0	0	0	1	100
		Gentamycin	1	100	0	0	0	0
		Nitrofurantoin	1	100	0	0	0	0
		Norfloxaxin	0	0	0	0	1	100
		Nalidixic acid	0	0	1	100	0	0
		Ofloxacin	0	0	0	0	1	100
6.	*Staphylococcus aureus* N=8	Amoxycillin	3	37.5	0	0	5	62.5
		Cephalexin	1	12.5	1	12.5	6	75
		Cotrimoxaxole	2	25	0	0	6	75
		Gentamycin	1	12.5	0	0	7	87.5
		Nitrofurantoin	0	0	0	0	8	100
		Norfloxaxin	3	37.5	1	12.5	4	50
		Nalidixic acid	8	100	0	0	0	0
		Ofloxacin	0	0	1	12.5	7	87.5
7.	*Staphylococcus saprophyticus* N=4	Amoxycillin	2	50	0	0	2	50
		Cephalexin	0	0	0	0	4	100
		Cotrimoxaxole	1	25	0	0	3	75
		Gentamycin	0	0	1	25	3	75
		Nitrofurantoin	1	25	0	0	3	75
		Norfloxaxin	1	25	1	25	2	50
		Nalidixic acid	4	100	0	0	0	0
		Ofloxacin	1	25	2	50	1	25

Chapter VI

6. DISCUSSION AND CONCLUSION

6. 1 DISCUSSION

This study was conducted among pregnant patients attending "Shree Birendra Hospital, Chhauni", Kathmandu, Nepal. Five hundred MSU samples were collected and subjected to reagent strip test for urinalysis and routine examination and then processed for culture and sensitivity test. The present study was conducted to find the prevalence of different uropathogens isolated in the asymptomatic pregnant patients and to determine the antibiotic susceptibility pattern of the isolated organisms.

Out of the total number of 500 pregnant women included in this study, 33 (6.6 percent) were identified by culture to have significant bacteriuria. Similar studies found culture positive result of 7.4% Jayalakshmi and Jayaram (2008), 4.5% in Abdullah and Al-Moslih (2005), 7.3% in Turpin *et al.* (2007) and 8.4% in Lavanya and Jogalakshmi (2002).

All 500 samples were from outdoor patients in this work. Out of total samples, 33 samples showed significant growth, giving 7 bacterial isolates, whereas majority of samples i.e. 277 showed no growth, 100 showed no significant growth and 90 samples showed mixed growths. High growth negative samples may be due to inclusion of all asymptomatic patients only. Despite giving instruction also mixed growth and insignificant growth was observed which may be due to improper method of sample collection. Reasons for multiple microorganisms, other than contamination, include fistulas, urinary retention, infected stones, or catheters (Nickel & Pidutti, 1992).

Traditionally, $> 10^5$ bacteria/ml of urine showing a single isolate is taken to indicate bacteriuria and distinguishes infection from contamination in asymptomatic patients (Orrett & Shurland, 1998; Graham, 2001). Among the total 33 bacterial isolates from

test, 21 (63.6 percent) were Gram negative rods and the remaining 12 (36.4 percent) were found to be Gram positive cocci.

In the present study, altogether 7 species of bacteria were isolated. *E.coli* accounts for 42.4 % of infection followed by *Staphylococcus aureus* (24.2 %), *Staphylococcus saprophyticus* (12.1%), *Citrobacter freundii* (9.1%), *Klebsiella oxytoca* (6.1%), *Enterobacter sps* (3%) and *Pseudomonas aeruginosa (*3%). In a study done by Hooton *et al.,* (1997) found the current prevailing theory regarding a reservoir for *E. coli* strains causing UTI is that they originate from the gastrointestinal tract flora. Pathogenic *E. coli* expresses specific adhesions such as P *fimbriae* and produce alpha and beta hemolysins

In a similar study conducted by Jones, 2009; he found *E.coli in* 80-85% of cultures followed by other pathogens *Klebsiella pneumoniae* (5%), *Proteus mirabilis* (5%), *Enterobacter* species, (3%), *S saprophyticus* (2%), Group B beta hemolytic *Streptococcus* (1%). The result is supported by similar study done by Turpin (2007) in which the dominant bacteria isolates were *E. coli* (37%) followed by *Staphylococcus aureus* (31%).

In a similar study done by Kattel *et al.* (2008), *Escherichia coli* (59.59%) was significantly the most predominant one followed by *Staphylococcus aureus* (12.56%), *Klebsiella* spp.(10.78%), *Enterococcus faecalis* (7.95%), *Pseudomonas aeruginosa* (5.01%), *Acinetobacter calcoaceticus* (1.09%) and others in the study of bacteriology of UTI.

Citrobacter freundii was the second principal isolate among Gram negative bacilli constituting 9.1 percent of the total bacterial isolates. In a similar study carried out by Shrestha *et al.* (2004) this species represented 3.8% of the total isolates. However this finding is not well supported by other studies because *Citrobacter freundii* has been isolated as etiological agent of UTI in very few cases in other studies. In many studies carried out by various workers, it has been established that more than 80.0% of the

urinary isolates belong to *Enterobacteriaceae* family, and as *C. freundii* also belongs to this family, so it would not be much surprising to isolate this organism as the second most common isolate in this study.

Among the Gram positive isolates, *S. aureus* was found to be the major isolate with 24.2 percent of the total isolates. This finding resembles the study done by Subedi (2004) who found 31.1% of infection due to *E. coli* followed by *Staphylococcus aureus* that caused 24.5 % of infection. In a similar study conducted by Pandey (2002), *E.coli* was found to be the predominant (35.4%) followed by COPS (22.3%) and CONS (18.8%). Presence of *Staphylococcus aureus* in urine often indicates pyelonephritis acquired via hematogenous spread, so a pure culture of *S. aureus* is considered to be significant regardless of number of colony forming units (Forbes *et al.*, 2002). A similar study by Goswami *et al.* (2001) 21.4% of *S. aureus* was isolated. Asymptomatic bacteriuria is several-fold more common among women and acute pyelonephritis is five to ten times more common in both sexes. Shrestha *et al.* (2004) conducted a study on UTI in female patients in Kathmandu and found 49.3% female patients with UTI, *E. coli* (52.9%) was the most predominant etiological agent followed by *S. epidermidis* (20.7%), *M. morganii* (6.7%), *C. freundii* (3.8%), *S. aureus* (2.9%) *and P. aeruginosa* (2.5%). Similarly, Subedi (2009) has shown same type of result in which *E.coli (*31.1%) was predominant followed by *Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella oxytoca.*

One case in the study found *P. aeroginosa. Ps. aeroginosa* is opportunistic pathogen. It is one of the primary causes of nosocomial infection. Mohammed *et al*., (2007) in his study in India found *P. aeroginosa* more prevalent in the middle age females. *P*. aeroginosa plays an important role in the bladder infection and is considered as primary pathogens in compromised host of Jones *et al*., (1999).

An early detection and treatment of ASB may be of considerable importance not only to forestall acute pyelonephritis and chronic renal failure in the mother, but also to reduce prematurity and fetal mortality in the offspring.

