CHAPTER-I

1. INTRODUCTION

Urinary Tract Infection (UTI) is the commonest bacterial infection prevalent in both male and female. UTI comprise a wide variety of clinical entities due to microbial invasion of tissues lining the urinary tract extending from the renal cortex to the urethral meatus. UTI is also defined as the presence of bacteria undergoing active multiplication in urine within and into the urinary drainage system and presence of more than 10^5 organisms/ml in the mid-stream sample of urine is considered as significant (Leigh, 1990; Jha and Bapat, 2005).

Similarly, infection of the adjacent structure such as prostrate and epididymis is also included in the definition of UTI. Infection may be expressed predominantly at a single site, kidney i.e. pelvis and cortex (pyelonephritis), pelvis and ureter (pyelitis), ureter (ureteritis), bladder (cystitis), prostrate (prostatitis) and urethra (urethritis) but the entire urinary tract is always at a risk of invasion by bacteria, once any one of its part is infected. As urethra is the common site for urinary tract and genital tract, urethritis is also included in sexually transmitted disease especially if the infection is caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

The urinary tract consists of the kidneys, ureters, bladder and urethra. All areas of the urinary tract above the urethra in a healthy human are sterile (Forbes *et al*, 2002). Bacteria may gain access to the urinary tract by the ascending route or the haematogenous route. The ascending route of infection is now believed the usual one, by which bacteria from the faecal flora spread to the perineum before ascending into the bladder. The spread is encouraged by faecal incontinence in the infant and by sexual activity, and possibly poor hygienic habits, in the adult. Brooks *et al*. (2004) reported that urine secreted in the kidney is sterile unless the kidneys as well as other organ of

tracts are infected. The urethra, however, contains a normal microbial flora, which contaminates urine in passage, so that voided urine may contain small numbers of bacteria in the absence of UTI. Because it is necessary to distinguish contaminating from etiologically important organisms, only quantitative urine examination can yield meaningful results.

According to Leigh (1990), UTI is more common in women than in men, at least partially because of the short female urethra and its proximity to the anus. The exact prevalence of UTIs is age and sex dependent. During the first year of life, UTIs are more common in males. However the incidence of UTIs among males is low after age 1 and until approximately age 60 when enlargement of the prostate interferes with emptying of the bladders. Therefore, UTI is predominantly a disease of females. Fowler (1990) found that the prevalence of infection correlates directly with age, increasing from about 1% to 6% between puberty and 60 years. Approximately 95% of infections are urinary re-infections, most occur in individuals who are healthy, and few are the cause of serious illness. Abyad (1991) reported that the average prevalence of asymptomatic bacteriuria is 6% during pregnancy due to anatomical and physiological changes such as change in hormone level. It is an important risk factor for acute pyelonephritis, hypertension, preeclampsia, fetal wastage, low birth weight and prematurity.

UTI is a serious health problem affecting millions of people each year. It is one of the most important causes of mortality and morbidity in the world affecting all age groups across the life span. Each year, UTIs account for about seven million office visits and another one million emergency department visits, resulting in about 100,000 hospitalizations (Urology channel, 2006). UTI is one of the most common bacterial infections encountered in clinical practice in Europe and North America. It is estimated that 150 million cases of UTI occur on a global basis per year resulting in more than 4 billion pounds (6 billion dollars) in direct health care expenditure (Harding and Ronald, 1994).

UTIis the commonest disease among Nepalese population. According to the annual report (2005/2006) published by department of health services, morbidity of UTI in Nepal is 144143. Geographical distribution of UTI in Mountain, Hill and Terai regions of Nepal are 16,175, 78,052 and 49,911 respectively. The figure may change if adequate laboratory facilities are available. It is not possible to establish diagnostic laboratory throughout the country because of physical, economical and geographical circumstances and conditions.

Nepal being a developing country it has about 66% (MoPE,2001) people illiterate who are not aware on health matters and don't have idea of hygienic living habit, so are always in the threat of infections caused by different types of organisms. Due to the present condition of education and economy of Nepalese, many people are not aware of their health. Incomplete antibiotic therapy and lack of counseling with physician is a common problem. . 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). To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory (Chakraborty, 2003). Carelessness during stay at hospital also increases the risk of getting UTI.

Antimicrobial resistance is now recognized as an increasingly global problem which was observed for the first time in *Escherichia coli* in 1940. The primary factor responsible for the development and spread of bacterial resistance is the injudicious use of antimicrobial agents (Tenvor and Hughes, 1996; Urassa *et al*., 1997). Much of the increase is occurring in acute uncomplicated cystitis (AUC), an infection that has traditionally been simple to treat. The current trend of rising trimethoprim-sulphamethoxazole and beta-lactam resistance rates is problematic. Of more concern, however, are the emerging issues of fluoroquinolone resistance and multidrug resistance (MDR) among community-acquired urinary isolates (Gupta, 2003). Antimicrobial resistance has been associated with an increased rate of clinical failure, and reports from

Canada and the US indicate that the prevalence of Cotrimoxazole resistance exceeds 15.0% and can be as high as 25.0% (Blondeau, 2004). Prior antimicrobial drug exposure is a strong risk factor for infection with antibiotic resistant Gram-negative bacteria among patients with UTIs (Joshua *et al*., 2002). The defining criterion for MDR in this study was resistance to ≥3 of the antibiotics belonging to different structural classes (Tuladhar, 2001; Shehabi *et al*., 2002).

A thesis submitted to central department of microbiology by Bijaya Kumar Dhakal on 'Prospective study of UTI based on culture and direct microscopy of urine along with the antibiotic sensitivity test of urinary pathogens' (1999) showed 89.47% of isolate were Ampicillin resistant similarly 71.05%, 63.16%, 15.79% and 57.89% were resistant to Nalidixicacid, Cotrimoxazole, Nitrofurantoin and Norfloxacin respectively. Similarly another thesis submitted to the central department of microbiology by Saphala Dhital on 'determination of antibiotic resistant gram negative urinary pathogen in pediatric patients at Kanti children's hospital'(2001) showed 80% of gram negative bacteria were resistant to Ampicillin, 72% resistant to Nalidixicacid, 70% resistant to Cotrimoxazole and 54% resistant to Chloramphenicol.

Plasmids confer extra chromosomal drug resistance. R plasmids have been found responsible for encoding the genes against one and often several antimicrobial drugs (Chakraborty, 2002; Forbes *et al*, 2002; Brooks *et al*, 2004).

The emergence of antimicrobial resistance is a complex problem driven by many interconnected factors, in particular the use and misuse of antimicrobials. Antimicrobial use, in turn, is influenced by interplay of the knowledge, expectations and interactions of prescribers and patients, economic incentives, characteristics of the health system(s) and the regulatory environment. The intensity of antimicrobial use together with populations highly susceptible to infection creates an environment which facilitates both the emergence and transmission of resistant organisms. Antimicrobial resistance is a predictable outcome of antimicrobial use. Acquired antimicrobial resistance is a

growing worldwide problem due to the increasing use of antimicrobials in humans, animals, and agriculture. Culture and the susceptibility test only reveal the trend of bacterial sensitiveness and resistance toward the antibiotics. Plasmid profiling being an epidemiological marker is used to study the antibiotic resistance worldwide. This also reveals the relatedness between the strains of a species. This study also puts light on the mechanism by which they are transferring resistance to the sensitive ones. Our research will be especially carried out to isolate the multi-drug resistant urinary bacterial pathogen from urinary tract infected patients and to study the relatedness by plasmid profiling.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To determine prevalence of multidrug resistant bacteria causing UTI and perform plasmid profiling.

2.2 SPECIFIC OBJECTIVES

- To isolate and identify the bacteria causing UTI.
- To perform antibiotic susceptibility testing of the isolated bacterial pathogens.
- To determine the prevalence of multi-drug resistant bacterial pathogen causing UTI.
- To determine the most prevalent multi-drug resistant bacteria causing UTI.
- To perform conjugation and transformation on different MDR isolates of *E.coli*.
- To determine minimum inhibitory concentration of antibiotics against donors and transconjugants.
- To study the relatedness of most common multi-drug resistant bacterial pathogen by plasmid profiling.

CHAPTER-III

3. LITERATURE REVIEW

3.1 UTI

UTI simply means the presence of bacteria undergoing multiplication in urine within the urinary drainage system (Leigh, 1990). From a microbiological perspective, UTI exists when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney, or prostate. UTI can be defined as a spectrum of disease involving microbial invasion of any of the genitourinary tissues extending from the renal cortex to urethral meatus (Singh, 1991). Two consecutive cultures should yield the same organism in counts of $\geq 10^5$CFU/ml of urine to confirm asymptomatic UTI (Johnson, 1991). In most instances, growth of more than 10^5 organisms per milliliter from a properly collected midstream "clean-catch" urine sample indicates infection (Stamm, 2003).

Bacteruria, in a non-catheterised patient is defined as the presence of more than 10^5 colony forming units (CFU) /ml of urine, with no more than two species of organisms isolated in culture (Leone *et al*., 2004). Urethritis caused by chlamydiae and gonococci is not included in the definition because of their unique characters and strict localization to the urethra and genital system (Pokharel, 2004).

In order to confirm UTI with reasonable confidence, the criteria of clinical features, significant 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. In the diagnosis of UTI, it is important to differentiate contamination from significant bacteriuria. In the 1950s, Kass studied adult women and established a threshold of 100,000 CFU per ml in a voided specimen as the standard to define a positive urine culture (Kass, 1956). He demonstrated that the presence of more than 10^5

colony forming units of bacteria per ml of urine in a single specimen indicated bacteriuria with a probability of greater than 80% which could be increased to more than 90% or upto 99% when 2 or 3 consecutive specimens were examined (Kalpana and Walter, 2003).

Hence, culture of microorgansims should be quantitative. The microbial count has to be interpreted in relation to the urinary dilution. These are usually reported as number of colony forming units (CFU) per millilitre of urine (Sen *et al*., 2006). Growth of any number of bacteria from urine obtained by suprapubic aspiration is considered a significant finding. However, even in these cases, diagnosis of an acute UTI is not certain due to the issue of colonization of the urinary tract known as asymptomatic bacteriuria. The definition and significance of asymptomatic bacteriuria has been a controversial topic since its identification in the 1950s and description by Kunin and colleagues (Kunin, 1970).

The relationship of 10^5organisms/ml is not true in all circumstances. Under conditions of water loading, low urinary p^H, high urea concentration or osmolarity, for example, it is less valid. A high fluid intake increases the rate of bladder emptying and lowers the bacterial count (Leigh, 1990). Specific definitions exist for certain categories of patients, such as those with spinal injuries or patients who require long-term catheterisation. The National Institute of Disability and Rehabilitation Research recommends the use of the threshold value of $\geq 10^2$ CFU/ml to diagnose UTI from catheter specimens, from individuals on intermittent catheterization and $\geq 10^4$ CFU/ml for clean void specimens from catheter-free males, using clean intermittent self-catheterization. According to their recommendations, any detectable growth of uropathogens from indwelling catheters or suprapubic aspirate is considered significant (Biering-Sorensen *et al*., 2001).

The accuracy of the findings on a culture of a midstream, "clean-catch" specimen of urine depends on how a positive culture is defined. When the traditional criterion, 100,000 bacteria per milliliter, is applied to a voided urine sample, the specificity is high, but the sensitivity is only about 50 percent. Lowering the threshold to 1000 bacteria per milliliter in the cases of young women with symptoms of cystitis raises the sensitivity considerably, with minimal reduction in specificity (Stamm *et al*., 1982).

In a study of febrile children, Hoberman *et al.* (1994) noted that a high proportion (65%) of cultures with colony counts between 10,000 and 49,000 grew mixed or gram-positive organisms suggestive of contamination. In young children, urine is frequently obtained by catheter, and 10,000 CFU/ml has often been considered the cutoff for defining UTI (Subcommittee on UTI, 1999).

3.2 PATHOGENESIS RELATED TO UTI

The urinary tract should be viewed as a single anatomic unit that is united by a continuous column of urine extending from the urethra to the kidney. Bacteria can invade and cause UTI via two major routes: ascending and hematogenous pathways. The great majority of bacterial infections, whether or not with symptoms, occur in the bladder (cystitis) after the ascending migration of bacteria from the urethra or perineum. Infection of the kidney may follow the hematogenous spread of bacteria, but more often the organisms ascend from the bladder via the ureter and the renal pelvis and calyces (Leigh, 1990; Forbes *et al.*, 2002).

3.3 URINARY TRACT AND BACTERIAL MULTIPLICATIONN IN URINE

Urine secreted in the kidney is sterile unless the kidney is infected. Uncontaminated bladder urine is also normally sterile. The urethra, however, contains a normal microbial flora which contaminates urine in passage, so that void urine may contain small numbers of bacteria in absence of UTI (Brooks *et al.*, 2004).

Under normal conditions the urine is sterile, but the proximity of the urethral meatus to the anus means that, particularly in women, small numbers of potentially pathogenic organisms are constantly entering the urethra and bladder. Although the urethra has a resident bacterial flora, these organisms do not commonly cause bladder infection in normal persons (Leigh, 1990). Human urine can support the bacterial growth due to its favorable chemical composition (Chernew *et al.*, 1962).

Infection of urinary tract occurs when bacteria capable of proliferating in urine get access into the tract because human urine contains no humoral or cellular defenses against bacterial growth (Fowler, 1990). Normal urine, unlike other body fluids, does not contain significant quantities of lysozyme or immunoglobulin, and any complement present is inactivated. Increased concentrations of IgA and IgG (Jodal *et al.*, 1979) and, IgM are found in adults and children with UTI but this is probably only a result of proteinuria and leakage of inflammatory exudates.

Phagocytosis of bacteria is impaired both by the absence of opsonins and the wide range of osmolarity in urine (Chernew and Braude, 1962). Organic acid present in urine may be bacteristatic according to the degree of their dissociation urea in high concentration was shown to be bactericidal against urinary tract pathogens but it is likely that its effect is mainly due to an increase in osmolarity, because bacterial inhibition also resulted from the addition of salts to give hyperosmolality (Asscher *et al*., 1968).

In the urinary tract there is dynamic culture system in which bacteria undergo multiplication while urine is continuously being added by glomerular filtration and lost by micturation. The bacterial population will be controlled by its growth rate and the balance between the speed of urinary flow and the volume of the system. Normal urine usually contains sufficient glucose to support maximum growth rate and any lowering of the pH is prevented by its buffering capacity. Glucose provides source of energy for the growth of urinary pathogenic bacteria (Leigh, 1990).