The prevalence of UTI during pregnancy increases with age (Jones, 2009). In this study, the majority of the patients belonged to age group 20-30 i.e 84.6 % of the total requests. The predominance of infection in females above 21 years old agreed with the report of Orrett and Shurland (1998). The high prevalence of infection in females is usually related to anatomical and pathogenic factors, e.g. the short length of the urethra hence lesser distance of bacteria ascending up the tract, lack of antimicrobial properties of prostatic fluid as in males, hormonal changes affecting the adherence of bacteria to the mucosa and urethra trauma during sexual intercourse. The latter factor accounts for the well recognised “honeymoon cystitis” that is associated with UTI infections. Recent infection has also been found to be a predisposing factor to UTI acquisition.

Most of infected pregnant women 7.1% (3/42) belonged to the group <20, 606% (28/423) belonged to age group 20-30 and 5.7% (2/35) belonged to more than 30. Statistically, there was no association between age and presence of asymptomatic bacteriuria. The studies conducted by Manandhar *et al*. (1995), Pandey (2002), Subedi (2004) found urine samples obtained from the female at the age group in between 20 to 30 have maximum infection. This age group seems to be sexually active, socioeconomically productive and culturally involved in marriage in our country. A number of studies suggest that sexual activity is an important factor in the pathogenesis of UTI in women.

The highest number of patients visiting antenatal clinic were from rural area 63%. The infection rate 7.6 % (14/185) was also higher in urban communities in comparison to rural community. There was no association between locality and presence of asymptomatic bacteriuria.

In the present study, highest number of urine samples were collected from urine samples of pregnant who married at the age group 20-30 and infectious urine samples having culture positive result were also more (8.4%) from those of the same age group. However the result was proved to be statistically insignificant. In a similar study done by Hazir (2007), maternal age was significantly lower in the women with a positive urine culture.

The study included more patients 63.8% who had their first pregnancy at the age group 20-30. Most of the patients who were found to have culture positive results were married at the age group more than 30(50%) followed by 20-30(7.2%) and less than 20(5%). This result was proved to be statistically significant.

Most of the patients were housewife (92.2%). 3 out of 16 (12.5%) people involved in service had positive urine culture and remaining 30 (6.5%) were housewives. There is no significant association (P value<0.05) between occupation and asymptomatic bacteriuria.

The highest number 276(55.2%) of infected pregnant women in this study were in multigravidae, which is similar to study done by Ghimire *et al.* (1995) and Pandey (2004). This is because increase in the physiological change in pregnancy increases the chances of UTI. This result is not statistically significant.

Also much of the infected urine samples were from parous women than nulliparous women. In the study conducted by Al-Haddad (2005), 75.6% of infected women had 1-3 children. Statistically, there is no significant association between parity and asymptomatic bacteriuria.

According to the trimester wise distribution of the patients in this study, much of the samples obtained were from pregnant women in the third trimester (41.2%). The highest number of culture positive result was found from the women in their second trimester, followed by first trimester and third trimester. Similar result was found in the study of

Pandey (2004) and Hazir (2007). In a study performed in Turkey, the prevalence of asymptomatic bacteriuria was more prevalent in the third trimester which is in contrast with our findings. However, there is no significant association between trimester and asymptomatic bacteriuria statistically.

In the present study, highest number of patients infected had secondary level of education (9.1%) followed by more than secondary level of education (5.5%) and primary level of education (1.7%). However none of illiterate patients were found to have infection. The result is statistically insignificant.

Out of 15 samples which were from women who had previous UTI, 4 i.e 25% had positive culture result and remaining 29 i.e 6% were from women who did not have previous UTI. The result is also statistically significant. This result is supported by similar study done by Fatima *et al*. (2006) who found 35.7% of culture positive ladies with past history of UTI.

The microscopic examination of urine was done by wet mount preparation and Gram stain. The intention of microscopy by wet mount preparation was to determine the number of white cells, red cells and epithelial cells in HPF (40 fields).

In this study, significant pyuria was observed in 4.2% (21/500) of requests. In this study, out of 479 cases of insignificant pyuria, only 29 (6.1%) showed culture positive results. Based on this result, the sensitivity and specificity of pyuria as a screening test for UTI were calculated as 12.1% and 96.4%. Positive predictive value of WBC count of ≥5/HPF for growth positive culture was found out to be 19.1%. In general, as the number of pus cells/HPF increases, the chance of getting culture positive results will also be higher. This pattern was also found in the present study.

In the study, all the samples which showed positive cultural test had insignificant haematuria. Out of total samples only 3 samples (0.6%) showed haematuria but none of them had bacteriuria on culture. Based on this, the sensitivity and specificity of

haematuria were calculated as 0% and 99.36% respectively. The predictive value of negative test (NPV) was found to be 99.3 %. Due to a higher quantity of false negative results seen, using microscopy of RBCs as a screening test of UTI was not found to be reliable. Schumann and Schweitzer (1991) suggested that the observation of 0-2 RBCs/HPF on the urinary sediment is normal both in males and females. But Wargotz *et al*. (1987) and Steward *et al*. (1995) reported that they are abnormal if the RBC count was ≥3/HPF.

Out of the total samples, 95.2% (476/500) showed insignificant epithelial cells count however among these 5.9% (28/476) showed significant bacteriuria. Similarly, 4.8% of total sample showed significant epithelial count and among these 20.8% (5/24) showed significant bacteriuria on culture. Based on this, the sensitivity and specificity of epithelial cell count was 15.15% and 95.93%. The positive predictive (PPV) value for significant epithelial account was 20.83% and negative predictive value (NNP) was 94.12%. Epithelial cell are normally present in urine specimen from female patients even when careful collection technique are used. Their presence does not invalidate the result in urine analysis and culture but the large number of epithelial cells may signify a specimen that contains greater quantity of organisms from vagina or perineum than UT. Epithelium cells are in urine as a result of normal exfoliation along UT (Schumann and Schweitzer, 1991).

Finding of leucocytes (>5 cells/HPF) is of great importance for urinary tract infection diagnosis, while erythrocytes and epithelial cells are of poor significance for urinary tract infection diagnosis (Merila *et al*., 1987).

In this study, reagent strip for urinanalysis was used to measure protein, pH and sugar in urine. In this study, out of total samples 461 were negative for albumin test, among them 26 (5.6%) showed significant bacteriuira on culture. While 7.8% of samples showed positive albumin test out of which 18% (7/39) showed significant bacteriuria. Based on this, sensitivity and specificity of albumin test was found to be 21.12% and

93.15% respectively. The PPV and NPV was 17.95% and 94.36%. There are various conditions in which protein (albumin) appear in urine, UTI is one of them. According to the North Thames Regional Guidelines for Diagnosis and Management of Urinary Tract Infection, if dipstick proteinuria is consistently more than 1+, then this may indicate UTI and a MSU specimen for culture should be taken. So, it could be concluded that detection of protein (albumin) in urine is also important for diagnosis of UTI and mid-stream urine sample should be cultured in cases when there is absence of significant pyuria.

In this study, all the patients were negative for urinary sugar test. The blood sugar even higher than upper level of normal range i.e. 120mg/dL is not excreted in urine unless the level increases to 180 mg/dL. The threshold of kidney to excrete sugar in urine is 180 mg/dL (Murray *et al.*, 2000).