3.4 PREDISPOSING FACTORS TO UTI

Gender and Sexual activity: Factors that may influence the risk of UTIinclude recent sexual intercourse, use of diaphragm with spermicide and delayed post-coital micturition. However, these factors have been identified primarily in small case–control studies reporting widely varying risk estimates (Adatto *et al*., 1979; Foxman *et al*., 1990). Rates of infection are high in postmenopausal women because of bladder or uterine prolapses causing incomplete bladder emptying; loss of estrogen with attendant changes in vaginal flora; loss of lactobacilli, which allows periurethral colonization with gram-negative aerobes, such as *E coli;* and higher likelihood of concomitant medical illness, such as diabetes (Nicolle and Ronald, 1987). However, the risk factors for healthy community- dwelling postmenopausal women have not been well described (Karlowsky *et al*., 2002). An important factor predisposing to bacteriuria in men is urethral obstruction due to prostatic hypertrophy (Stamm, 2003). During the neonatal period, infection is 3 to 5 times greater in males than in females (Bergstrom *et al*., 1968; Drew and Acton, 1976).

Pregnancy: Pregnancy causes anatomic and hormonal changes that favor development of UTI (Baron and Finegold, 1990). UTI during pregnancy pose particular risks for both mother and child. It increases the risk for premature birth, infant mortality and later chronic kidney disease. UTI occurring in the first and third trimester of pregnancy increase the risk for mental retardation and developmental delay in the infant from 1.2% to 2.0%. About 2.0-11.0% of pregnant women have asymptomatic bacteriuria in early pregnancy. The higher prevalence occurs in women of lower socioeconomic status and those with a past history of UTI. From 13.0% to 27.0% of women with asymptomatic bacteriuria in early pregnancy will experience acute pyelonephritis later in pregnancy (Nicolle, 1994). Infants of women who harbor *Ureaplasma urealyticum* also have increased risk for respiratory infections (Todar, 2002).). This predisposition to upper tract infection during pregnancy results from decreased ureteral tone decreased ureteral peristalsis and temporary incompetence of the vesicoureteral valves (Stamm, 2003).

Bacterial Virulence Factors: The usual initiating mechanism in bacterial infection involves bacterial adhesions to specific molecules on cell surfaces, followed by invasive disease. Initially, bacterial adhesion occurs via bacterial fimbriae in the case of gram negative bacteria, while gram positive bacteria adhere more frequently via extracellular polysaccaharides (Roberts, 1996). Adherence of bacteria to epithelial cells plays an important role in the colonization and infection of mucous membranes (Smith, 1977). It is suggested that an excretion of excess calcium in the urine may lead to increased bacterial adherence to uroepithelial in vivo and an increased potential for UTI (Apicella and Sobota, 1990).

The epithelial cells of the human urinary tract contain glycoconjugate receptors for a pathogenic bacterium. Most *E. coli* isolated from the urine of patients with acute pyelonephritis recognize and bind to human uroepithelial cell membrane glycolipids containing the sequence gal- alpha 1-4 gal. Not all strains of *E. coli* are equally capable of infecting the intact urinary tract. Bacterial virulence factors markedly influence the likelihood that a given strain once introduced into the bladder, will cause UTI. Most *E. coli* strains that cause symptomatic UTI in non-catheterized patients belong to a small number of specific O, K, and H serogroups (Stamm, 2003). Numerous investigations suggest that the strains of *E. coli* that causes UTI possess certain virulence factors that enhance their ability to colonize and invade the urinary tract. Some of these virulence factors include increased adherence to vaginal and uroepithelial cells by bacterial surface structures (adhesions, in particular, pili), a-hemolysin production and resistance to serum-killing activity (Forbes *et al*., 2002).

Uropathogenic *E. coli* (UPEC) causes 90.0% of UTI in anatomically-normal, unobstructed urinary tracts. The adhesin that has been most closely associated with uropathogenic *E. coli* is the P fimbriae. UPEC usually produce siderophores that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce hemolysins which are cytotoxic due to formation of transmembranous pores in host cells. Another factor thought to be involved in the

pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement-dependent bactericidal effect of serum (Todar, 2002).

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

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

Catheters and Hospitalizations: The urinary tract is the most common site of nosocomial infection (stamm, 1993). This is the most common nosocomial infection; 80% of infections are associated with the use of an indwelling bladder catheter (Emmerson *et al*., 1996). UTIs are the second most common nosocomial infections in ICUs in Europe and the first in the United States (Richards *et al*., 1999). Indwelling urinary catheters, which are used in 15% to 25% of short-term care patients during their hospitalization, confer a predisposition to bacteriuria (Saint and Chenoweth, 2003).

Most of these infections 66% to 86% follow instrumentation of the urinary tract, mainly urinary catheterization (Martin and Bookrajin, 1962). Catheter associated UTIs are

caused by a variety of pathogens, including *E.coli*, *Klebsiella* spp., *Proteus* spp., *Enterococcus*, *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., and *Candida* spp. Urinary tract pathogens such as *Serratia marcescens* and *Pseudomonas cepacia* have special epidemiological significance (Maki *et al*., 2000). Many of these organisms are part of the patients' endogenous bowel flora, but they can also be acquired by cross contamination from other patients or hospital personnel or by exposure to contaminated solutions or non- sterile equipments (McLeod, 1958; Selden *et al.*, 1971).

Over 20.0% of hospital-acquired infections are of urinary tract and about 75.0% of these follow the use of catheters in the urinary tract. Catheterized patients who develop diarrhea are nine times more likely to develop UTIs than are patients without diarrhea (Leigh, 1990). Bacteriuria develops in at least 10.0 to 15.0% of hospitalized patients with indwelling urethral catheters. The risk of infection is about 3.0 to 5.0% per day of catheterization (Stamm, 2003).

In a study by Tuladhar *et al.* (1990) in Tribhuvan university teaching hospital showed that UTIis the most prevalent nosocomial infection which accounted 62.7% of total nosocomial infection. Similarly *E.coli* was the dominant etiological agent (48%) followed by *Pseudomonas* spp. (25.8%), *Klebsiella* spp. (22.5%) and *Staphylococcus aureus* (3.2%) to cause nosocomial UTI. Adverse consequences include local and systemic morbidity, secondary bloodstream infection, death, a reservoir of drug-resistant microorganisms and increased health care costs (Saint *et al*., 2002). Among catheterized patients, clinical manifestations of UTI (pain, urgency, dysuria, fever, and leukocytosis) are uncommon even when bacteriuria or funguria is present and are no more prevalent with positive urine culture results than with negative results(Tambyah and Maki, 2000).

The current centres for disease control and prevention (CDC) definitions stratify nosocomial UTI into symptomatic, asymptomatic and other infections of urinary tract (Horan and Gaynes, 2004).

Kidney Stones: Kidney stones, in some cases, can cause obstruction followed by infection, particularly pyelonephritis. Symptoms of severe UTI in people with a history of kidney stones may indicate obstruction of the urinary tract, which is a serious condition. Formation of infectious urinary calculi is the most common complication accompanying UTI by members of the genus *Proteus* supported by other studies (Torzewska *et al*., 2003). Recent studies have shown that men have higher risk of forming renal stone than women (Yagisawa *et al.*, 1999).

In a study on bacteriology of urinary calculi in relation to UTI, out of 52 patients, 37.0% patients had calculi associated UTI with *E. coli* and *P. mirabilis* being the most common causative microorganisms (Nass *et al*., 2001). Kumar (2003) found that the prevalence of Renal Stone (RS) was higher without UTI (44.4%) than those with UTI (27.8%) in males. In case of females, the result showed 17.6% and 5.1% in cases with and without UTI.

Diabetes: UTI is an important clinical problem for people with diabetes. The number of bacteria in the urine of daibetic patients was significantly higher than in that of non diabetic controls due to high level of glucose. UTI is 2-3 times more common in adult diabetic patients than in non-diabetics (Leigh, 1990). There is an increased prevalence of asymptomatic bacteriuria in diabetic women, but not in diabetic men (Zhanel *et al*., 2000). On a population basis, diabetic women, depending on age, are 6-24 times more likely than non-diabetic women to be admitted for acute pyelonephritis; and diabetic men are 3.4-17 times more likely than their non-diabetic counterparts to be admitted for the same condition (Nicolle *et al*., 1996).The risk for symptomatic complicated UTIs may also be higher in people with diabetes. In fact, certain UTI-related abscesses are reported only in patients with diabetes. These patients are also at higher risk for fungal-related UTIs. The suggested mechanisms of an increased susceptibility to UTI are decreased antibacterial activity due to ‘sweet urine’, defects in neutrophil function and increased adherence to uroepithelial cells (Todar, 2002).

Renal transplantation: UTIs are the most common infections following renal transplantation. Their importance is debated. Some reports suggest that UTIs are mostly benign, while other suggests that they may induce graft loss. About 80.0% of patients with cellular rejection had a UTI, suggesting that UTI might trigger a graft rejection (Takai *et al*., 1998). UTI is an important cause of morbidity in renal transplant recipients. Around 50.0% of patients suffer from at least one episode of the infection during the first 6 months post transplant (Part *et al*., 1985). About 20.0% of UTIs occurs during the first year of transplantation. Female recipients have significantly more UTI than males (Russel *et al*., 2000).

Other factors: These include obstruction to free flow of urine due to tumor, stricture, stone or prostatic hypertrophy, neurogenic bladder dysfunction, vesicoureteral reflux (VUR) etc. (Stamm, 2003).

3.5 ETIOLOGICAL AGENTS OF UTI

Bacteria of only a limited number of species are able to initiate infection in the normal urinary tract, but members of many other species cause infection in patients with an abnormality of the urinary tract, in catheterized patients and in those receiving antimicrobial treatment (Forbes *et al*., 2002).

E. coli, the most common member of the family Enterobacteriaceae, accounts for 75–90% of all UTIs in both inpatients and outpatients (Gupta *et al*., 2002). In a study in India asymptomatic bacteriuria was observed in 10.57% with female preponderance over male. The maximum isolates were *E.coli* (32.8%). Followed by *Klebsiella pneumoniae* (22.4%) and *S. aureus* (15.1%) (Kumar *et al.*, 2002). In another study from Aurangabad, in a combined population group of indoor as well as outdoor patients, *Klebsiella* was found to be the commonest followed by *E.coli, P.aeruginosa* and *S.aureus* (Bajaj *et al*.,1999).

In an acute uncomplicated UTI, *E. coli* is the predominant organism causing the infection across all age groups. Other aerobic gram-negative rods such as *Klebsiella* spp., *Proteus* spp., *Citrobacter* spp., *Acinetobacter* spp., *Morganella* spp. and *P. aeruginosa* are also frequently isolated. Gram-positive bacteria such as *Enterococci* and *S. aureus*, as well as yeast are important pathogens in complicated UTI (Kalpana and Walter, 2003).

Sharma (1983) studied UTIof children admitted in Kanti Bal hospital whose age varies from 4 days to 14 years. During the study *E.coli* was found to be the commonest organism (48%) followed by *Klebsiella* spp. (19%), *Proteus* spp. (16%), *Streptococcus faecalis* (13%) and *Citrobacter* spp. (4%). *E.coli* (53.3%) was the commonest isolate followed by *Klebsiella* spp (16%), *Staphylococcus* spp. (12.1%), *Proteus* spp. (5.75), *P. aeruginosa* (5.7%), *Citrobacter freundii* (3.8%), *Streptococcus* spp. (1.8%), *Salmonella typhi* (0.9%), and *Providencia* spp. (0.9%) in a study by Manandhar (1996).

Prevalence of *E.coli* in urine was found much higher (52.54%) as compared to *Klebsiella* spp. (40.70%), *Proteus* spp. (6.76%) (Ghimire, 1995). Similar study done by Gautam in 1998 found *E.coli* as the most predominant pathogen (57%) followed by *Klesiella pneumoniae* (24%0 *Proteus* spp. (10%), *P. aeruginosa* (1.7%), *Salmonella typhimurium* (1.7%), *Shigella boydii* (1.7%), *Streptococcus faecalis* (1.7%) and *S. aureus* (1.7%).

Other less frequently isolated agents are Gram negative bacilli, such as *Acinetobacter* and *Alcaligenes* spp., other *Pseudomonas* spp., *Citrobacter* spp. and beta-hemolytic streptococci. Gram positive pathogens such as *Streptococcus faecalis*, *S. saprophyticus* and group B streptococci can also infect the urinary tract. UTIs due to *S. faecalis* are usually associated with the use of instruments or catheterization. Novobiocin resistant *S. saprophyticus* is a true primary pathogen of the urinary tract, which is responsible for 20% of urethritis and cystitis in sexually active but otherwise healthy young women (Leigh, 1990; Forbes *et al.*, 2002). *Salmonella typhi* and *Salmonella paratyphi* can be

found in the urine of about 25% of patients with enteric fever from the third week of infection (Cheesbrough, 2000). Candida infection may occur in diabetic and immunocompromised patients. Rarer infecting organisms include *Streptococcus agalactiae*, *Streptococcus milleri*, other Streptococci and *Gardnerella vaginalis* (Collins *et al*., 1986).

3.6 CATEGORIZATION OF UTI

Different forms of UTIs and their definition given by Wilhelm and Edson, 1987 are tabulated in table 1.

Table 1: Bcteriuric syndromes * and their definition

Syndromes	**Definition**
Lower UTI	Lower urinary tract symptoms (dysuria, urgency, frequency, suprapubic pain) and urine culture with $\geq 10^3$ bacteria /ml
Acute cystitis	Lower urinary tract symptoms and urine culture with $\geq 10^5$bacteria /ml
Acute urethral syndrome	Lower urinary tract symptoms and 10^2 to 10^5 bacteria /ml or sexually transmitted agent (e.g. Neisseria gonorrhoeae, Chlamydia trachomatis, herpesvirus) or no identifiable pathogen
Acute pyelonephritis	Upper urinary tract symptoms (fever, rigors, flank pain, nausea, prostration) and urine culture with $\geq 10^5$ bacteria/ml
Asymptomatic bacteriauria	No symptoms and urine culture with $\geq 10^5$ bacteria/ml
Recurrent bacteriuria	No symptomsor recurrent lower urinary tract symptoms and urine culture with $\geq 10^2$ bacteria/ ml
Relapse	Recurrent infection with same bacterial strain
Reinfection	Recurrent infection with different bacterial strain
Complicated bacteriuria	Urine culture with ≥105 bacteria/ml and associated structural abnormality of the urinary tract (e.g., involvement with urinary tract stones or catheter), may be asymptomatic.

*These syndromes are usually associated with pyuria (≥8 leucocytes/ mm^3 of uncentrifuged urine)

The classification of UTI given by Norrby (1990) is shown in table 2.

Table 2 Classification of UTI

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

Patients with significant bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria (Schaeffer, 1998).The presence of specific symptoms for UTI, including dysuria, frequency, urgency, suprapubic discomfort, and flank pain, should lead to screening. However, young children with UTI may present with nonspecific symptoms, such as poor feeding, vomiting, irritability, jaundice (in newborns), or fever alone, and a broader approach to screening may be appropriate (Subcommittee on UTI, 1999).