The bacterial culture study shows that over 57.1% of all organisms grown were resistant to amoxycillin. However, in more than 95% of the cases, the organism was sensitive to third generation cephalosporin and gentamycin (Bahadi, 2010).

Among the common antibiotics used against all Gram negative isolates, Nitrofurantoin was the drug of choice as 19(90.5%) isolates were found to be susceptible to this drug followed by Gentamycin with a susceptibility of 85.7 %. This result is supported by Jha and Bapat (2005) in which 92.5% of urinary isolates were susceptible to Gentamycin. *E.coli* was found to be 100% sensitive to this nitrofurantoin. Most of the Gram negative isolates, i.e. 12 (57.1%) were resistant to Amoxycillin and Nalidixic acid had 42.9% resistance to gram negative organisms isolated.

Similarly, in gram positive isolates also nitrofurantoin was the most effective drug having susceptibility of 91.7%. Gentamycin and cephalexin had equal susceptibility of 83.3%. amoxicillin had resistance value of 41.7% and norfloxacin was 33.3% resistant.

6.2 CONCLUSION

The prevalence of asymptomatic bacteriuria in pregnant women attending Shree Birendra Hospital, Chhauni is 6.6%. Majority of patients with positive urine culture were in their second trimester. There is significant association between asymptomatic UTI in pregnant women and the presence of previous UTI. Also there is significant association between asymptomatic UTI in pregnant women and the women with higher age of their first pregnancy. The prevalence of UTI was higher in multigravidae women than in primigravidae women. Out of total 500 urine samples taken, 33 showed positive result in culture. 7 different species of bacteria were isolated. *Escherichia coli* was the predominant organism followed by *Staph aureus* and other organisms. In this study the most sensitive antibiotics were nitrofurantoin and gentamycin.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. Out of 500 MSU samples processed from antenatal patients, in 33 samples significant growth was observed while 90 samples with mixed growth, 277 samples with no growth and 100 samples with non significant growth were observed.
2. Frequency of positive growth of isolates was found to be higher in women belonging to the age group less than 20.
3. Rate of urinary tract infection was found to be higher in patients of urban area when compared with rural area.
4. Most of the patients were housewives and the maximum positive culture result i.e 30 out of 33 was also obtained from the patients involved in this group.
5. The higher number of positive cases had married at the age of 20 to30.
6. Most of the pregnant patients who had positive urine culture were pregnant for the first time at the age of more than 30.
7. 20 out of total 33 positive urine culture results were from multigravidae women.
8. Among total 33 of positive urine culture, higher number i.e 16 was from parous women who gave birth once. This is quite different than that of nulliparous women, 14 of whom had positive result of urine culture.
9. Majority of patients with positive urine culture were in their second trimester.
10. Most of the patients with positive urine culture attained secondary level of education.
11. Among the total 500 samples, 95.8% (479/500) showed significant pyuria, however among these 6.1% (29/479) gave positive culture results. Similarly, 21 (4.2%) samples showed insignificant pyuria, and among these 19% (4/21) gave positive culture result.
12. The higher number of positive cases 25% (4/16) had previous UTI.

13. Out of total samples, 99.4% (497/500) of samples showed insignificant haematuria, however among these 6.6% (33/497) showed significant bacteriuria. Similarly, 0.6% of samples showed significant haematuria but among these none showed significant bacteriuria
14. Out of the total samples, 95.2% (476/500) showed insignificant epithelial cell count however among these 5.9% (28/476) showed significant bacteriuria. Similarly, 4.8% of total sample showed significant epithelial count and among these 20.8% (5/24) showed significant bacteriuria on culture
15. The predominant bacteria causing UTI among antenatal patients were found to be the Gram negatives (N=21) which constituted 63.64% Gram positive bacteria (N=12) constituted only 36.36%.
16. Altogether 7 different species of bacteria were isolated from the growth positive cultures. *Escherichia coli* was found to be the most predominant isolate (42.4%). followed by *Staphylococcus aureus* (24.2%), *Staphylococcus saprophyticus* (12.1%), *Citrobacter freundii* (9.1%), *Klebsiella oxytoca* (6.1%), *Enterobacter* sps (3%) and *Pseudomonas aeruginosa* (3%).
17. Nitrofurantoin was found to be the most effective drug against both the Gram negative bacteria and gram positive bacteria.

7.2 RECOMMENDATIONS

1. All pregnant women should have their urine tested for asymptomatic bacteriuria in each trimester to identify most cases.
2. Pregnant women who had previous UTI should be screened for asymptomatic UTI.
3. Antibiotics should be used only on the basis of Laboratory results.

REFERENCES

Abdullah AA and Al-Moslih MI (2005) Prevalence of asymptomatic bacteriuria in Sharjah, united Arab emirates. 11:5-6

Aboderin OA (2009) Antimicrobial resistance in Escherichia coli strains from urinary tract infections J Natl Med Assoc. 101:1268-73

Abyad A (1991) Screening for asymptomatic bacteriuria in pregnancy: urinalysis vs urine culture. J Fam Pract. 33:471-474

Acharya VN, Jadav SK.(1980) Urinary tract infection: current status. J Postgrad Med. 26:95

Asscher AW, Sussman M and Weisser R (1968) Bacterial growth in human urine. In "Urinary Tract Infections". Edited by Francis O'Grady and Brumfitt W., Oxford University Press, London, New York & Toronto. pp 3-14

Al-Haddad AM (2005) Urinary tract infection among pregnant women in Al-Mukalla district, Yemen, East Mediterr Health J. 11:505-10

Astal ZY and Sharif FA (2002) Relationship between demographic characteristics and community acquired urinary tract infection. Eastern Mediterranian Health J. 8:1

Bahadi E, Kabbaj D, Elfazazi H, Abbi R, Hafidi MR, Hassani MM, Moussaoui R, Elouennass M, Dehayni M and Oualim Z (2010) Urinary tract infection in pregnancy. Saudi J Kidney Dis Transpl. 21:342-4

Brooks D (2004) The management of suspected urinary tract infection in general practice. Am Fam Physician. 69:157

Boscia JA, Abrutyn E and Kaye D (1987) Asymptomatic bacteriuria in elderly persons: treat or do not treat? Ann Intern Med. 106:764–766

Cardozo L (1995) Urinary Tract Disorder in Pregnancy Dewhurst's Textbook of Obsterics and Gynacology for Postgraduates, 5th edition Black well science Limited, John street London

Chakraborty P (2001) A Text Book of Microbiology, 1st ed. New Central Book Agency (P) Ltd, Calcutta

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

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

Chernew I and Brande AI (1962) Depression of phagocytosis by solute in concentration found in the kidney and urine. J. Clin, Invest. 41: 1945-1953

Chilton L (2008) Infections and Antimicrobial Resistance in the Elderly Living in Long-Term Care Settings. Geriatrics and Aging. 11:582-588

Chongsomchai C (1992) Screening for asymptomatic bacteriuria in pregnant women; urinalysis versus urine culture. Journal of the Medical Association of Thailand. 82:369–73

Collee JG, Fraser AG, Marmion BP and Simmons A (1999) Mackie and McCartney Practical Medical Microbiology 14th ed. Churchill Livingstone, USA

Collins LE, Clarke RW and Maskell R (1986) Streptococci as Urinary Pathogens. Lancet. 2:497-481

Collier L, Balows A and Sussman M (1998) Topley and Wilson’s Microbiology and Microbial infections (9th ed). London: Oxford University Press3:601-21

Conolly A and Thorp JM (1999) Urinary Tract Infection in Pregnancy, Urol Clin North Am 26:779-78

Delzell, JE Jr, Lefevre, ML (2000) Urinary tract infections during pregnancy. Am Fam Physician. 61:713.