The magnitude of symptoms from unirnary tract infection may be more pronounce in men because of concurrent infection of the prostrate grand. In one study only 14 of 280 sexually active young men with symptoms suggestive of bacteriuria actually had UTI

(Barnes *et al.*, 1986). Hence, bacteriuria in men is almost always symptomatic and may lead to infection to prostrate, epididymis or testes (Fowler, 1990).

About 20 percent of young women with an initial episode of cystitis have recurrent infections. Occasionally, such recurrences are due to a persistent focus of infection, but well over 90 percent of recurrences in young women are episodes of exogenous reinfection, typically months apart (Nicolle and Ronald, 1987). Acute cystitis is a common, morbid, and costly medical problem of adult women (Stamm *et al.*, 1993; Foxman *et al*., 2000).

Complicated UTIs are those that occur in a patient who has a functionally, metabolically, or anatomically abnormal urinary tract or that are caused by pathogens that are resistant to antibiotics (Hooton and Stamm, 1991).

Infection in domiciliary practice are usually uncomplicated (Medical) and affects mainly women, the majority of whom are otherwise healthy. In hospital , on the other hand , infections are often complicated (Surgical) , and many are consequences of interference to the free flow of urine by residual inflammatory changes , obstructive uropathy , calculi, or neurological lesion of invasive procedures such as urinary tract investigation , surgical operations and particularly catheterization (Kunin, 1987; Leigh, 1990).

3.7 LABORATORY DIAGNOSIS OF UTI

A urine sample from patient with a suspected UTI is the most common type of specimen received by most clinical microbiological laboratories. The schedule for routine examination should therefore be carefully determined with a view to obtaining the necessary diagnostic information with the greatest possible economy of labour and resources (Collee *et al*., 1996).

3.7.1 Methods of Specimen Collection and Transport

Prevention of contamination by normal vaginal, perineal and anterior urethral flora is the most important consideration for collection of a clinically relevant urine specimen. 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 culture, microscopy and biochemical analysis. For the isolation and identification of bacteria in urine sample, the sample collection is very important. Generally, four types of urine samples are made available for laboratory investigation depending upon the situation/condition of the patient. They are mid-stream urine (MSU), straight catheterized urine, supra-pubic bladder aspirated urine and urine from indwelling catheter.

Most urine samples submitted for microbiological examination are in the form of MSU where it is hoped that the flushing action of urine will cleanse the urethra and limit contamination by urethral commensals (Gillespie, 1994). Although slightly more invasive, straight catheterization may allow collection of bladder urine with less urethral contamination but need a physician or other trained health professionals. If good aseptic techniques are used, supra-pubic bladder aspiration can be performed with little risk to get contamination-free urine in premature infants, infants, small children, and pregnant women and other adults with full bladders. In catheterized patients, urine should be collected directly from the catheter and not from the collection bag because organisms can multiply there, obscuring the true relative numbers (Forbes *et al.*, 2002).

Since urine itself is a good culture medium, all specimens should be processed by the laboratory within 2 hours of collection, or be kept refrigerated at 4^0C until delivery to the laboratory and processed no longer than 18 hours after collection (Vandepitte *et al*., 2003).Transport medium that can be used for urine specimens are 1.8% boric acid, sodium chloride or polyvinylpyrolidine (Pokharel, 2004).

3.7.2 Screening Procedures

As many as 60.0-80.0% of all urine specimens received for culture may contain no etiologic agents of infection or contain only contaminants. A wide range of screening techniques has been developed for detection of UTI so that time, reagents and money of the laboratory is saved. Of these, the Gram stain is the easiest, least expensive, and probably the most sensitive and reliable screening method (Forbes *et al.,* 2002).

3.7.3 Urinalysis

A urinalysis involves a physical and chemical examination of urine. In addition, the urine is centrifuged to allow sediments containing blood cells, bacteria, and other particles to collect. This sediment is then examined under a microscope. A urinalysis includes the observation of the urine for color, cloudiness, acidity and white blood cells (WBC) counts. A high WBC count in the urine is referred to as *pyuria*. Pyuria is usually sufficient for a diagnosis of UTI in non-hospitalized patients if well correlated with standard symptoms (or just fever in small children). Microscopy is a valuable adjunct in the diagnosis of urinary infections (Gillespie, 1994). Alongside bacteriuria, finding of significant pyuria is of great importance for UTI diagnosis and it strengthens further the microscopic diagnosis, while RBCs and epithelial cells are of very poor significance for UTI diagnosis (Merila *et al.,* 1987).

Pus cells: These are round 10-15 µm in diameter cells that contains granules. In urinary infections they are often found in clumps. Normal urine may contain a few white cells (<5/HPF) (Cheesbrough, 1984). The visualization of leucocytes, principally neutrophils, is indicative 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 urethrea or may be due to dehydration, stress and fever (Godkar, 2001). Pyuria is considered significant if ≥5 pus cells are seen per HPF in urine sediments.

Red blood cells (RBCs): Red blood cells are found in small numbers in normal urine. In normal male and female, occasional red cells (0-2/HPF) may be seen on microscopic examination of the sediment. The finding of RBC counts ≥3/HPF is considered as abnormal (Froom *et al*., 1986; Wargotz *et al.*, 1987). Haematuria may be found in urinary schistosomiasis, bacterial infections, acute glomerulonephritis, sickle cell disease, leptospirosis, infective endocarditis, calculi (stones) in the urinary tract, malignancy of the urinary tract and hemorrhagic conditions. The number may exceed during renal disease, post streptococcal glomerulonephritis, lower urinary tract disease, other disease including appendicitis, salphingitis, malaria, sub-acute bacterial endocarditis etc. (Cheesbrough, 1984).

Epithelial cells: It is normal to find a few epithelial cells in urine. These cells are nucleated and vary in size and shape. When seen in large number, however, they usually indicate inflammation of the urinary tract or vaginal contamination of the specimen (Cheesbrough, 1984). Wargotz *et al*. (1987) reported that greater than or equal to five squamous epithelial cells per high power field is considered as abnormal. Normally few cells (3-5/HPF) from genitourinary tract can be found in urine due to sloughing off of old cells. 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).

3.7.4 Chemical Examination of Urine

Chemical tests for bacteriuria are of value in large population-screening programmes. These include detection of protein and glucose in the urine, nitrate reductase (Greiss) test, leukocyte esterase test and triphenyltetrazolium-chloride reduction test (Leigh, 1990). 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, vaginal secretions or smegma. Whilst it is an indicator of renal disease, detection of the 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.

3.7.5 Bacteriological Examination of Urine

Bacteriological culture of the urine is the only accurate way of diagnosing bacteriuria. Quantitative or semi-quantitative techniques depending upon the resources of the laboratory are to be preferred. 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, 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 (Leigh, 1990).

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 it over a plate of 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 0.001 ml loopful of urine yields 400 colonies, the count per ml will be 10^5, or just indicative of significant bacteriuria (Collee *et al*., 1996).

3.7.6 Antibiotic 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. As antibiotics are concentrated in urine to higher levels than are found in the tissues, high-content test discs should be used.

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 (2001), 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.

World Health Organization (WHO) recommended modified Kirby- Bauer disk diffusion technique is used by most laboratories to test routinely for antimicrobial susceptibility. Using this test, antimicrobial resistance is detected by allowing the antibiotics to diffuse from a point source, commonly in the form of an impregnated filter paper disc, into an agar medium that has been seeded with the test organism. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level for the test strain. Following incubation, the diameter of the zone of inhibition around each disc is measured in millimeters (Collee *et al*., 1996).

3.8 BACTERIAL RESISTANCE TO ANTIBIOTICS

Resistance can be defined as the temporary or permanent ability of an organism and its progeny to remain viable or multiply under environmental conditions that would destroy or inhibit other cells (Denyer *et al*., 2004).

Antibiotic resistance is an emerging problem worldwide. It is true to say that early treatment failures with antibiotics did not represent a significant clinical problem because other classes of agents, with different cellular targets, were available. It is the emergence of multiple resistances, i.e. resistance to several types of antibiotic agent that is causing major problems in the clinical practice today. Several factors drove this situation in the 1970s and 1980s, including the introduction of extended-spectrum agents and advances in medical techniques, for example, in organ transplantation and cancer chemotherapy. The net result has been a huge selective pressure in favor of multiple resistant species. Notable Gram positive organisms include Methicillin-resistant *Staphylococccus aureus* (MRSA) and coagulase-negative staphylococci (CONS), Glycopeptide-moderate sensitive *S. aureus* (GISA), Vancomycin-resistant *Enterococcus* (VRE) species and Penicillin non-susceptible *Streptococcus pneumoniae* (PNSSP). Concerns among the Gram negative organisms include multidrug-resistant *P.*

aeruginosa, *Stenotrophomonas maltophila* and *Acinetobacter baumannii* and members of the Enterobacteriaceae with extended-spectrum beta-lactamases (ESBLs) (Smith, 2004).

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

Drug resistance may be a natural or an acquired characteristic of a microorganism.

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

Acquired resistance: Acquired drug resistance may result from mutation, adaptation or gene transfer. Spontaneous mutations occur at low frequency. Rapid mutation can occur and there is clearly a heavy selective pressure resulting from the overuse of antibiotics in medical practice. The probability that a mutation arises will be proportional to the number of target sites within the gene. In *E. coli*, mutations in the *gyr*A gene, encoding the Gyr A subunit of topoisomerase II and leading to fluoroquinolone resistance have been identified in at least seven locations in the *par*C gene, encoding a subunit of topoisomerase IV, have been observed. As a consequence, the prediction that the

mutation rate would be higher in *gyr*A than *par*C is correct. Indeed, the opposite is true for fluoroquinolone resistance in *S. pneumoniae* (Todar, 2002; Smith, 2004).

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

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

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

Mechanism of Antimicrobial Resistance

There are many different mechanisms by which microorganisms might exhibit resistance to drugs.

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.

Permeability of the Outer Membrane: The complex structure of the outer envelope of Gram-negative bacteria ensures that they are intrinsically less sensitive than Gram-positive bacteria to a variety of antibiotics such as Benzylpenicillin, Methicillin, Macrolides, Lincomycin, and Vancomycin.

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).

Multiple drug resistance

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

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

Mobile gene cassettes and integrons: Many Gram negative resistance genes are located in gene cassettes. One or more of these cassettes can be integrated into a specific position on the chromosome termed as integron. Thus, integrons are genetic elements that recognize and capture multiple mobile gene cassettes (Smith, 2004). Although integrons by themselves are not mobile, due to their presence in plasmids and transposons, they can be transferred horizontally. Integrons for these reasons a major mechanism for the spread and maintenance of MDR (Fluit *et al*., 2000).

Chromosomal multiple-antibiotic resistance (Mar) locus: The multiple-antibiotic resistance (*mar*) locus was first described in *E. coli* by Stuart Levy and collegues at

Tufts University and has since been recognized in other enteric bacteria. The locus consists of two divergently transcribed units, *mar*C and *mar*RAB (Smith, 2004).

Multidrug efflux pump: A multidrug efflux pump can excrete a wide range of compounds where there is often little or no chemical similarity between the substrates (Denyer *et al*., 2004).

3.9 GLOBAL SCENARIO

Antimicrobial resistance is now recognized as an increasingly global problem which was observed for the first time in *E. coli* in 1940. The primary factor responsible for the development and spread of bacterial resistance is the injudicious use of antimicrobial agents (Urassa *et al.,* 1997).

The clinical management of UTI is complicated by the increasing incidence of infections caused by strains of *E. coli* that are resistant to commonly used antimicrobial agents. In studies in the US, the rates of resistance to Trimethoprim–Sulfamethoxazole among *E. coli* isolates from women with UTIs ranged from 15.0-18.0 % (Kahlmeter, 2000).

Although UTI is not usually thought of as a disease associated with community-wide outbreaks, certain multidrug-resistant, uropathogenic lineages of *E. coli* have exhibited epidemic behavior. *E. coli* O15:K52:H1 caused an outbreak of community-acquired cystitis, pyelonephritis, and septicemia in South London in 1987 and 1988 (Phillips *et al*., 1988) and is an endemic cause of UTI in Barcelona, Spain (Prats *et al*., 2000).

With the possible exception of Nitrofurantoin, each antimicrobial class introduced for the treatment of UTI over the decades has encountered problems with resistance. In the last decade, resistance to TMP/SMX has reached levels of clinical significance. In the last 30 years, TMP/SMX resistance rates have increased from nearly 0% to roughly 18.0% across the United States. Resistance to Ciprofloxacin may follow a similar trend. In less than 20 years, Ciprofloxacin resistance rates among UPEC have increased to 2.5-

5.0% in the US. The increasing prevalence of resistance has had an impact on antibiotic prescribing (Stamm, 2003).

Resistance of bacteria to drugs used to treat AUC is a problem in countries outside the US. Particularly high rates of resistance to Cotrimoxazole have been reported in Israel (31.0%), Spain (32.0%) and Bangladesh (60.0%). Although the prevalence of resistance to Ciprofloxacin and other fluoroquinolones has generally remained low, it has reached 18.0% in Bangladesh and 4.0% in Israel. Resistance to Norfloxacin is 13.0% in Spain (Gales *et al*., 2000).

In a study conducted during 1999 in US hospitals, the percentage of strains of each species exhibited an MDR phenotype were 1.7% for *E. coli*, 3.0% for *K. pneumoniae* and 7.7% for *Proteus mirabilis*. For *E. coli* and *K. pneumoniae*, the most prominent MDR phenotypes were resistance to Cephalothin, Cotrimoxazole and Ciprofloxacin. For *Proteus mirabilis*, the prominent MDR phenotype was resistance to Ciprofloxacin, Cotrimoxazole and Nitrofurantoin (Selman *et al*., 2000). Perhaps the most significant change in resistance among uropathogens has been the increase in the prevalence of resistance to Trimethoprim–Sulfamethoxazole (TMP–SMX), the current drug of choice in the United States for empirical therapy for uncomplicated UTI in women. In addition, TMP–SMX resistance has been associated with concurrent resistance to other antibiotics, resulting in multidrug-resistant uropathogens (Sahm *et al*., 2001).

In many locales of northern Israel, the prevalence of resistance to Trimethoprim-Sulfamethoxazole (TMPSMZ) among uropathogens exceeds the 10 to 20% threshold at which authorities suggest using alternative empirical regimens, such as a fluoroquinolone, Nitrofurantoin, or Fosfomycin (Raz *et al.*, 2002).

Increasing resistance to Ampicillin and Trimethoprim/sulfamethoxazole in *E. coli*, the main causative pathogen of UTIs (UTIs), has been demonstrated in urinary tract isolates obtained from patients visiting their general Practitioners (Kahlmeter G, 2000). Prior

antimicrobial drug exposure is a strong risk factor for infection with Trimethoprim–Sulfamethoxazole-resistant Gram-negative bacteria among patients with UTIs (Joshua *et al.,* 2002).