Department of Health Services (1996/97) Annual Report. His Majesty Government

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

Faro S and Fenner DE (1998) Urinary tract infections. Clin Obstet Gynecol. 41:744-54

Fatima N,Yasmin S and Ishrat S (2006) Prevalence and complication of asymptomatic bacteriuria during pregnancy, Professional Med J. 13: 108-112

Fihn SD (1992) Lower urinary tract infection in women. Current opinion in Obest and Gynecology. 4:571-578

Fihn SD (2003) Clinical practice. Acute uncomplicated urinary tract infection in women. N Engl J Med. 349:259-66

Fitzgerald M (2002) Urinary Tract Infection: Providing the best care. www.medscape.com/viewarticle/436592_5 9

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

Fowler JE and Mariano M (1990) Immunologic response of the prostate to bacteriuria and bacterial prostates: Antigen specific immunoglobulin in men with bacterial prostatitis. J Urol. 131:363

Froom P (2004) What Is an Abnormal Test Strip Urinary Erythrocyte Concentration? Clinical Chemistry. 50:673-675

Froom P, Gross M and Ribak J (1986) The effect of age on prevalence of asymptomatic microscopic hematuria Am. J Clic. Path. 86;656-567

Ghimire P (1995) Prevalence of bacteriuria and UTI in Nepali women. Msc dissertation submitted to the Central Department of Microbiology, T.U., Kathmandu

Ghimire P, Sharma AP and Adhikari M (1995) Prevalence of Urinary Tract Infection In pregnant woman of Nepal, Journal of Nepal Medical Association 33:232-235

Giespella WA (1987) Urinary tract infection in young women, with special reference to Staphylococcus saprophyticus. J Clin Pathol 31:348-350

Gillespie WA, Sellin MA, Gill P, Stephens M, Tuckwell LA and Hilton AL (1978) Urinary tract infection in young women, with special reference to *Staphylococcus saprophyticus* J Clin Pathol, 31: 348–350

Giuliani A, Tamussino K, Basver A, Haas J and Petru E. (2002) The impact of body mass index and weight gain during pregnancy on puerperal complications after spontaneous vaginal delivery, Wien Klin Wochenschr. 114:383-6

Godkar PB (1994) Text book of Medical Laboratory Technology. First edition. Bhalani Publishing House, Mumbai. 404-48

Goswami R, Bal CS, Tejaswi S, Punjabi GV, Kapil A and Kochupillai N (2001) Prevalence of urinary tract infection and renal scars in patients with diabetes mellitus. Diabet Res Clin Pract 53:181-186

Goya N, Tanabe K and Iguchi Y (1997) Prevalence of urinary tract infection during outpatient follow up after renal transplantation. Infect 25:101-105

Graham JC and Galloway A (2001) ACP Best Practice No 167: The laboratory diagnosis of urinary tract infection. Journal of Clinical. Pathology. 54; 911-919.

Gratacos E, Torres PJ, Villa J, Alonso P and Cararach V (1994) Screening and treatment of asymptomatic bacteriuria in pregnancy to prevent pyelonephritis. J Infect Dis. 169: 1390-1392

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

Hanson LA, Fasth A, Jodal U, Kaijser B and Svanborg EC (1981) Biology and pathology of urinary tract infections Clin Pathol. 34: 695–700

Hazhir S (2007) Asymptomatic Bacteriuria in Pregnant Women. Urology Journal. 4:24-7

Herm FB, Hooton TM and Stamm WE (2003) Pathogenesis and managemaent of bacterial urinary tract infections in adult patients with diabetes mellitus. International Journal of antimicronial agents. 22:S35-S43

Hooton TM and Stamm WE (1997) Diagnosis and treatment of uncomplicated urinary tract infection. Infect Dis Clin North Am. 11:551-581

Hugo WB and Russel AD (1993) Pharmaceutical Microbiology, 5th edition, Blackwell Scientific Publications, UK

Jackson E and Fowler JR (1990) Urinary tract infectio and inflammation. Diabetologia croatica. 31:2

Jayalakshmi J and Jayaram VS (2008) Evaluation of various screening tests to detect asymptomatic bacteriuria in pregnant women. Indian J Pathol Microbiol. 51:379-81

Jha N and Bapat SK (2005) A study of sensitivity and resistance of pathogenic microorganisms causing UTI in Kathmandu valley. Kathmandu University Medical Journal. 3:123-129

Johnson JR (1991) Virulence factors in Escherichia coli urinary tract infection. Clin Microbiol Rev. 4:80-128

Jones RN, Kugler KC, Pfaller MA, Winokur PL (1999) Characterisation of pathogens causing urinary tract infections in hospitals in the North American: survelliance program. Journal of Diagnosis micribiology infection Disease. 35:55-63.

Jones LA (2009) Urinary tract infection in pregnancy. Am J Obstet Gynecol. 113:346-52

Kaitz AL (1961) Urinary concentrating ability in pregnant women with asymptomatic bacteriuria. J Clin Invest. 40:1331

Kass EH (1960) Bacteriuria and pyelonephritis of pregnancy. Arch Intern Med. 105:194

Kattel HP, Mishra SK, Acharya J, Shah AS, Rijal B P, Pokhrel B P (2009) Relationship Between Pyuria and Bacteriuria in Suspected Urinary Tract Infection Journal of Nepal Association for Medical Laboratory Sciences. 10:1

Kattel HP, Acharya J, Mishra SK, Rijal BP and Pokhrel BM (2008) Bacateriology of urinary tract infection among patients attending tribhuvan university teaching hospital Kathmandu, Nepal Journal of Nepal Association for Medical Laboratory Sciences 9:1 pg 25-29

Kaye D and Sobel JD (1986) The Role of Bacterial Adherence in Urinary Tract Infections in Elderly Adults geronj.oxfordjournals.org. 42:29-32

Lavanya SV and Jogalakshmi D (2009) Asymptomatic bacteriuria in antenatal women. Indian J Med Microbiol. 20:105-6.

Leigh DA (1990) UTI. In: Smith GR and Easmon CSF, In (eds.) Topley and Wilson's Principles of Bacteriology, Virology and Immunity, Bacterial Diseases, 8th ed.. Frome and London: Butler and Tanner Ltd. 3:197-214

Lucas, Cunningham FG (1993) Urinary infection in pregnancy. Clin Obstet Gynecol. 36:855-68.