The published in vitro surveillance data from centers across the United States and Canada indicate that approximately 10 to 25% of urinary tract isolates of *E. coli* from female outpatients are resistant to Trimethoprim-Sulfamethoxazole (SXT) (Karlowsky *et al.,* 2002).

An understanding of the microbiological basis for the emergence of antibiotic resistance among pathogenic *E. coli* strains is needed to guide efforts to interrupt this process. At this time, it is unknown to what extent today's drug-resistant clinical *E. coli* strains are simply antibiotic-resistant versions of the same virulent clones of extraintestinal pathogenic *E. coli* that constitute most of the susceptible bacterial population (Johnson, 1991).

The levels of antibiotic resistance in Dakar appeared dramatic and worrisome with resistance rates ranging from 18.6% for fluoroquinolones to 73.6% for Ampicillin (Tenvor and Hughes, 1996).

Much of the data is available for community acquired infections. This may be different from that of hospital acquired infections. Since patterns of antibiotic resistance in a wide variety of pathogenic organisms may vary even over short periods and depend on site of isolation and on different environments, periodic evaluation of antibacterial activity is needed to update this information. (Jones and Thornsberry, 1982).

In a study of urinary isolates among indoor patients, high percentage of strains showed resistance to Cotrimoxazole. First generation cephalosporins were effective for *E.coli*, while newer Aminoglycosides like Amikacin and third generation Cephalosporins were found to be effective against *K. pneumoniae* and *P.aeruginosa,* and ineffective against

Acinetobacter spp.. Amongst the outdoor patients, more than 50% patients showed *E.coli* as the commonest isolate which was 70%-80% resistant to Cotrimoxazole and Aminopencillin, however, first generation Cephalosporins, Nitrofurantoin and Norfloxacin were effective but in cases where UTI was associated with agents other than *E.coli,* Amikacin and third generation Cephalosporins were found to be effective (Gupta *et al.,* 2002).

Among urinary isolates of *E. coli* from female outpatients in the United States, national resistance rates to SXT were relatively consistent (14.8 to 17.0%) from 1995 to 2001 but demonstrated considerable regional and institutional variation in 2001. Fluoroquinolone resistant isolates of *E. coli* from urine were frequently multidrug resistant (Karlowsky *et al*., 2006).

A Canadian National Surveillance Study showed that Ampicillin, Cotrimoxazole, Mecillinam, Nitrofurantoin and Ciprofloxacin mean resistance rates for 2,000 urinary tract isolates collected from outpatients across Canada in 1998 were 41.1%, 19.2%, 14.7%, 5.0% and 1.8% respectively. For *E. coli* isolates alone (n=1,681), comparable rates were 41.0%, 18.9%, 7.4%, 0.1% and 1.2 % respectively. The majority of *E. coli* isolates resistant to Ampicillin, Cotrimoxazole or Ciprofloxacin were susceptible (MIC, <16µg/ml) to Mecillinam (Zhanel *et al*., 2000).

In continuous surveillance of routine samples from five Dutch laboratories to study resistance to the antibiotics most commonly prescribed for UTI in the Netherlands, namely Norfloxacin, Amoxicillin, Trimothoprim and Nitrofurantoin from 1989 to 1998 in >90000 *E. coli* isolates; it was found that resistance to Norfloxacin increased from 1.3% in 1989 to 5.8% in 1998 (Goettsch *et al.*, 2000).

The analysis of all pertinent results in the Surveillance Network Data-base-USA from 1 January to 30 September 2000 found that 7.1% of *E. coli* was MDR. Among the MDR isolates, 97.8% were resistant to Ampicillin, 92.8% to Cotrimoxazole, 86.6% to

Cephalothin, 38.8% to Ciprofloxacin and 7.7% to Nitrofurantoin. Rates of MDR were demonstrated to be higher among males (10.4%) than females (6.6%), among patients >65 years of age (8.7%) than patients <17 (6.8%) and 18 to 65 (6.1%) years of age, and among inpatients (7.6%) than outpatients (6.9%) (Sahm *et al.*, 2001).

The analysis of susceptibility testing data from the Surveillance Network Database-USA (n=286,187) from 1995 to 2001 found out that the resistance rates among *E. coli* isolates to Ampicillin range, 36.0 to 37.4% per year. Similarly Cotrimoxazole range, 14.8 to 17.0%, Ciprofloxacin range, 0.7 to 2.5%, and Nitrofurantoin range, 0.4 to 0.8% per year that varied only slightly over this 7-year period. It was found that in 2001, Cotrimoxazole resistance among *E.coli* isolates was >10% in all nine US Bureau of the Census regions (Karlowsky *et al*., 2002).

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

The ECO.SENS study done at 252 community healthcare centres in 16 countries in Europe plus Canada showed that resistance in *E. coli* occurred most frequently to Ampicillin (30.0%) and Sulphonamides (29.0%), followed by Trimethoprim (15.0%), Cotrimoxazole (14.0%) and Nalidixicacid (5.0%) but was low to Co-amoxiclav, Mecillinam, Cefadroxil, Nitrofurantoin, Fosfomycin, Gentamicin and Ciprofloxacin, all at <3.0% (Kahlmeter, 2003).

A retrospective study on all of the bacterial strains isolated from the urine of outpatients who attended the Pasteur Institute of Bangui with a suspected UTI between January 2000 and April 2002 found that more than 84.0% of isolates were

Enterobacteriaceae: *E. coli* (55.6%), *K. pneumoniae* (16.9%), *Citrobacter diversus* (4.2%), *Salmonella* spp. (3.5%) and other Enterobacteriaceae (4.2%). A high percentage of the Enterobacteriaceae were resistant to Amoxicillin and Cotrimoxazole although most remained susceptible to Ciprofloxacin (Hadiza *et al*., 2003).

In a prospective, multicenter study conducted between March and July 2002 in 15 microbiology laboratories located in nine autonomous regions of Spain, the susceptibility rates of *E. coli* were 97.9% for Fosfomycin, 95.8% for Cefixime, 94.3% for Nitrofurantoin, 90.8% for Amoxicillin-Clavulanic acid and 77.2% for Ciprofloxacin. Overall fluoroquinolone resistance was near 23.0%, but this rate varied significantly according to sex, age, type of urinary infection and geographic region (Andreu e*t al*., 2005).

The study performed with isolates from community-acquired UTIs collected from 15 centres representing six different geographic regions of Turkey showed that *E. coli* was the causative agent in 90.0% of the uncomplicated UTIs and in 78.0% of the complicated UTIs ($p<0.001$). About 17.0% of *E. coli* strains isolated from uncomplicated cases and 38.0% of *E. coli* strains isolated from complicated UTI were found to be resistant to Ciprofloxacin (Arslan *et al.*, 2005).

A study done in various geographic regions in the US and Canada revealed that out of 1142 *E. coli* isolates, resistance rates were: Ampicillin (37.7%), Cotrimoxazole (21.3%), Ciprofloxacin (5.5%), Levofloxacin (5.1%) and Nitrofurantoin (1.1%). This study reported higher rates of antibiotic resistance in US versus Canada outpatient urinary isolates (Zhanel *et al.*, 2005).

An Italian study conducted during 2004 revealed that the overall prevalence of *E. coli* was 85.3%. *K. pneumoniae, S. saprophyticus, P. mirabilis*, *E. faecalis* and other rare species were far less represented. Determination of the antibiotic susceptibility pattern of the entire collection of *E. coli* (512 organisms) revealed that among the drugs

analyzed Ampicillin was the least active molecule with only 62.5% of the strains being inhibited. Amoxicillin-Clavulanate and Cefuroxime displayed a higher potency 87.7% and 89.2% repectively. Nitrofurantoin (96.7%) and Fosfomycin (98.6%) were the most potent drugs (Fadda *et al*., 2005).

A study carried out from 1998 to 2003 in Manisa in the western part of Turkey, the range of resistance of *E. coli* to Ampicillin was found to be 47.8 to 64.6% and that to Cotrimoxazole was 37.1 to 44.6% during the study period. About 24.5% of isolates of *E. coli* (216 of 880) were found to be MDR. Among the MDR isolates, 100.0% were resistant to Ampicillin and Cotrimoxazole, 97.2% to Amoxicillin-Clavulanate, 87.5% to Cefazolin, 80.6% to Ciprofloxacin, 74.1% to Gentamicin, 33.3% to Nitrofurantoin and 30.6% to Cefuroxime. MDR ratios were found to be 19.6% in 1998, 21.5% in 1999, 25.0% in 2000, 29.2% in 2001, 26.8% in 2002 and 27.7% in 2003 (Kurutepe *et al*., 2005).

A prospective clinico-microbiological study including all clinically diagnosed patients with community acquired acute cystitis attending a tertiary care teaching hospital over a period of 3 years was conducted and >35.0% of the urinary *E. coli* isolates were resistant to the fluoroquinolones, which were found to be the most commonly used empirical antibiotics in acute cystitis. Resistance was minimum against Nitrofurantoin (9.3%) and Amikacin (11.0%). More than 80.0% of the fluoroquinolone-resistant strains were found to be sensitive to Nitrofurantoin (Biswas *et al*., 2006).

3.10 NEPALESE SCENARIO

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

Cotrimoxazole and Ciprofloxacin respectively. Similarly 94.5%, 60.0%, 38.0%, 44.0% and 0% of the isolated *Klebsiella* spp. were found to be resistant to same antibiotics respectively (Ghimire, 1995).

E. coli was found as the most predominant pathogen (57.0%) followed by *K. pneumoniae* (24.0%), *Proteus* spp. (10.0%), *P. aeruginosa* (1.7%), *Salmonella typhimurium* (1.7%), *Shigella boydii (*1.7%), *Streptococcus faecalis* (1.7%) and *S. aureus* (1.7%). In vitro susceptibility test of these pathogens showed that almost all isolates were susceptible to Nitrofurantoin (88.0%), followed by Ciprofloxacin (81.0%), Nalidixicacid (69.0%) and Chloramphenicol (60.0%) whereas Cotrimoxazole and Amoxicillin were least effective antibiotics against these bacterial isolates (Gautam , 1997).

E. coli (47.4%) was the most predominant bacteria followed by *Klebsiella* spp. (13.2%), *S. aureus* (10.5%) and *P. aeruginosa* (7.9%). In vitro susceptibility test showed that Nitrofurantoin (84.2%) was only the effective drug followed by Norfloxacin (28.9%) and Ampicillin (10.5%) against the bacterial isolates (Dhakal, 1999).

E. coli was the most predominant pathogen (78.0%) followed by *K. pneumoniae* (9.0%), *Proteus* mirabilis (2.0%), *P. aeruginosa* (2.0%), *Citrobacter* spp. (2.0%) and *Enterobacter* spp. (1.0%). With regards to antibiotic susceptibility pattern, 80.0% of the Gram negative bacteria were resistant to Ampicillin, 72.0% to Nalidixic acid, 70.0% to Cotrimoxazole and 54.0% to Chloramphenicol. Norfloxacin (73.0%) was most active quinolone; while resistance to Amikacin was 29.0%. Overall resistance to Ciprofloxacin, Nitrofurantoin and Gentamicin was 32.0% (Dhital, 2000).

Rai *et al*. (2000) found that *E. coli* (61.8%) was the most predominant pathogen followed by *K. pneumoniae* (12.2%) and *S. aureus* (12.2%). With regards to antibiotic susceptibility pattern, Cephalexin (100.0%) was the most effective drug for Gram positive bacteria, followed by Nitrofurantoin (93.8%), Ciprofloxacin (85.7%),

Cotrimoxazole (50.0%) and Norfloxacin (50.0%). Likewise, Nitrofurantoin (77.3%) was the drug of choice in UTI for Gram negative bacteria, followed by Gentamicin (59.1%) and Cotrimoxazole (40.9%).

Tuladhar (2001) reported that in 1947 urine specimens, culture positive were found in 517 (26.6%) of which MDR bacterial strains were detected in 122 (23.6%) cases in which *E. coli* 72 (13.1%), *Klebsiella* spp. 20 (3.9%) and *S. aureus* 13 (2.1%) were the predominants. Out of 1479 urine specimens of hospitalized patients, there were 230 culture positive cases of which MDR bacterial strains were detected in 81 (35.2%) cases in which the most predominants were *E. coli* 51 (22.2%), *Klebsiella* spp. 14 (6.1%) and *S. aureus* 5 (2.2%).

E .coli was the most common isolate accounting for 77.5% of all bacterial isolates and was followed by *Proteus* spp., *Klebsiella* spp. *and Staphylococcus* spp. Ciprofloxacin was found to be most effective antibiotic against *E. coli* followed by Nalidixic acid. *Proteus* spp. was 100.0% susceptible to Nalidixic acid and Gentamicin. *Saphylococcus* spp. was susceptible to Nitrofurantoin (100.0%), Cotrimoxazole (100.0%) and Norfloxacin (60.0%) (Chhetri *et al*., 2001).

In a study done at NPHL, it was found that urine samples of kidney transplant patients showed 15.0% positive growth. *E. coli* (46.7%) was the most predominant bacteria causing UTI followed by *Klebsiella* spp. (13.3%), *Pseudomonas* spp. (13.3%), *S. aureus* (13.3%), *Proteus* spp. (3.3%), *Citrobacte*r spp. (3.3%), *Streptococcus faecalis* (3.3%) and *M. morganii* (3.3%). Gentamicin and Amikacin (100.0%) were the most effective drugs against Gram negative bacteria (Ghimire *et al.*, 2004).

Karki *et al.* (2004) showed that five bacteria isolated were *E. coli* (33.3%), *Proteus* spp. (27.7%), *Klebsiella* spp. (16.6%), *S. aureus* (8.8%) and *P. aeruginosa* (1.1%). Nitrofurantoin was the most effective drug against all bacterial isolates.

In a study done at Kathmandu Model Hospital, it was found that the predominant bacteria causing UTI were the Gram negative isolates constituting 88.2% among them 67.9% were MDR strains whereas Gram positive bacteria constituted only 11.8% out of which 38.9% were MDR strains (Shrestha, 2005).

Similarly a study carried out at TUTH, *E. coli* was the most common isolate accounting for 61.2% of all bacterial isolates followed by *Klebsiella* spp. (9.2%), *P. aeruginosa* (7.1%), *Enterococcus faecalis* (6.1%), *S. aureus* (6.1%) and *Proteus mirabilis* (4.1%). Gram negative bacilli showed best susceptibility towards Ceftazidime (80.7%) followed by Nitrofurantoin (79.5%). Multidrug resistance (MDR, resistant to two or more than two classes of antibiotics) was observed in 68.4% of the total isolates and it was 61.7% in case of *E. coli* and 66.7% in *S. aureus* (Manandhar, 2005).