Manandhar S (1996) Microbiology of urinary tract infection:A hospital based study. Msc Dissertation submitted to the Central Department of Microbiology, T.U, Kathmandu

Manandhar T (2005) Antibiotic susceptibility profile of bacterial pathogens in urinary tract infection with special reference to extended spectrum beta lactamase (ESBL) producing strains. Msc Dissertation submitted to the Central Department of Microbiology, T.U, Kathmandu

Merila M, Raisanen S, Ylitalo P and Eskelinen S (1987) Microscopic estimation of bacteria and cells in urine: II. A clinical study on the application of the theoretical consideration to clinical practice. Int Urol Nephrol. 19:109-114

Mulvey MA, Schilling JD, Martinez JJ and Hultgren SJ (2000) Bad bugs and beleaguered bladders: Interplay between uropathogenic Escherichia coli and innate host defenses. 97:8829-8835.

Mittal P and Wing DA (2005) Urinary tract infections in pregnancy. Clin Perinatol. 32:749-64.

Moghadas AJ and Irajian G (2009) Asymptomatic urinary tract infection in pregnant women. Iranian journal of pathology 4:105-108

Mohammad M (2002) Laboratory aspects of asymptomatic bacteriuria in pregnancy. Southeast Asian journal of tropical medicine and public health 33:575–80.

Mohammad M, Ghasemi E, Mokhayeri H, Pournia Y and Boroun H (2010) Antimicrobial resistance patterns of E. coli detected from hospitalized urine culture samples. Asian J. Biol. Sci, 3:195-201.

Murray RK, Granner DK, Mayes PA and Rhodwell VW (2000) Herper's Biochemistry 25th ed. Prentice Hall International (USA) Ltd

Naber KG (2000) Treatment options for acute uncomplicated cystitis in adults, Journal of antimicrobial chemotherapy, 46(S1): 23-27

Nicolle LE (1994) Screening for asymptomatic bacteriuria in pregnancy. In: Goldbloom RB (ed.) The Canadian Guide to Clinical Preventive Health Care. Ottawa: Canada Communication Group. 100-107

Nicolle LE (2003) Asymptomatic Bacteriuria: when to screen and when to treat Infect. Dis. Clinc. North Am 17;(2)367-94

Nicolle LE (2000) Asymptomatic Bacteriuria — Important or Not? The new Eng J of medicine 343:1037-1039

Orette FA and Shurland SM (1998) The changing patterns of antimicrobial susceptibility of urinary pathogens in Trinidad. Singapore Journal Medicine 39: 256-259.

O'Sullivan DJ, Fitzerald MG, Meynell MJ and Malins JM (1960) A simplified method for the quantitative bacterial culture of urine. J Clin Pathol. 13:527–528.

Pandey R (2002) A hospital based study of urinary tract infection among women visiting antenatal clinic of Tribhuwan University Teachin Hospital. Msc dissertation submitted to central department of Mictobiology, TU, Kathmandu, Nepal.

Parker AS, Cerhan JR, Lynch CF, Leibovich BC, and Cantor KP (2004) History of Urinary Tract Infection and Risk of Renal Cell Carcinoma. American Journal of Epidemiology. 159:42-48

Parsons CL and Mulholland SG (1978) Antibacterial activity of bladder surface mucin duplicated by exogenous glycosaminoglycan (heparin). Am J Pathol. 93:423–432.

Patterson A (1987) Bacteriuria in pregnancy. Infect Dis Clin North Am. 1:807-22.

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

Griffin K (2011) Pregnancy weight matters www.gatesheadhealth.nhs.uk

Regier L (2007) Drugs for urinary tract infections. RxFiles, http://www.rxfiles.ca

Regmi S (2003) Evaluation of Nitrite test in detecting urinary tract infection. Msc dissertation submitted to central department of Miicrobiology, TU Kathmandu, Nepal

Saidi, Delaporte V, Lechevallier E. (2005) Urological problems encountered during pregnancy. Prog Urol. 15:1-5.

Sharma PR (1983) Urinary infection- The infection that matters. J Inst Med 5:19-22

Sobel JD and Kaye D (1986) Utility of urine cultures in the emergency department. Annals of Emergency Medicine. 15:302-307

Stamm WE, Hooton TM. (1993) Management of urinary tract infections in adults. New England journal of medicine. 329:1328–34.

Stamm WE. (2003) Urinary tract infections and Pyelonephritis. In:Braunwald E, Hauser SL, Fauci AS, Longo DL, Kasper DL and Jameson JL Harrison's Principles of Internal Medicine, 15th ed. McGraw Hill companies, Inc. 2:1620-1623

Stewart D, Hervey Y, Downie CT and Scott CJ (1995) Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Tropical Journal of Pharmaceutical Research. 2:223-228

Stenqvist K, Sandberg T, Lidin-Janson (1987) Virulence factors of Escherichia coli in urinary isolates from pregnant women. J Infect Dis. 156:870.

Stenqvist K, Dahlen-Nilsson I and Lidin-Janson (1989) Bacteriuria in pregnancy:1.Frequency and risk of acquisition. Am J Epidemiol. 129:372-79.

Steward DK, Wood GL and Cohen R (1985) Urinary tract infection in adults Am J Infect Control. 13:154-60

Subedi (2004) A hospital based study on urinary tract infection among pregnant women visiting Lumbini Zonal hospital Butwal. MSc dissertation submitted to central department of Microbiology, TU, Kathmandu, Nepal.

Subedi M, Basnyat SR and Acharya SD (2009) Urinary Tract Infection in Pregnancy and its Correlation with Nitrite Test Department of Microbiology, Amrit Campus,Thamel, Kathmandu, Nepal JNHRC. 7:80-83

Sweet RL (1977) Bacteriuria and pyelonephritis during pregnancy. Semin Perinatol. 1:25

Todar K (2002) Todar's Online Textbook of Bacteriology. http://www.textbook of bacteriology. net

Tugrul S, Oral O, Kumru P, Kose D, Alkan A and Yildirim G (2005) Evaluation and importance of asymptomatic bacteriuria in pregnancy. Clin Exp Obstet Gynecol. 32:237-40

Tuladhar NR, Banjade N, Pokharel BM, Rizal B, Manandhar R, Shrestha S, Shah A and Chaurasia S (2003) Antimicrobial resistant bacterial strains from inpatients of Tribhuvan University teaching hospital Kathmandu. J Inst Med. 25:19-26

Tuladhar NR, Shrestha HG and Nakanishi (1989).Urinary pathogens and their sensitivity to various antimicrobial drugs in Nepal – two year and five months study. J Ins Med. 11:1-8.

Turpin, MinkahB, Danso KA and Frimpong EH (2007) Asymptomatic Bacteriuria in Pregnant Women Attending Antenatal Clinic at Komfo Anokye Ghana Medical Association 41: 26–29

Stamm WE and Hooton TM (1993) Management of Urinary Tract Infections in Adults 329:1328-1334

Wadland W and Plante D (1989) Screening for asymptomatic bacteriuria in pregnancy. Fam Phy. 29:372-376.

Wilkie ME, Almond MK, Marsh FP (1992) Diagnosis and management of urinary tract infection in adults BMJ 305:1137-41

Wargotz ES, Hyde and Karcher DS (1987) Urine sediment analysis by the yellow automated urinanalysis workstation. Am J Clin Pathol 88:746-748

Warren JW (1997) Catheter-associated urinary tract infections. Infect Dis Clin North Am. 11:609-22

Wing AJ, Brunner FP, Brynger H, Chantler C, Donckerwolcke RA, Gurland HJ, Hathway RA and Jacobs C (1978) Combined report on regular dialysis and transplantation in Europe, VIII, 1977. In, "Proceedings of the European Dialysis and Transplant Association". Dialysis Transplantation Nephrology. 15: 3-77

Whalley R (1967) Bacteriuria of pregnancy. Amer. J. Obstet. Gynaecol. 97: 723-738

APPENDIX-I

QUESTIONNAIRE

1. Date ……
2. S.N …….
3. Name: … … … … … … … … … … … … … … …
4. Age: … … … … … … … …… … … … …
5. Address: … … … … … … … … … … … … … …
6. Occupation:
    1. Agriculture
    2. Service
    3. Housewife
    4. Business
    5. Other
7. Education
    a. Illiterate
    b. Primary
    c. Secondary
    d. More

8. Age at marriage:
    a. <20 yrs
    b. 20-30 yrs
    c. >30 yrs
9. Age at first pregnancy
    a. <20 yrs
    b. 20-30 yrs
    c. >30 yrs
10. Gravida ……..
11. Parity ……

12. POG
    a. 1st trimester
    b. 2nd trimester
    c. 3rd trimester
13. Previous UTI

a. YES

b. NO

Examination

Weight……..

Height ……..

Investigation

Epithelial cells ……..

Pus cell ……

RBC ……..

Urine culture: …

Antibiotic sensitivity test:

Antibiotics used	Resistant	Intermediate	Sensitive

APPENDIX-II

I. COMPOSITION AND PREPARATION OF DIFFERENT CULTURE MEDIA

The culture media used were from Hi-Media Laboratories Pvt. Limited, Bombay, India. (All compositions are given in grams per liter and at 25^0C temperature)

1. Blood agar (BA)

Blood agar base (infusion agar) + 5-10% sheep blood

Ingredients	gm/liter
Beef heart infusion	500.0
Tryptose	10.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25^0C) 7.3±0.2

42.5 grams of the blood agar base medium was suspended in 1000 ml distilled water and sterilized by autoclaving at 121^0C (15lbs pressure) for 15 minutes. After cooling to 40-50^0C, 50 ml sterile defibrinated sheep blood was added aseptically and mixed well before pouring.

2. Cystein Lactose Electrolytes Deficient media (CLED)

Ingredients	gm/liter
Peptic digest of animal tissue	4.0
Caesin enzyme hydrolysate	4.0
Beef extract	3.0
Lactose	10.0
Bile salt	1.5
L- cystein	0.128
Brommothymol blue	0.02
Agar	15.0

36.15 grams of powder was suspended in 1000 ml distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15lbs pressure) for 15 minutes.

3. Mueller Hinton Agar (MHA)

Ingredients	gm/liter
Beef, Infusion form	300.0
Casein Acid Hyrolysate	17.5
Starch	1.5
Agar	17.0
Final pH (at 25^0C) 7.4±0.2	

38 grams of the medium was suspended in 1000 ml distilled water and the medium was warmed to dissolve. 10 ml was distributed in test tubes and sterilized by boiling in water bath for 10 minutes.

4. Nutrient Agar (NA)

Ingredients	gm/litre
Peptone	10.0
Sodium Chloride	5
Beef Extract	10.0
Yeast Extract	1.5
Agar	12.0
Final pH (at 25^0C) 7.4±0.2	

37 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

5. Nutrient Broth (NB)

Ingredients	gm/litre
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Final pH (at 25^0C) 7.4±0.2	

13 grams of the medium was dissolved in 1000 ml distilled water and autoclaved at 121˚C for 15 minutes.

6. Sabouraud Dextrose Agar (SDA)

Ingredients	gm/litre
Mycological peptone	10.0
Dextrose	40.0
Agar	15.0
Final pH (at 25^0C)	5.6±0.2

65 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

II. Biochemical Test Media

1. MR-VP Medium

Ingredients	gm/litre
Buffered Peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final pH (at 25^0C)	6.9±0.2

17 grams was dissolved in 1000 ml distilled water. 3 ml of medium was distributed in each test tube and autoclaved at 121^0C for 15 minutes.

2. Hugh and Leifson's Medium

Ingredients	gm/litre
Tryptone	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Bromothymol Blue	0.08
Agar	2.0
Final pH (at 25^0C)	6.8±0.2

9.4 grams of the medium was rehydrated in 1000 ml cold distilled water and then heated to boiling to dissolve completely. The medium was distributed in 100 ml amounts and sterilized in the autoclave for 15 minutes at 15 lbs pressure (121^0C). To 100 ml sterile medium aseptically added 10ml of sterile Dextrose and mixed thoroughly and dispensed in 5 ml quantities into sterile culture tubes.

3. Sulphide Indole Motility (SIM) medium

Ingredients	gm/litre
Beef Extract	3.0

Peptone	30.0
Peptonized Iron	0.2
Sodium Thiosulphate	0.025
Agar	3.0

Final pH (at 25^0C) 7.3±0.2

36 grams of the medium was suspended in 1000 ml distilled water and dissolved completely. Then it was distributed in tubes to a depth of about 3 inches and sterilized.

4. Simmon's Citrate Agar

Ingredients	**gm/litre**
Magnesium Sulfate	0.2
Mono-ammonium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Agar	15.0
Bromothymol Blue	0.08

Final pH (at 25^0C) 6.8±0.2

24.2 grams of the medium was dissolved in 1000ml distilled water. 3ml medium was distributed in test tubes and sterilized by autoclaving at 121^0C for 15 minutes. After autoclaving tubes containing medium were tilted to form slant.

5. Triple Sugar Iron (TSI) Agar

Ingredients	**gm/litre**
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0

Final pH (at 25^0C) 7.4±0.2

65 grams of the medium was dissolved in 1000ml of distilled water and sterilized by autoclaving at 15 lbs (121^0C) pressure for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of thickness.

6. Christensen Urea Agar

Ingredients	gm/litre
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Dipotassium Phosphate	1.2
Mono-potassium Phosphate	0.8
Phenol Red	0.012
Agar	15.0

Final pH (at 25^0C) 7.4±0.2

24 grams of the medium was suspended in 950 ml distilled water and sterilized by autoclaving at121^0C for 15 minutes. After cooling to about 45^0C, 50 ml of 40% urea was added and mixed well. Then 5 ml was dispensed in test tube and set at slant position.

III. Staining and Test Reagents

1. For Gram's Stain

(a) Crystal Violet solution

Crystal Violet	20.0 g
Ammonium Oxalate	9.0 g
Ethanol or Methanol	95 ml

Distilled Water (D/W) to make 1 litre

Preparation: In a clean piece of paper, 20 gm of crystal violet was weighed and transferred to a clean brown bottle. Then, 95 ml of ethanol was added and mixed until the dye was completely dissolved. To the mixture, 9 gm of ammonium oxalate dissolved in 200 ml of D/W was added. Finally the volume was made 1 litre by adding D/W.