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

In a study done at NPHL, *E. coli* was the most common isolate accounting for 43.3% of all bacterial isolates followed by *S. aureus* (23.3%) and *Klebsiella* spp. (16.6%). Amoxycillin was found to be most effective antibiotic against *E. coli* followed by Nalidixic acid and Nitrofurantoin. Similarly, Ampicillin was found to be the most effective antibiotic against *S. aureus* whereas amoxycillin and Norfloxacin were equally effective against *Klebsiella* spp. (Jha and Bapat, 2005).

In a study carried out by Shrestha (2007) at NPHL, multidrug resistance (MDR) was observed in 45.0% (36/80) of total bacterial isolates. Multidrug resistance was found to be 51.3% (20/39) in *E. coli* and that in *K. pneumoniae* was 33.3 % (5/15). Higher rate of MDR

was found in males (57.7%, 15/26) than in females (38.8%, 21/54). Among the MDR *E. coli* isolates, 100.0%, 90.0% and 65.0% were resistant to Ampicillin, Norfloxacin and Cotrimoxazole respectively. Among the MDR *K. pneumoniae* isolates, 100.0% were resistant to Ampicillin, Cotrimoxazole and Norfloxacin.

3.11 ELECTROPHORESIS

The term electrophoresis describes the migration of a charged particle under the influence of an electric field. Under the influence of an electric field these charged particles will migrate either to the cathode or to the anode, depending on the nature of their net charge (Wilson and Walker, 2000).

3.12 PLASMID PROFILING

In a study of plasmid analysis of representative *E. coli* isolates showed the presence of a wide range of plasmid sizes, and there were no consistent relationships between plasmid profiles and resistance phenotypes. A large, transferable R-plasmid of 28 kb was found in most *E. coli* isolates (67%) that were resistant to at least Ampicillin, Cotrimoxazole and Tetracycline. This R-plasmid reservoir may contribute to the spread of multiple antibiotic resistances. These R-plasmids were found in *E. coli* isolates from both hospitalized patients and non-hospitalized patients at similar rates (47% and 53% respectively) (Shehabi *et al.*, 2002).

In a study done by Aibinu (2007) in multidrug resistant E. coli, Plasmid analysis of isolates showed detectable plasmids with size ranging between 21, 771 – 27, 444kb.

Low-level quinolone resistance was found to be transferable by a plasmid discovered in a clinical isolate of *K. pneumoniae* from Birmingham, Ala.. The gene responsible, named *qnr*, proved to be distinct from previously identified quinolone resistance genes (Tran and Jacoby, 2002).

In a study done by Georgnaras *et al.* (2001) showed that all (50) but one of the strains contained between one and six plasmids, with sizes ranging from 1.5 to 89 kb. One, two, or four plasmids were harbored by almost equal proportions of the strains (24, 28, and 24%, respectively).

Shears and others found that the resistance transfer pattern in Enterobacteriaceae among Sudanese children was most commonly mediated by plasmids. In a recent study of *E. coli* isolated from patients with UTIs in the Sudan, the most common resistance pattern—to Ampicillin, Tetracycline, and Chloramphenicol, observed in 94%, 85%, and 54% of the isolates respectively—was found to be transferable by a plasmid (Musa and Shears, 1998).

CHAPTER-IV

4. MATERIALS AND METHODS

This study was conducted at Kathmandu Model Hospital and Central Department of Microbiology, Tribhuvan University, Kirtipur from May 2007 to January 2008. During this period, a total of 710 mid stream urine samples from patients suspected of UTI were collected and processed according to the standard laboratory methods. Plasmid extraction and analysis of MDR strains were carried out in the research laboratory of central department of microbiology.

4.1 MATERIALS

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

4.2 METHODS

4.2.1 Urine sample collection

The patients were given a clean, dry and sterile and leak proof container and requested for 5-10 ml mid-stream urine sample. Before providing the container, each patient was instructed properly for the collection of sample. The samples were processed as soon as possible. Detailed guidelines for collection of clean catch mid-stream urine are mentioned in Appendix-VI.

4.2.2 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, sex, signs and symptoms (dysuria, frequency, urgency, fever, stomach pain etc.), antibiotics used or not and the macroscopic examination (color, appearance) of urine was also performed before processing.

4.2.3 Urine sample evaluation

Before proceeding, the urine samples were evaluated in terms of their acceptability. Considerations included proper labeling, visible signs of contamination and any transportation delays in getting the urine samples to the laboratory. A properly labeled sample contained patient's full name, date and time of collection. Single urine sample was collected from each patient so bacteriological culture was performed first followed by the routine microscopic observation.

4.2.4 Macroscopic examination

The specimen obtained in laboratory was observed for its color and appearance and reported accordingly.

4.2.5 Microscopic examination

Ten (10) ml of urine sample was taken in a clean sterile centrifuge tube and centrifuged at 3000 rpm for 10 min. The supernatant was discarded. The sediment was then examined by wet mount preparation.

Wet mount preparation: Microscopic examination of urinary sediments by wet preparation includes the detection of WBC (pus cells) and RBC. Number of WBC and RBC were estimated as number per HPF i.e. 40X objective of microscope.

4.2.6 Urine culture

Semi-quantitative culture technique was used to culture urine specimens and to detect the presence of significant bacteriuria by standard methods (Cheesbrough, 1984). An inoculating loop of standard dimension was used to take up approximately fixed (±10% error was accepted) and known volume (0.001ml) of mixed uncentrifuged urine was inoculated on the surface of 5% Blood Agar (BA) and MacConkey Agar (MA). Urine specimen was thoroughly mixed to ensure uniform suspension of bacteria before inoculating the agar plates. The inoculated MA and BA plates were aerobically incubated overnight at 37º C.

The bacterial count was reported as described by Cheesbrough (2000):

- Less than 10^4 organisms/ml or no growth, (not significant or contamination or no growth)
- 10^4-10^5 CFU/ml (mean infection or contamination, repeat sample)
- More than 10^5 organisms/ml, significant bacteriuria (UTI)

4.2.7 Identification of isolates

The growth plates showing significant bacterial growth were proceeded for further study. Plate showing no bacterial growth, mixed growth and bacterial growth of insignificant number were excluded from the study. Identification of significant isolate was done by using microbiological techniques as described in the Bergey's manual which involves morphological appearance of the colonies, staining reactions and biochemical properties. Standard protocol provided by Cheesbrough (1984) and Collee *et al.* (1996) was followed for identification of bacteria isolated from urine specimens.

Pure culture for identification: 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 MA 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, oxidase, other biochemical and antibiotic susceptibility test. The Gram-staining procedure is mentioned in the Appendix-IV.

Biochemical Test: 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, oxidase, growth on MSA, Bile-esculin 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, Triple Sugar Iron (TSI) test, Urease test, Nitrate test, Motility test, Sulphide production test and Gas production test.

The composition and preparation of biochemical media and reagents used in the biochemical test are mentioned in the Appendix-III. The procedure for performing biochemical tests are mentioned in Appendix-V.

4.2.8 Antibiotic susceptibility testing

The antimicrobial susceptibility testing of the isolates towards various antimicrobial disks was done by modified Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory and Standards Institue (CLSI-M100-S16, 2007) using Mueller Hinton agar (MHA).

- 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 Petri-dish.
- Using a sterile inoculating loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into Mueller Hinton broth tube and was incubated at 37ºC for 2-4 hrs.
- After incubation, the turbidity of the suspension was matched with the turbidity standard of McFarland tube number 0.5.
- Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture technique. The plate was left for about 5 minutes to let the agar surface dry.

- Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) was placed, evenly distributed on the inoculated plates, not more than 6 discs were placed on a 90 mm diameter Petri-dish.
- After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and compared with standardized zone interpretative chart provided by the company.

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

4.2.9 Quality control

To obtain reliable microbiological result, it is necessary to maintain quality control. Quality of each test was maintained by using standard procedures. The quality of each agar plates prepared was by incubating one plate of each lot on the incubator. Control strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used for the standardization of the Kirby-Bauer test and also for correct interpretation of zone of diameter. Quality of sensitivity tests was maintained by maintaining the thickness of MHA at 4 mm and the pH at 7.2-7.4. Similarly antibiotics discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures.

4.2.10 Purity Plate

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

4.2.11 Preservation of MDR isolates Of *E. coli*

All the MDR isolates were preserved in media containing Trypticse soy broth. The

composition of preservation media is mentioned in appendix III.

4.2.12 Extraction of plasmids DNA from MDR *E.coli*

The plasmid DNA was extracted from the overnight culture (mid log phase) of MDR *E.coli*, grown in Lauria Bertani broth, supplemented with proper antibiotics. The plasmid was extracted according to protocols described by Sambrook *et al*. (2001) and using commercial Kits procured from Bangalore genei. The detailed protocols are mentioned in appendix IX. The composition of Lauria Bertani broth is mentioned in appendix III. The preparation of solutions for extraction of plasmid is mentioned in appendix III.

4.2.13 Conjugation between MDR *E.coli* (donor) and *E.coli* HB101

MDR *E. coli* sensitive to Streptomycin were selected and grown for conjugation study, similarly recipient strain *E.coli* HB101 (streptomycin resistant) was grown and conjugation mixture was made accordingly described by Phornphisutthimas *et al*. (2007). After incubation the transconjugants were selected on suitable antibiotic plate. The detailed protocol for conjugation is mentioned in appendix IX.

4.2.14 Transformation of plasmid DNA in recipient *E.coli* (TB1)

The plasmid DNA extracted were selected and transformed in *E.coli* TB1 (sensitive to all antibiotics but resistant to streptomycin) according to the protocols described by Sambrook *et al*. (2001). The detailed protocols are mentioned in appendix IX.

4.2.15 Determination of minimum inhibitory concentration (MIC) of donors and transconjugants

MICs were determined by two fold broth macrodilution method recommended by CLSI-M7-A7(2007). The inocula of 4×10^5 CFU were prepared from suitably diluted overnight broth culture at 37^0C. Positive growth controls were kept for each isolates and *E.coli* 25922 of known MIC was also included in each test as control for antibiotic potency. The detailed protocol for this test is mentioned in appendix IX.

4.2.16 Extraction of plasmids DNA from Transconjugant and Transformant

Extraction of plasmid DNA was done by alkaline lysis method as described by Sambrook *et al*. (2001)

4.2.17 Electrophoresis of plasmid DNA from MDR E.coli, transconjugant and transformant

The electrophoresis was done in standard agarose in 70 volts for 3 hours. Then the agarore slab was visualized under the UV transilluminator. The detailed protocol for electrophoresis is mentioned in appendix IX.

4.2.18 Determination of size of DNA by semi log plot

Making standard curve: Using a ruler, we measured the distance traveled from the well (in cm) by each illuminated band. The number of base pairs (of marker DNA) of each illuminated band versus the distance traveled was plotted on Semi-log paper. No. of base pairs was plotted on the log scale, and cm migrated on the linear scale. The best line through these points was drawn to generate a standard curve. From this curve the size of each band was determined.

4.2.19 Decontamination of ethidium bromide

The procedure for decontamination of ethidium bromide remaining in the agarose slab is mentioned in the appendix IX.

4.2.20 Statistical analysis

Chi-square test was used to determine significant association of bacteriuria between genders, MDR strains and gender. Odds ratio was used to find the association between previous antibiotic use and drug resistance. Test of present work are shown in Appendix- X.

CHAPTER- V

5. RESULTS

This study was conducted among patients suspected of UTI(UTI), attending at Kathmandu Model Hospital, Kathmandu, Nepal. Seven hundred and ten urine samples were collected from patients visiting at the hospital during the study period.

5.1 MICROBIAL ANALYSIS OF URINE SAMPLES

5.1.1 Culture results

Out of 710 samples, 219 samples (30.85 %) showed significant growth (i.e. $>10^5$ organisms per ml of urine sample), 307 samples (43.23 %) were sterile and 184 samples (25.92 %) produced insignificant growth. The results are shown in table 3 and figure 1.

Table 3: Urine culture result

Total no. of samples	Culture results	No.	%
710	**Significant growth**	**219**	**30.85**
	No significant growth	**184**	**25.92**
	No growth	**307**	**43.23**

5.1.2 Gender and source of UTI positive samples

Out of 219 UTI positive, 20.99% (46/219) were male patients while 79.01% (173/219) were female patients. Similarly in the UTI positive, 86.30% (189/219) were out-patients and 13.70% (30/219) were in-patients. The results are shown in table 4.

Table 4: Gender and source of UTI positive samples

Source	Male	%	Female	%	Total	%
Out-patient	33	15.06	156	71.24	189	**86.30**
In-patient	13	5.93	17	7.77	30	**13.70**
Total	**46**	**20.99**	**173**	**79.01**	**219**	**100**

5.1.3 Age wise distribution of growth positive culture

Highest number of growth positive result (39.73%) was observed in age group 20-30 which was followed by age group 30-40 (21.47%). The highest numbers of male patients showing positive urine culture falls in age group 20-30 (5.48%) which was followed by age group 30-40(3.65%). Similarly the highest numbers of female patients showing positive urine culture falls in age group 20-30 (28.77%) which was followed by age group 30-40(14.15%). The results are shown in Appendix –XIV.

5.1.4 Distribution of microorganisms

Out of 219 UTI positive cultures, 11 genera of organisms were identified. *E.coli* (81.28%) was isolated in larger number which was followed by *C. freundii (*3.67%). The least numbers were of *S. aureus*, *Salmonella* Typhi and *Enterobacter cloacae*, 0.46% each. The results are shown in table 5.

Table 5: Distribution of microorganisms

Organisms Isolated	Total No. of Isolates	No.	%
Acinetobacter spp.	219	2	0.91
Citrobacter diversus		3	1.37
Citrobacter Freundii		8	3.67
Enterobacter aerogens		3	1.37
Enterobacter cloacae		1	0.46
Escherichia coli		178	81.28
Klebsiella oxytoca		2	0.91
Klebsiella pneumoniae		4	1.83
Morganella morganii		2	0.91
Proteus mirabilis		3	1.37
Pseudomonas aeruginosa		2	0.91
Salmonella Typhi		1	0.46
Staphylococcus aureus		1	0.46
S. epidermidis		4	1.83
S. saprophyticus		2	0.91
Streptococcus faecalis		3	1.37
Total		**219**	**100**

5.1.5 Age wise distribution of bacterial isolates

Among the total of 219 isolates, 69 belonged from the patients of age-group 20-29, whereas 37 isolates were obtained from patients of age-group 30-39. *E. coli* the most predominant of the species was isolated chiefly from patients of age-group 20-29 as well as 60-69 and 30-39. The results are shown in Appendix-XIV.

5.1.6 Bacterial isolates on source and gender of patients

Out of 173 isolates from female patients, *E. coli* was predominant with 67.12% (147/219). Similarly, out of 46 isolates from male E. coli was predominant with 14.15% (31/219). In both in-patient and out- patient *E. coli* was predominant. Results are shown in table 6.