(b) Lugol's Iodine

Potassium Iodide	20.0 g
Iodine	10.0 g
Distilled Water	1000 ml

Preparation: To 250 ml of D/W, 20 gm of potassium iodide was dissolved. Then 10 gm of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1 litre by adding D/W.

(c) Acetone-Alcohol Decoloriser

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

Preparation: To 25 ml D/W, 475 ml of absolute alcohol was added, mixed and transferred into a clean bottle. Then immediately, 500 ml acetone was added to the bottle and mixed well.

(d) Safranin (Counter Stain)

Safranin	10.0 g
Distilled Water	1000 ml

Preparation: In a clean piece of paper, 10 gm of safranin was weighed and transferred to a clean bottle. Then 1 litre D/W was added to the bottle and mixed well until safranin dissolved completely.

3. Normal saline

Sodium Chloride	0.85 g
Distilled Water	100 ml

Preparation: The sodium chloride was weighed and transferred to a leak-proof bottle premarked to hold 100 ml. Distilled water was added to the 100 ml mark, and mixed until the salt was fully dissolved. The bottle was labeled and stored at room temperature.

TEST REAGENTS

a. For Catalase test

Catalase Reagent (3% H_2O_2)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

Preparation: To 97 ml of D/W, 3 ml of hydrogen peroxide was added and mixed well.

b. For Oxidase Test

Oxidase Reagent (impregnated in Whatman's No. 1 filter paper)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD)	1 gm
Distilled Water	100 ml

Preparation: This reagent solution was made by dissolving 1 gm of TPD in 100 ml D/W. To that solution strips of Whatman's No. 1 filter paper were soaked and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

c. For Indole Test

Kovac's Indole Reagent

Isoamyl alcohol	30 ml
p-dimethyl aminobenzaldehyde	2.0 g
Hydrochloric acid	10 ml

Preparation: In 30 ml of isoamylalcohol, 2 g of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to that, 10 ml of conc. HCl was added and mixed well.

d. For Methyl Red Test

Methyl Red Solution

Methyl red	0.05 g
Ethyl alcohol (absolute)	28 ml
Distilled Water	22 ml

Preparation: To 28 ml ethanol, 0.05 gm of methyl red was dissolved and transferred to a clean brown bottle. Then 22 ml D/W was added to that bottle and mixed well.

e. For Voges-Proskauer Test (Barritt's Reagent)

Solution A

α-Naphthol	5.0 g
Ethyl alcohol (absolute)	100 ml

Preparation: To 25 ml D/W, 5 g of *α*-Napthol was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

Solution B

Potassium hydroxide	40.0 g
Distilled Water	1000 ml

Preparation: To 25 ml D/W, 40 gm of KOH was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

f. Mac Farland standard 0.5

It is prepared by adding 0.6 ml of 1% w/v barium chloride solution to 99.4 ml of 1% v/v solution of sulphuric acid.

APPENDIX-III

A. Gram-staining Procedure

First devised by Hans Christian Gram during the late 19th century, the Gram-stain can be used effectively to divide all bacterial species into two large groups: those that take up the basic dye, crystal violet (Gram-positive) and those that allow the crystal dye to was out easily with the decolorizer alcohol or acetone (Gram-negative). The following steps are involved in Gram-stain:

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with crystal violet stain and allowed to remain without drying for 10-30 seconds.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.
7. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer. Thicker smear requires more aggressive decolorizing.
8. The slide was flooded with counter stain (safranin) for 30 seconds and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 1000X.

B. Standardization of Loop

The wire loop was calibrated by the following procedures

1. A small container (e.g., bijou bottle of distilled water) was weighed.
2. Using a loop of about 3 mm internal diameter, 100 loopfuls of water was removed onto a blotting paper.
3. The container and water was reweighed.
4. The difference in weight was calculated.
5. The volume held by the loop was the difference in weight divided by 100.
6. The procedure was performed three times and the average of the three results was taken.
7. Thus the calibrated loop having 3 mm internal diameter held 0.002ml of MSU specimen.

APPENDIX-IV

Method of collection of midstream urine

It cannot be overemphasized that considerable importance is attached to the method of collection of urine specimens, transport to the laboratory and the initial efforts by the laboratory to screen and culture the urine. It is the responsibility of laboratory to provide patient with sterile, wide mouthed, glass or plastic jars, beakers or suitable

receptacles. They should have tight- fitting lids or be covered with papers or foils prior to sterilization by dry heat or autoclaving.

Whenever possible, the first urine passed by the patient at the beginning of the day should be sent for examination. This specimen is the most suitable for culture, microscope and biochemical analysis.

Midstream urine (MSU) for microbiological examination is as follows

WOMEN

A woman who is ambulatory should:

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

2. Undress in a suitable room spread the labia and cleanse the vulva and labia thoroughly using sterile cotton gauze pads and warm soapy water wiping from front to rear.

3. Rinse thoroughly with warm water and dry with a sterile cotton gauze pad. During the entire process the patient should keep the labia separated and not touch the cleansed area with fingers.

4. Pass urine, discarding the first part of the stream. Collect the remaining urine in the sterile container, closing the lid as soon as the urine has been collected.

5. Hand the clean- catch midstream urine, in the closed container, to the health personnel for prompt delivery to the laboratory.

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

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

APPENDIX-V

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

1. Equipments:

Incubator

Autoclave

Refrigerator

Microscope

Centrifuge

Weighing Machine

1. **Microbiological media (Hi-Media)**

Nutrient Agar
Urease Agar
Simmons Citrate Agar
Mueller-Hinton Agar
TSI Agar
Nutrient broth
Mac Conkey Agar
Blood Agar
MR-VP Broth

2. **Chemicals/ Reagents**

3% hydrogen peroxide
Acetone-alcohol
Safranine
Normal Saline
Blood plasma
Methyl red
Mineral oil
NNNN-tetramethyl paraphenyl diamine dihydrochloride
Crystal violet
Gram's iodine
Barium chloride
Sulphuric acid
Barrit's reagent
Kovac's reagent
Glycerol
Glucose oxidase kit

3. **Antibiotics Discs (Hi-Media)**

Amoxycillin (10 mcg)
Gentamicin (10 mcg)
Nitrofurantoin (300 mcg)
Cephalexin (30 mcg)
Novobiocin (5 µg)
Nalidixic acid (30µg)
Cotrimoxazole (1.25 mcg)
Norfloxacin (10 µg)

4. **Miscellaneous**

Glasswares
Forceps, Droppers
Blotting paper, cotton, Tissue paper
Distilled water
Inoculating loop,
Lysol
Immersion oil
Sticker

ZONE SIZE INTERPRETATIVE CHART

Antimicrobial Agents used	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Amoxycillin	Am	10µg	13	14-17	18
Cephalexin	CP	30 µg	14	15-17	18
Cotrimoxazole	Co	1.25/23.75µg	10	11-15	16
Gentamicin	G	10 µg	12	13-14	15
Ofloxacin	Of	5 µg	12	13-15	16
Norfloxacin	Nfx	10 µg	12	13-16	17
Nalidixic Acid	Na	30µg	13	14-18	19
Nitrofurantoin	Nf	300µg	14	15-16	17

(Source: Product Information Guide, Hi-Media Laboratories Pvt. Limited, Bombay, India).