Table 6: Distribution of bacterial isolates on source and gender of patients

Organisms	Male				Female				Total	
	Out- Patient		In-Patient		Out-Patient		In-Patient			
	No.	%	No.	%	No.	%	No.	%	No.	%
Acinetobacter sps	0	0	0	0	1	0.46	1	0.46	2	0.91
C. diversus	1	0.46	0	0	2	0.91	0	0	3	1.37
C. fruendii	1	0.46	3	1.37	2	0.91	2	0.91	8	3.65
Enterobacter cloacae	0	0	0	0	1	0.46	0	0	1	0.46
E.aerogens	1	0.46	0	0	2	0.91	0	0	3	1.37
E. coli	24	10.96	7	3.19	133	60.73	14	6.39	178	81.28
K. oxytoca	1	0.46	0	0	1	0.46	0	0	2	0.91
K. pneumoniae	0	0	0	0	4	1.83	0	0	4	1.83
M. morganii	0	0	0	0	2	0.91	0	0	2	0.91
P.mirabilis	1	0.46	0	0	2	0.91	0	0	3	1.37
P. aeruginosa	0	0	1	0.46	1	0.46	0	0	2	0.91
Salmonella typhi	1	0.46	0	0	0	0	0	0	1	0.46
S. aureus	1	0.46	0	0	0	0	0	0	1	0.46
S.epidermidis	1	0.46	1	0.46	2	0.91	0	0	4	1.83
S. saprophyticus	0	0	0	0	2	0.91	0	0	2	0.91
Streptococcus faecalis	1	0.46	1	0.46	1	0.46	0	0	3	1.37
Total	**33**	**15.09**	**13**	**5.94**	**156**	**71.21**	**17**	**7.76**	**219**	**100**

5.1.7 Correlation of pyuria with culture result (N=189)

Out of 189 samples, 72.49% (137/189) of samples showed insignificant pyuria, however among these, 2.92% (4/137) of samples gave positive culture results. Similarly, 52 (27.51%) of total samples showed significant pyuria, and among these 84.61% (44/52) samples gave positive culture results. The results are shown in table 7.

Table 7: Correlation of pyuria with culture result (N=189)

Pyuria	Culture positive (%)	Culture negative (%)	Total (%)
Significant (≥5WBC/HPF)	44 (84.61)	8 (15.39)	**52 (27.51)**
Insignificant (<5WBC/HPF)	4 (2.92)	133 (97.08)	**137 (72.49)**
Total	**48 (25.39)**	**141 (74.61)**	**189 (100.0)**

5.2 ANTIBIOTIC SUSCEPTIBILITY PATTERN

5.2.1 Antibiotic susceptibility pattern of Gram negative bacteria

Among the common primary antibiotics used against urinary bacterial isolates, Nitrofurantoin and Norfloxacin were the drug of choice with a susceptibility of 82.77% each. Most of the bacterial isolates, i.e. 120 (57.43%) were resistant to Amoxycillin. Among the antibiotics used secondarily Amikacin (82.14%) was the drug of choice which was followed by Chloramphenicol (67.85%). The results are shown in table 8.

Table 8: Antibiotic susceptibility pattern of Gram Negative bacteria

Antibiotic used	No. of isolates	Sensitivity pattern					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Amoxycillin	207	87	42.02	0	0	120	57.98
Cefixime	209	141	67.46	0	0	68	32.54
Cefotaxime	209	143	68.42	0	0	66	31.58
Ciprofloxacin	209	133	63.63	2	0.95	74	35.42
Co-trimoxazole	209	115	55.02	0	0	94	44.98
Nitrofurantoin	209	173	82.77	6	2.87	30	15.64
Norfloxacin	209	132	63.15	1	0.47	76	36.38
Ofloxacin	209	136	65.07	1	0.47	72	34.46
Amikacin	56	46	82.14	3	5.35	7	12.31
Ceftazidime	56	0	0	0	0	56	100
Ceftriaxone	56	0	0	0	0	56	100
Chloramphenicol	56	38	67.85	0	0	18	32.15
Gentamicin	56	13	23.21	0	0	43	76.79

5.2.2 Antibiotic susceptibility pattern of Gram positive bacteria

Among the Gram positive isolates, 70% were susceptible to Co-trimoxazole and Cloxacillin. Amoxicillin, whereas all the 4 (100%) isolates were susceptible to

Vancomycin. Cephalexin was found to be the least effective as only 40% isolates were sensitive. The results are shown in table 9.

Table 9: Antibiotic susceptibility pattern of Gram Positive Bacteria

Antibiotic used	No. of isolates	Sensitivity pattern					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Amoxycillin	10	6	60	0	0	4	40
Cephalexin	10	4	40	0	0	6	60
Ciprofloxacin	10	5	50	0	0	5	50
Cloxacillin	10	7	70	0	0	3	30
Co-trimoxazole	10	7	70	0	0	3	30
Erythromycin	10	5	50	0	0	5	50
Norfloxacin	10	5	50	0	0	5	50
Vancomycin	4	4	100	0	0	0	0

5.2.3 Antibiotic susceptibility pattern of *E.coli*

Among the primary antibiotics evaluated, *E. coli* was found to be highly susceptible towards Nitrofurantoin (89.9%) which was followed by Cefixime and Cefotaxime (69.7%). Most of the *E. coli* isolated was resistant to Amoxicillin (55.6%). Among secondary antibiotics, Amikacin was the drug of choice with susceptibility of 87.5%. Ceftazidime and Ceftriaxone showed least activity. The results are shown in table 10.

Table 10: Antibiotic susceptibility pattern of *E.coli*

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible					
		No.	%	No.	%	No.	%
E. coli N=178	Amoxycillin	79	44.4	0	0	99	55.6
	Cefixime	124	69.7	0	0	54	30.3
	Cefotaxime	124	69.7	0	0	54	30.3
	Ciprofloxacin	114	64	0	0	64	36
	Co-trimoxazole	99	55.6	0	0	79	54.4
	Nitrofurantoin	160	89.9	4	2.2	14	7.9
	Norfloxacin	113	63.5	0	0	65	36.5
	Ofloxacin	115	64.6	0	0	63	35.4
N=48	Amikacin	42	87.5	3	6.3	3	6.3
	Ceftazidime	0	0	0	0	48	100
	Ceftriaxone	0	0	0	0	48	100
	Chloramphenicol	35	72.9	0	0	13	27.1
	Gentamicin	13	27.1	0	0	35	72.9

5.2.4 Antibiotic susceptibility pattern of *Citrobacter* spp.

Among the primary antibiotics evaluated, *Citrobacter* spp. was found to be highly susceptible towards ofloxacin (54.4%) which was followed by Nitrofurantoin (45.4%), Norfloxacin (36.4%) and Cefotaxime (36.4%). Most of the *Citrobacter* spp. isolated was resistant to Amoxicillin, Cefixime and Co-trimoxazole (72.8%). Among secondary antibiotics, Chloramphenicol was the drug of choice with susceptibility of 40 %. Ceftazidime, Gentamycin and Ceftriaxone showed least activity. The results are shown in table 11.

Table 11: Antibiotic susceptibility pattern of *Citrobacter* spp.

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Citrobacter spp. N=11	Amoxycillin	3	27.2	0	0	8	72.8
	Cefixime	3	27.2	0	0	8	72.8
	Cefotaxime	4	36.4	0	0	7	63.6
	Ciprofloxacin	3	27.2	2	18.4	6	54.4
	Co-trimoxazole	3	27.2	0	0	8	72.8
	Nitrofurantoin	5	45.4	1	9.2	5	45.4
	Norfloxacin	4	36.4	1	9.2	6	54.4
	Ofloxacin	6	54.4	1	9.2	4	36.4
N=5	Amikacin	1	20	0	0	4	80
	Ceftazidime	0	0	0	0	5	100
	Ceftriaxone	0	0	0	0	5	100
	Chloramphenicol	2	40	0	0	3	60
	Gentamicin	0	0	0	0	5	100

5.2.5 Antibiotic susceptibility pattern of *Klebsiella* spp.

Among the primary antibiotics evaluated, *Klebsiella* spp. was found to be highly susceptible towards Ciprofloxacin, Norfloxacin and Ofloxacin (83.3%) which were followed by Co-trimoxazole, Cefixime and Cefotaxime (66.6%). Most of the *Klebsiela* spp. isolated was resistant to Amoxicillin (100%). Among secondary antibiotics, Amikacin and Chloramphenicol were the drug of choice with susceptibility of 100% each. Ceftazidime, Gentamicin and Ceftriaxone showed least activity. The results are shown in table 12.

Table 12: Antibiotic susceptibility pattern of *Klebsiella* spp.

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Klebsiella spp. N=6	Amoxycillin	0	0	0	0	6	100
	Cefixime	4	66.6	0	0	2	33.4
	Cefotaxime	4	66.6	0	0	2	33.4
	Ciprofloxacin	5	83.3	0	0	1	16.7
	Co-trimoxazole	4	66.6	0	0	2	33.4
	Nitrofurantoin	1	16.7	1	16.7	4	66.6
	Norfloxacin	5	83.3	0	0	1	16.7
	Ofloxacin	5	83.3	0	0	1	16.7
N=1	Amikacin	1	100	0	0	0	0
	Ceftazidime	0	0	0	0	1	100
	Ceftriaxone	0	0	0	0	1	100
	Chloramphenicol	1	100	0	0	0	0
	Gentamicin	0	0	0	0	1	100

5.2.6 Antibiotic susceptibility pattern of *Enterobacter* spp. and *Salmonella* Typhi

Among the primary antibiotics evaluated, *Enterobacter* spp. was found to be highly susceptible towards Ciprofloxacin, Norfloxacin and Ofloxacin (100%) which were followed by Nitrofurantoin, Cefixime and Cefotaxime (75%). Most of the *Enterobacter* spp. isolated was resistant to Amoxicillin (50%). Among the primary antibiotics evaluated, *Salmonella typhi* was found to be highly susceptible towards Co-trimoxazole, Cefixime and Cefotaxime (100%). Amoxicillin, Nitrofurantoin, Norfloxacin and Ciprofloxacin showed the least activity. The results are shown in table 13.

Table 13: Antibiotic susceptibility pattern of *Enterobacter* spp. and *Salmonella*Typhi

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Enterobacter spp. N=4	Amoxycillin	2	50	0	0	2	50
	Cefixime	3	75	0	0	1	25
	Cefotaxime	3	75	0	0	1	25
	Ciprofloxacin	4	100	0	0	0	0
	Co-trimoxazole	3	75	0	0	1	25
	Nitrofurantoin	3	75	0	0	1	25
	Norfloxacin	4	100	0	0	0	0
	Ofloxacin	4	100	0	0	0	0
Salmonella Typhi N=1	Amoxycillin	0	0	0	0	1	100
	Cefixime	1	100	0	0	0	0
	Cefotaxime	1	100	0	0	0	0
	Ciprofloxacin	0	0	0	0	1	100
	Co-trimoxazole	1	100	0	0	0	0
	Nitrofurantoin	0	0	0	0	1	100
	Norfloxacin	0	0	0	0	1	100
	Ofloxacin	0	0	0	0	1	100

5.2.7 Antibiotic susceptibility pattern of *P. aeruginosa*

Among the antibiotics evaluated, *P. aeruginosa* was found to be highly susceptible towards Amikacin. Pseudomonas was found resistant to Ceftazidime, Ceftriaxone (100%). The results are shown in table 14.

Table 14: Antibiotic susceptibility pattern of *P. aeruginosa*

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
P. aeruginosa N=2	Cefixime	0	0	0	0	2	100
	Cefotaxime	1	50	0	0	1	50
	Ciprofloxacin	0	0	0	0	2	100
	Co-trimoxazole	0	0	0	0	2	100
	Nitrofurantoin	2	100	0	0	0	0
	Norfloxacin	0	0	0	0	2	100
	Ofloxacin	0	0	0	0	2	100
	Amikacin	2	100	0	0	0	0
	Ceftazidime	0	0	0	0	2	100
	Ceftriaxone	0	0	0	0	2	100
	Chloramphenicol	0	0	0	0	2	100
	Gentamicin	0	0	0	0	2	100

5.2.8 Antibiotic susceptibility pattern of *Proteus mirabilis*

Among the primary antibiotics evaluated, *Proteus mirabilis* was found to be highly susceptible towards Cefixime, Cefotaxime, Ciprofloxacin, Norfloxacin and Ofloxacin (100%) which were followed by Co-trimoxazole, and Amoxicillin (66.6%). Most of the *Proteus mirabilis* isolated was resistant to Nitrofurantoin (66.6%). The results are shown in table 15.

Table 15: Antibiotic susceptibility pattern of *Proteus mirabilis*

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Proteus mirabilis N=3	Amoxycillin	2	66.6	0	0	1	33.4
	Cefixime	3	100	0	0	0	0
	Cefotaxime	3	100	0	0	0	0
	Ciprofloxacin	3	100	0	0	0	0
	Co-trimoxazole	2	66.6	0	0	1	33.4
	Nitrofurantoin	1	33.4	0	0	2	66.6
	Norfloxacin	3	100	0	0	0	0
	Ofloxacin	3	100	0	0	0	0

5.2.9 Antibiotic susceptibility pattern of *M. morganii* and *Acinetobacter* spp.

Among the primary antibiotics evaluated, *M. morganii* was found to be highly susceptible towards Cefixime, Cefotaxime, Co-trimoxazole, Ciprofloxacin, Norfloxacin and Ofloxacin (100%) which were followed by Nitrofurantoin (50%). Most of the *M. morganii* isolated was resistant to Amoxicillin (100%).

Among the primary antibiotics evaluated, *Acinetobacter* spp. was found to be highly susceptible towards Ciprofloxacin (100%) which was followed by Amoxicillin, Ofloxacin, Norfloxacin, Co-trimoxazole, Cefixime and Cefotaxime (50%). Nitrofurantoin showed the least activity (100%). The results are shown in table 16.

Table 16: Antibiotic susceptibility pattern of *M. morganii* and *Acinetobacter* spp.

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
M. morganii	Amoxycillin	0	0	0	0	2	100
N=2	Cefixime	2	100	0	0	0	0
	Cefotaxime	2	100	0	0	0	0
	Ciprofloxacin	2	100	0	0	0	0
	Co-trimoxazole	2	100	0	0	0	0
	Nitrofurantoin	1	50	0	0	1	50
	Norfloxacin	2	100	0	0	0	0
	Ofloxacin	2	100	0	0	0	0
Acinetobacter spp.	Amoxycillin	1	50	0	0	1	50
N=2	Cefixime	1	50	0	0	1	50
	Cefotaxime	1	50	0	0	1	50
	Ciprofloxacin	2	100	0	0	0	0
	Co-trimoxazole	1	50	0	0	1	50
	Nitrofurantoin	0	0	0	0	2	100
	Norfloxacin	1	50	0	0	1	50
	Ofloxacin	1	50	0	0	1	50

5.2.10 Antibiotic susceptibility pattern of CONS and *S. aureus*

Among the antibiotics evaluated, CONS was found to be highly susceptible towards Amoxicillin, Ciprofloxacin, Erythromycin and Norfloxacin (83.3%) which were followed by Co-trimoxazole, Cephalexin and Cloxacillin (66.6%). Single *S. aureus* was isolated which was confirmed to be MRSA and was found resistant to all antibiotics tested except Vancomycin. The results are shown in table 17.

Table 17: Antibiotic susceptibility pattern of CONS and *S. aureus*

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
CONS N=6	Amoxycillin	5	83.3	0	0	1	16.7
	Cephalexin	4	66.6	0	0	2	33.4
	Ciprofloxacin	5	83.3	0	0	1	16.7
	Cloxacillin	4	66.6	0	0	2	33.4
	Co-trimoxazole	4	66.6	0	0	2	33.4
	Erythromycin	5	83.3	0	0	1	16.7
	Norfloxacin	5	83.3	0	0	1	16.7
S. aureus(MRSA) N=1	Amoxycillin	0	0	0	0	1	100
	Cephalexin	0	0	0	0	1	100
	Ciprofloxacin	0	0	0	0	1	100
	Cloxacillin	0	0	0	0	1	100
	Co-trimoxazole	0	0	0	0	1	100
	Erythromycin	0	0	0	0	1	100
	Norfloxacin	0	0	0	0	1	100
	Oxacillin	0	0	0	0	1	100
	Vancomycin	1	100	0	0	0	0

5.2.11 Antibiotic susceptibility pattern of *Streptococcus faecalis*

Streptococcus faecalis showed susceptibility towards Co-trimoxazole (100%) and Amoxicillin (33.3%). This organism was found to resistant to other antibiotics evaluated. The results are shown in table 18.

Table 18: Antibiotic susceptibility pattern of *Streptococcus faecalis*

Organisms isolated	Antibiotics used	Antibiotic Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Streptococcus faecalis N=3	Amoxycillin	1	33.4	0	0	2	66.6
	Cephalexin	0	0	0	0	3	100
	Ciprofloxacin	0	0	0	0	3	100
	Cloxacillin	0	0	0	0	3	100
	Co-trimoxazole	3	100	0	0	0	0
	Erythromycin	0	0	0	0	3	100
	Norfloxacin	0	0	0	0	3	100
	Vancomycin	3	100	0	0	0	0

5.3 ANALYSIS OF MDR ISOLATES

5.3.1 Distribution of MDR on bacterial isolates

Most of the bacteria isolated were found to be resistant to ≥3 classes of drugs (41.55%) and were considered MDR. It was found that 82 (37.44%) were found to be susceptible to all antibiotics evaluated in the study and 24 (10.95%) isolates were resistant to 1 drug and 22 (10.04%) isolates were resistant to 2 drugs. Among the MDR strains, 38.20 % (79/178) of *E. coli were* found to be MDR. The results are shown in table 19.

Table 19: Distribution of MDR on bacterial isolates

Organisms	**Total no. of isolates**	**Resistant to**					
		0 drug	**1 drug**	**2 drugs**	**≥3 drugs**	**MDR Strains**	
						≥3 classes of drugs	**%**
Acinetobacer spp.	2	0	1	0	1	**1**	**50.0**
Citrobacter spp.	11	2	1	0	8	**8**	**72.72**
CONS	6	3	0	0	3	**3**	**50.0**
E. coli	178	74	20	16	68	**68**	**38.20**
Enterobacter spp.	4	2	0	1	1	**1**	**25.0**
K. oxytoca	2	0	1	0	1	**1**	**50.0**
K.pneumoniae	4	0	0	3	1	**1**	**25.0**
M. morganii	2	0	0	2	0	**0**	**0**
P. mirabilis	3	1	1	0	1	**1**	**33.33**
P. aeruginosa	2	0	0	0	2	**2**	**100**
S. Typhi	1	0	0	0	1	**1**	**100**
S. aureus	1	0	0	0	1	**1**	**100**
S.faecalis	3	0	0	0	3	**3**	**100**
Total	**219**	**82**	**24**	**22**	**91**	**91**	**41.55**

5.4.2 Distribution of MDR on gender and source of patients

Among 46 positive growths in male, 58.69% were MDR. Among 173 growths positive females, 36.99% were MDR. The results are shown in table 20.

Table 20: Distribution of MDR on gender and type of patients

Pattern of drug Susceptibility	**Male**		**Female**		**Total**
	Out-patient	**In-patient**	**Out-patient**	**In-patient**	
MDR	17 (36.95)	10 (21.73)	53 (30.63)	11 (6.35)	**91 (41.55)**
Non MDR	5 (10.86)	1 (2.18)	37 (21.38)	3 (1.73)	**46 (21.01)**
Sensitive	11 (23.91)	2 (4.35)	66 (38.15)	3 (1.73)	**82 (37.44)**
Total	**33**	**13**	**156**	**17**	**219**

MDR- Multidrug resistant

5.4.3 Age wise distribution of MDR

The highest number of MDR was found in age group 80-89 (100%) which was followed by 20-29 (52.29%). The lowest number of MDR was found in age group >90 followed by 10-19. The results are shown in table 21.

Table 21: Distribution of MDR on age of patients

Age of the patient	Total no. of isolates	Pattern of drug resistance	
		Multidrug resistance (%)	Non-multidrug resistant (%)
0-9	7	2 (28.57)	2 (28.57)
10-19	11	1 (9.09)	5 (45.45)
20-29	69	26 (37.68)	13 (18.84)
30-39	37	15 (40.54)	7 (18.91)
40-49	25	12 (48)	4 (16)
50-59	17	9 (52.29)	6 (35.29)
60-69	30	14 (46.66)	5 (16.66)
70-79	17	8 (47.05)	4 (23.52)
80-89	4	4 (100)	0

5.4.4 Antibiotic resistance pattern of MDR *E.coli* isolates

As shown in table 27, among the MDR *E. coli* isolates, 94.11% (64/68) were resistant to Amoxicillin, 95.58% (65/68) to Norfloxacin, 86.76% (59/68) to Cotrimoxazole, 92.64% (63/68) to ofloxacin and Ciprofloxacin, 17.64% (12/68) to Nitrofurantoin, 79.41% (54/68) to cefotaxime and 77.94% (53/68) to cefixime. The result is given in table 22.

Table 22: Antibiotic resistance pattern of MDR *E. coli* isolates

Antibiotics used	MDR *E. coli* isolates	Isolates resistant to antibiotics	
		No.	Percentage
Amoxycillin	68	64	94.11
Cefixime		53	77.94
Cefotaxime		54	79.41
Ciprofloxacin		63	92.64
Cotrimoxazole		59	86.76
Nitrofurantoin		12	17.64
Norfloxacin		65	95.58
Ofloxacin		63	92.64
Amikacin	48	3	6.25
Ceftazidime		48	100
Chloramphenicol		13	27.08
Ceftriaxone		48	100
Gentamicin		35	72.91

5.5 PLASMID ANALYSIS

5.5.1 Profiles of plasmids from MDR E.coli

Plasmids were extracted from 29 MDR *E.coli* selected and run in agarose gel which showed 14 types of plasmid profiles of size ranging from 2 to 51Kb.Twelve isolates showed single plasmid, 2 showed double plasmids. Similarly 6, 6, 1 and 1 isolates contained 3, 4, 5 and 7 plasmids respectively. The most repetitive plasmid was 32.5 Kb. The results are shown in Appendix-XII and photograph 4.

5.5.2 Plasmid profiles and resistance patterns of donors and transconjugants

Ten isolates which were resistant to commonally used antibiotics but sensitive to streptomycin were tested by conjugation experiment. Most of the transconjugants seemed to acquire the resistant properties during resistance transfer study through conjugation. It can be seen that all (10) isolates posses conjugative type of plasmids and transferred resistance phenotypes successfully to recipient *E.coli* HB101. In most of the transconjugants, plasmids having 32.5 Kb was found to be transferred. This plasmid could be responsible for transfer of antibiotic resistance. U27, U29 transferred 38 Kb

plasmid, U26, U6, U25, U14, U24, U9, U12 transferred 32.5 Kb plasmid while U10 transferred 51 Kb plasmid. The results are shown in table 23 and photograph 5.

Table 23: Plasmid profiles and resistance patterns of donors and transconjugants

Donors	Resistance pattern of donors	Resistance pattern of transconjugants	Size of plasmids in donors	Size of plasmids in transconjugants
U6	Am,Cf,Ce,Cfx,Co,Of, Nx, Na,Ca,Ci	Am,Cf,Co,Of,Nx, Na, Cz	32.5	32.5
U14	Am,Cf,Ce,Cfx,Co,Of, Nx,Na,Ca,C,G,Ci	Am,Cf,Of,Nx, Na, Cz	32.5	32.5
U27	Am,Cf,Co,Nx,Na,Of,Ca,Ci	Am,Cf,Co,Of,Nx, Na, Cz	38	38
U24	Am,Cf,Ce,Cfx,Co,Of, Nx,Na,Ca,G,Ci	Am,Cf,Co,Of,Nx, Na, Cz	32.5,5.4	32.5
U9	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,C,G,Ci	Am,Cf,Co,Of,Nx, Na, Cz	32.5, 4.5, 2.5, 1.8	32.5
U10	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	Am, Cz	51	51
U26	Am,Cf,Ce,Cfx,Co,Of,Nx,Na,Ak,Ca, G,Ci	Am,Cf,Co,Of,Nx, Na, Cz	32.5, 7.5	32.5,7.5
U29	Am,Cf,Co,Nx,Ca,Ci	Am	38	38
U25	Am,Cf,Ce,Cfx,Co,Of,Nx,Na,C,Ca,G, Ci	Am,Cf,Co,Of,Nx, Na, Cz	32.5, 7.5, 4.7	32.5,7.5
U12	Am,Cf,Ce,Cfx,Co,Of,Nx,Na,Ca,Ci	Am,Cf,Co,Of,Nx, Na, Cz	51,32.5, 10, 4.7, 3.9	51,32.5,10

5.5.3 Conjugation frequency

The conjugation frequency was determined in terms of per ml of donors used. It was found that the chance of transferring Trimethoprim and Norfloxacin resistance through conjugation was higher compared to the other antibiotics used. The results were shown in table 24.

Table 24: Conjugation frequency of transconjugants on different selective media

Donors	Approximate no. of donors (Cfu/ml)	No. of transconjugants on selective plates containing Streptomycin						Conjugation frequency (Transconjugants /ml of Donor cells)
		Tr	Nx	A	Cf	G	C	
U6	5×10^8	112	324	5	400	0	0	2.24×10^{-7} (Tr) 0.648×10^{-8}(Nx) 1×10^{-7}(A) 0.8×10^{-8} (Cf)
U14	5×10^8	0	5	0	40	0	0	0.1×10^{-7}(Nx) 0.8×10^{-7}(Cf)
U27	5×10^8	125	132	16	176	0	0	2.5×10^{-7} (Tr) 1.32×10^{-7} (Nx) 0.32×10^{-7} (A) 1.76×10^{-7} (Cf)
U24	5×10^8	55	20	0	25	0	0	1.1×10^{-7} (Tr) 0.4×10^{-7} (Nx) 0.5×10^{-7} (Cf)
U9	5×10^8	60	105	0	60	0	0	1.2×10^{-7} (Tr) 2.1×10^{-7} (Nx) 1.2×10^{-7} (Cf)
U10	5×10^8	0	0	TNTC	0	0	0	
U26	5×10^8	20	15	100	25	0	0	0.4×10^{-7} (Tr) 0.3×10^{-7} (Nx) 2×10^{-7} (A) 0.5×10^{-7} (Cf)
U29	5×10^8	50	0	TNTC	0	0	0	1×10^{-7} (Tr)
U25	5×10^8	30	5	TNTC	15	0	0	0.6×10^{-7} (Tr) 0.1×10^{-7} (Nx) 0.3×10^{-7} (Cf)
U12	5×10^8	552	568	TNTC	30	100	100	1.10×10^{-6} (Tr) 1.136×10^{-6} (Nx) 0.6×10^{-7} (Cf) 2×10^{-7} (C) 2×10^{-7} (G)

5.5.4 Transformation

Transformation was carried out using purified plasmids. Three out of five competent cells ***(E.coli* TB1**) were successfully transformed. The transformed cells showed resistance to fewer antibiotics. The results are shown in table 25.

Table 25: Antibiotic profile of transformants

Donors Plasmid from	Recipient	Antibiotic resistant profile of Transformants
U10	*E.coli* TB1	A,G
U21		A, Co
U9		A,G

5.5.5 Susceptibility of donors and transconjugants

The donors and transconjugants showed high degree of tolerance to the antibiotics. The resistance transferred through conjugation was stronger than resistance transferred through transformation. Most of the donors and transconjugants showed high degree of antibiotic resistance. Most of the donors and transconjugants had the minimum inhibitory concentration >1024 µg/ml. The results are shown in appendix-xi.

5.6 Statistical analysis

The chances of getting UTIamong female gender was found statistically significant ($p<0.05$). The analysis of MDR shows the higher percentages of in-patients have multidrug resistant uropathogens compared to out patients which was also found statistically significant ($p<0.05$). Similarly the chances of having MDR uropathogen were statistically significant when observed among male gender ($p<0.05$).

Table 26: Statistical analysis of the results

N=710 / **Independent Variables**		**χ^2 value**	**P value**
Gender	**UTI**	5.27	<0.05
Type of patients	**Multidrug resistance**	4.23	<0.05
Gender	**Multidrug resistance**	4.61	<0.05
Gender	**No. of isolates V_S MDR strains**	6.68	<0.05
Antibiotic use (n=44)	**Drug resistance**	Odds ratio	1.56

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

Nepal is one of the developing country in South Asia with comparatively poor health status due to illiteracy, lack of hygienic and sanitary knowledge, malnutrition, economic status and lack of advanced techniques in medical practices. So, people are usually victimized by many infectious diseases. According to annual report published by Department of Health Services (2005/2006), the morbidity of UTI in Nepal is 0.57% of the total population. UTIs due to multidrug-resistant bacteria are well known problems (Weiner *et al*., 1999).

The present study was conducted to isolate bacteria causing UTI and determine the prevalence of MDR bacterial isolates among the patients visiting KMH, Kathmandu. Seven hundred and ten mid-stream urine samples were collected from patients visiting KMH and subjected to routine examination and then processed for culture. The results obtained were tabulated in the previous chapter. In this chapter, the results are discussed and compared with the findings of other investigators.

Out of total 710 urine samples, 272 (43.23%) urine samples showed no growth, 184 (25.92%) showed no significant growth and 219 (30.85%) samples showed significant growth. A similar study were carried out by Ling *et al*. (1992), Levett (1993), Manandhar (1996), Obi *et al*. (1996), Gautam (1998), Dhakal (1999) and Chhetri *et al*. (2001) showed low number of growth positivity. The low growth positive rate observed in this study might be due to inclusion of every patients requesting for urine culture regardless of their illness and symptoms. As stated by Manandhar (1996), the possible cause of low rate of growth positivity is that the samples might be from patients under treatment, infection due to slow growing organisms or due to those organisms that were

not able to grow on the routine culture media used. However, very low growth positivity (4.6%) has also been reported (Talukder, 1987).