APPENDIX-VI

Morphology and cultural characteristics of bacteria isolated from urine sample

BACTERIA	MORPHOLOGICAL CHARACTERISTICS	CULTURAL CHARACTERISTICS
Escherichia coli	Gram negative rod of 1-3µm×0.4-0.7µm size, aerobic and anaerobic, nonsporing, motile, noncapsulated	On BA: Large 1-4 mm in diameter, grayish white, moist, smooth, convex and opaque. The colonies may appear mucoid and and some strains are haemolytic. On CLED: Yellow colonies due to lactose fermentation, opaque with slightly deeper coloured center.
Citrobacter species	Gram negative motile bacilli	On NA: smooth, convex, non pigmented colonies with 2-4 mm diameter. On CLED: Late lactose fermentingor

		non lactose fermenting colonies.
Klebsiella species	Gram negative, short and thick rod of 1-2µm ×0.8µm size, nonsporing, nonmotile and capsulated.	Large dome shaped moist and usually viscid or mucoid colonies when cultured on BA and CLED. Most Klebsiella species are lactose fermenting.
Pseudomonas aeruginosa	Gram negative slender rod of 1.5-3 µm × 0.5µm size, nonsporing, motile with a single polar flagellum, most strains produce slime, strict aerobe.	Six different colonial types of Pseudomonas aeruginosa are encountered (Philips, 1969). Type 1 is the most common: colonies are large, low convex, rough in appearance and often oval, sometimes surrounded by a thin serrated skirt of growth. Type 2 colonies are small, domed and smooth and are described as coliform like. Colony type 3 and 4 are small and appear rough and rugose respectively. The mucoid alginate – producing type 5

		colony results in merging colonial growth after overnight incubation, the drawf colony type 6 is the smallest colony form. It has characteristic sweet musty odour and the distinctive blue- green appearance due to fluorescein and pyocyanin pigment On BA: Large, flat colonies showing haemodigestion. On CLED: Green with rough periphery.
Staphylococcus aureus	Gram positive, spherical cocci, 0.8-1 µm in diameter, non sporing , facultative anaerobe , non motile, except for rare strains non capsulated. They	On BA: Large, 2-4 mm diameter. Circular, smoothwith glistening surface, entire edge, soft butyrous consistence and opaque and pigments appearance. The pigmentation is golden yellow to cream coloured.

	are arranged in characteristics grape like clusters or in small groups, pairs, singles and short chain(less than five cocci in line).	Some strains are beta haemolytic when grown aerobically. On CLED: Small (pin head size), 0.1-0.5mm, pink or pink orange due to lactose fermentation. Some strains are non-lactose fermenting.
CONS	Morphologically similar to *Staphylococcus aureus*	On CLED: yellow to white colored colonies.

APPENDIX-VII

Table: Distinguishing reactions of the members of Enterobacteriaceae

Species	Test/ substrate											
	lac	Mot	gas	ind	VP	cit	PDA	ure	lys	H_2S	inos	ONPG
E. coli	+	+	+	+	-	-	-	-	+	-	-	+
Shigella groups A, B, C	-	-	-	±	-	-	-	-	-	-	-	-
Sh. sonnei	-	-	-	-	-	-	-	-	-	-	-	+
Salmonella (most serotypes)	-	+	+	-	-	+	-	-	+	+	±	-
S. typhi	-	+	-	-	-	-	-	-	+	+	-	-
S. paratyphi A	-	+	+	-	-	-	-	-	-	-	-	-
C. freundii	±	+	+	-	-	+	-	±	-	±	-	+
C. koseri	±	+	+	+	-	+	-	±	-	-	-	+
K. pneumoniae	+	-	++	-	+	+	-	+	+	-	+	+
K. oxytoca	+	-	++	+	+	+	-	+	+	-	+	+
E. aerogenes	+	+	++	-	+	+	-	-	+	-	+	+
E. cloacae	+	+	+	-	+	+	-	±	-	-	-	+
Hafnia alvei	-	+	+	-	+	-	-	-	+	-	-	+
Serratia marcescens[b]	-	+	±	-	+	+	-	-	+	-	±	+
P.mirabilis	-	+	+	-	±	±	+	++	-	+	-	-
P. vulgaris	-	+	+	+	-	-	+	++	-	+	-	-
M. morganii	-	+	+	+	-	-	+	++	-	±	-	-
Providencia rettgeri	-	+	-	+	-	+	+	++	-	-	+	-
P. stuartii	-	+	-	+	-	+	+	±	-	-	+	-
P. alcalifaciens	-	+	+	+	-	+	+	-	-	-	-	-
Yersinia enterocolitica[c]	-	-	-	±	-	-	-	±	-	-	±	+
Y. pestis	-	-	-	-	-	-	-	-	-	-	-	±

Y. pseudotuberculosis	-	-	-	-	-	-	-	+	-	-	-	±

[a] lac, inos, fermentation of lactose, inositol; mot, motility; gas, gas from glucose; ind, indole production; VP, Voges-Proskauer; cit, Citrate utilization (Simmons'); PDA, phenylalanine deaminase; ure, urease; lys, lysine decarboxylase; H_2S, H_2S produced in TSI agar; ONPG, metabolism of *o*-nitrophenyl-β-D-galactopyranoside.

[b] Some strains of Serratia marcescens may produce a red pigment

[c] *Yersinia* are motile at 22°C.

{Key: +, ≥85% of strains positive; -, ≥ 85% of strains negative; 16-84% of strains are positive after 24-48 hour at 36°C}

(Source: Collee *et al*, 1996)

APPENDIX-VIII

CALCULATION OF SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUES

Tests	True positive (a)	False positive (b)	False negative (c)	True negative (d)
WBC Counts	4	17	29	450
Epithelial cells counts	5	19	28	448
Albumin tests	7	32	26	435

CALCULATION OF SENSITIVITY
Sensitivity can be calculated as
Sensitivity = True positive (a)/ True positive (a) +False negative (c) x 100%

Sensitivity of WBC count = 12.12
Sensitivity of Epithelial cells count = 15.15
Sensitivity of Albumin Test = 21.12

CALCULATION OF SPECIFICITY
Specificity can be calculated as
Specificity = True negative (d)/ True negative (d) + False positive (b) x100%

Specificity of WBC count = 96.35
Specificity of Epithelial cells count = 95.93
Specificity of Albumin Test = 93.15

CALCULATION OF PREDICTIVE VALUE OF POSITIVE TEST (PPV)

Predictive value of positive Test (PPV) can be calculated as
PPV = True positive (a)/True positive (a) +False positive (b) x 100%

PPV of WBC Count = 19.05
PPV of Epithelial Cells = 20.83
PPV of Albumin Test = 17.95

CALCULATION OF PREDICTIVE VALUE OF NEGATIVE TEST (PPN)

Predictive value of negative Test (PPN) can be calculated as
PPN = True negative (d)/True negative (d) +False negative (c) x 100%

PPN of WBC Count = 93.95
PPN of Epithelial Cells =94.12
PPN of Albumin Test = 94.36