Females are more susceptible to UTI than males (Leigh, 1990). The present study also supports this fact where the rate of growth positivity was found to be 33.52% (173/516) in females and 23.71% (46/194) in males. This higher growth positivity seen in females was found to be statistically significant ($p<0.05$) and is attributed to their anatomical structure (short urethra and proximity to anal orifice) leading to easy access for coliform bacilli. This correlation was found to be similar to that stated by Leigh (1990), Baron and Finegold (1990), Fowler (1991), Collee *et al*. (2002). This result confirms and expands the previous findings of Steenberg *et al*. (1969), Jha and Yadav (1992), Chhetri *et al*. (2001), Jha and Bapat (2005) and Rajbhandari and Shrestha (2002) in Nepal. The relative infrequency of UTIin men may be attributable to the length of the male urethra and the bactericidal activity of the prostatic fluid (Leigh, 1990).

In this study, age group of 20-29 years showed highest percentage of growth positivity. High-infected females also belonged to the same group. This finding correlates to the results of Steenberg *et al.* (1969) in Denmark, Manandhar (1996), Dhakal (1999), Rajbhandari and Shrestha (2002), Shrestha (2005), Jha and Bapat (2005) and Shrestha (2007). The females of this age group are sexually active and are of childbearing age. A number of studies suggest that sexual activity is an important factor in the pathogenesis of UTI in women. It had been reported that the prevalence in nuns and unmarried women is considerably lower than in married women (Leigh, 1990). These studies also support the fact that the sexual activities predispose an increase in incidence of UTI in sexually active ages. Among males, highest growth positivity was found among age group of 20-29 years. The result did not correlate with the report of Leigh (1990), Forbes *et al*, (2002) which may be due to culmination of samples in this age group.

Among the total 219 bacterial isolates, 207 (94.50%) were Gram negative bacilli, 2 (0.90%) were Gram negative coccobacilli and only 10 (4.60%) were found to be Gram positive cocci. In a study done by Karki *et al.* (2004), 91.1% of the isolates were Gram

negative bacilli and 8.8% of them were Gram positive cocci. Similarly a study done by Shrestha (2007), 93.8% of the isolates were Gram negative and 6.2% of them were Gram positive cocci. In a similar study performed by Blomberg *et al.* in Tanzania in 1996, out of 107 urinary isolates, 66.36% constituted Gram negative isolates. The higher incidence of UTI by Gram negative bacteria was also accounted in the study done by Okada *et al*. (1994) in Japan, Manandhar (1996) and Dhakal (1999) in Nepal. From our study and the study by other workers we can conclude that the high incidence of UTI is caused by Gram negative bacteria in comparison to Gram positive. This statement is further supported by Vorland *et al*., 1985; Kosaki *et al*., 1987 and Ayhan *et al*., 1988.

Altogether 16 different bacterial isolates were found in this study. Among the isolates, *E. coli* (81.28%) was found to be the most predominant organism followed by *Citrobacter spp.* (5.02%), *Klebsiella spp.* (2.74%), CONS (2.74%), *Enterobacter spp.* (1.81%), *Proteus mirabilis* (1.37%), *Streptococcus faecalis* (1.37%), *Acinetobacter* spp (0.91%), *P. aeruginosa* (0.91%), Salmonella typhi (0.46%) and *S. aureus* (0.46%).

Higher prevalence of *E. coli* seen in this study also resembled the study done by various other workers viz: Sharma *et al.* (1983), Tuladhar *et al*. (1989), Jha and Yadav (1992), Manandhar (1996), Dhakal (1999), Chhetri *et al*. (2001) and Shrestha (2007) in Nepal. The result is also in harmony with the study done at international context: Steenberg *et al.* (1969), Fowler (1990), Leigh (1990), Kosakai (1990), Kahlmeter (2000) and Farrell *et al*. (2003). In an acute uncomplicated UTI, *E. coli* is the predominant organism causing the infection across all age groups. Other aerobic gram-negative rods such as *Klebsiella* species, *Proteus* species, *Citrobacter* species, *Acinetobacter* species, *Morganella* species and *P. aeruginosa* are also frequently isolated. Gram-positive bacteria such as Enterococci and *S. aureus*, as well as yeast are important pathogens in complicated UTI (Kalpana *et al*., 2003).

E. coli can bind to the glycoconjugate receptor (Gal α 1| 4 Gal) of the uroepithelial cells of human urinary tract so it can initiate infection itself. *E. coli* is isolated in 90.0% of infections and strains are characterized by unique virulence determinant, the p pilus (Gal-Gal receptor) (Johnson, 1991). *E. coli* is the most predominant organism to colonize the urethral meatus (Schaeffer and Chmiel, 1983) and perineum (Leigh, 1990) before ascending to the bladder. This ability of *E. coli* may be the reason to be the most frequent organism to cause UTI in both sexes all over the world.

E. coli infection is high in female as compared to male. In this study also, *E. coli* infection was found to be 83.14 % in females whereas 16.85 % in males (out of total *E. coli* isolates). Similar type of result was found by Vorland *et al.* (1985), Kosakai *et al*. (1990), Gautam (1998), Dhakal (1999) and Shrestha (2007).

Citrobacter spp. was isolated as the second commonest pathogen in frequency causing UTI, which accounted for 5.02 % (11/219). The finding of this study is not in harmony with the study done by Gautam (1998), Astal *et al*. (2002), Hadiza *et al*. (2003), Kumari *et al.* (2005), Manandhar *(*2005), Zhanel *et al*. (2005) and Das *et al*. (2006). But this finding is consistent with overall result in a sense Gram negative bacteria are the major source of UTI.

In the present study, the incidence of *K. pneumoniae* was found to be 1.83% and that of *K. oxytoca* was 0.91%, which follows the statement of Fowler (1990)-"*K. pneumoniae* is the primary pathogen in the genus although *K. oxytoca* may also cause bacteriuria." hundred percent of *K. pneumoniae* was isolated from female patients only.

Proteus mirabilis accounted for 1.37% of total bacterial isolates. *Proteus* spp. produces urease resulting in rapid hydrolysis of urea with liberation of ammonia. Thus in UTI with *Proteus*, the urine becomes alkaline promoting stone formation and making acidification virtually impossible. The rapid motility of *Proteus* may also contribute to

its invasion of the urinary tract (Brooks *et al.*, 2004). Formation of infectious urinary calculi is the most common complication accompanying UTI by members of the genus *Proteus* supported by earlier studies (Li *et al.*, 2002; Torzewska *et al.*, 2003).

Four isolates of *Enterobacter* spp. were isolated during the study period. Most of (3/4) the isolates belonged to female patients. Two isolates of *Acinetobacter* spp. was isolated during the study period. This finding is in harmony with the study done by Modi and Erch (2006) in which *Acinetobacter calcoaceticus* accounted for 1.37%.

Among Gram positive bacteria, CONS was found to be the most predominant with 2.74% of the total isolates. The present finding agrees with the studies done by Dhakal (2002) and Shrestha (2005). This organism is the most predominant species colonizing the urethra and the perineum in both sexes. Furthermore, it is an opportunistic pathogen and can cause infection when the immune system is impaired. *S. aureus* constituted only 0.46% of total isolates in this study. Presence of this organism in urine often indicates pyelonephritis acquired via hematogenous spread, so a pure culture of *S. aureus* is considered to be significant regardless of the number of CFUs (Forbes *et al*., 2002).

Microscopic observation of the urine was done by wet mount preparation. The purpose of microscopy by wet mount preparation was to determine the number of white cells and red cells. Finding of ≥5WBCs/HPF is of great importance, while erythrocytes and epithelial cells are of poor significance for UTI diagnosis (Merila *et al*., 1987). Eisinger *et al*. (1997) has suggested that the finding of >10WBC/HPF in urine sediments predicts a positive urine culture and hence indicates UTI. But other many workers (Wargotz *et al*., 1987; Ziloski and Smucker, 1989; Abyad *et al.*, 1991 and Chakraborty, 1995) concluded that pyuria is significant if >5 leucocytes are seen per HPF.

In this study, significant pyuria was observed in 27.51% (52/189) of requests. In this study, Out of 137 cases of insignificant pyuria, only 4 showed culture positive while remaining 133 showed culture negative results. Based on this result, the sensitivity and

specificity of pyuria as a screening test for UTI were calculated as 91.66% and 94.32% respectively. Positive predictive value of WBC count of ≥5/HPF for growth positive culture was found out to be 84.61%.

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 this study and was equally occurred in both sexes. As mentioned in the earlier text, bacteriuria without significant pyuria often occur in cases of asymptomatic patients, patients with diabetes, enteric fever or bacterial endocarditis whereas significant pyuria with sterile bacterial cultures occur in patients with prior antibiotic use, pregnancy, renal tuberculosis (abacterial pyuria) corticosteroid administration, analgesic nephropathy, renal calculi or in the presence of bacteria that are not able to grow in the media used.

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

In this study, Nitrofurantoin (82.77%) was found to be the most effective antibiotic against Gram negative bacteria. In a similar study carried out by Dhakal (1999) at Tribhuvan University Teaching Hospital, 84.21% of urinary isolates were susceptible to Nitrofurantoin. Most of the bacteria were sensitive except few strains of *E.coli*, *Klebsiella*, *Enterobacter* and *Citrobacter*. This finding is supported by the findings of Arosio *et al*. (1979), Jha and Yadav (1992), Levett (1993) and Gautam (1998). In this study, Nitrofurantoin moderately inhibited the growth of *Proteus mirabilis* (33.33%) as reported the same result by Modi and Erch (2006). Nitrofurantoin should be considered

as drug of choice for acute, uncomplicated UTI, particularly in view that it continues to show such low in-vitro resistance (Obi *et al*., 1996).

On the other hand, Amoxicillin (among primary antibiotics evaluated) was found to be the least effective drug against Gram negative bacteria (57.98% resistant). In this study 55.6% of *E. coli* and 100% of *K. pneumoniae* were found to be resistant to Amoxicillin. Resistant to Amoxicillin was also observed by various other researchers (Arosio *et al.,* 1978; Obi *et al.,* 1996). Similar results were found by Sharma (1983) and Bomjan (2005), in which Amoxicillin resistance was present in more cases with *E. coli.*

Resistance to β-lactam antimicrobial agents in ***E. coli*** is primarily mediated by ß-lactamases, which hydrolyze the β-lactam ring and thus inactivate the antibiotic. The classical TEM-1, TEM-2, and SHV-1 enzymes are the predominant plasmid-mediated β-lactamases of gram-negative rods (Livermore, 1995). In addition to this mechanism, there are more than seven efflux systems in *E. coli* that can export structurally unrelated antibiotics; these multidrug resistance efflux pump (MDR pump) systems contribute to intrinsic resistance for toxic compounds such as antibiotics, antiseptics, detergents, and dyes (Sulavik *et al*., 2001).

Among the antibiotics evaluated secondarily, Amikacin was found to be effective (82.14%) against Gram negative bacteria. Ceftazidime was found to be least effective (100%). This result was different from the findings of Bomjan (2005).

About 44.98% and 36.38% of Gram negative bacteria were resistant to Co-trimoxazole and Norfloxacin respectively. Perhaps the most significant change in resistance among uropathogens has been the increase in the prevalence of resistance to trimethoprim–sulfamethoxazole (TMP–SMX), the current drug of choice in the United States for empirical therapy for uncomplicated UTI in women. In addition, TMP–SMX resistance has been associated with concurrent resistance to other antibiotics, resulting in multidrug-resistant uropathogens (Zhanel *et al*., 2000; Sahm *et al*., 2001). The published

in vitro surveillance data from centers across the United States and Canada indicate that approximately 10 to 25% of urinary tract isolates of *E. coli* from female outpatients are resistant to trimethoprim-sulfamethoxazole (SXT) (Karlowsky *et al*., 2002).

Similarly, resistance of Ciprofloxacin and Ofloxacin against Gram negative bacteria was found to be 35.42% and 34.46% respectively. In contrast to this result, Norfloxacin has been recommended as highly effective antimicrobial (Esko and Renkonen, 1985; Chattopadhyay and Mandal, 1993). It has been suggested that fluoroquinolones (Ciprofloxacin, Norfloxacin and Ofloxacin) are a logical choice for empirical therapy of uncomplicated UTIs (Tice, 1999). However, the widespread use of fluoroquinolones for such a common infection raises concerns regarding the possibility of accelerated development of resistance (Warren *et al.,* 1999). Fluoroquinolone resistance among Gram negative bacteria is found predominantly among MDR isolates suggesting that fluoroquinolone resistance will be maintained and perhaps accelerate even if other antimicrobials are used (Friedrich *et al.,* 1999).

Since the use of fluoroquinolone antibiotic in clinical practice was introduced about a decade ago, quinolone-resistant *E. coli* (QREC) strains are being isolated with increasing frequency. Eom *et al*. in 2002 reported that the incidence of QREC increased steadily from 14.4% to 21.3% during 5 years from 1996 to 2000 in Korea. In their study they found that the multidrug resistance rate of QREC was much higher (38.3%) than those of quinolone susceptible isolates (18.8%). Mutations at the target site appear to be the major mechanism for fluoroquinolone resistance in *E. coli*; point mutations conferring resistance are localized to particular portions of *gyrA*, which is a gyrase subunit gene, and *parC*, which encodes a topoisomerase subunit. In the case of *E. coli*, mutations in *gyrA* are predictive of major differences in the level of resistance, irrespective of mutations in *parC* (Ozeki *et al*. 1997).

In our study, both the isolates of *P. aeruginosa* were found to be MDR-strains, and one was isolated from in patients and another from out patient Rates of colonization with *P.*

aeruginosa increase in hospitalized patients, particularly in those who have been hospitalized for extended periods of time and/or have received broad-spectrum antimicrobial therapy or cancer chemotherapy. These increasing resistance rates have greatly limited the number of therapeutic choices (Livermore, 2002).

To evaluate the activity of several antipseudomonal agents, a data analysis from The Surveillance Network (TSN) Database-USA from 1999 to 2002 was carried out by Flamm *et al*. (2002) and found that MDR-strains (resistance to three or more antimicrobial agents) accounted for 24.9% of all isolates.

In our study, both isolates of *P. aeruginosa* were resistant to Ciprofloxacin. Li *et al*. (1994) showed that active efflux played a role in the resistance, to various non- β -lactam agents, of *P. aeruginosa* strains, and de-energization by the addition of a proton conductor increased the accumulation level to that expected for equilibration across the cytoplasmic membrane. Their study also suggested the involvement of an active efflux mechanism also in the resistance to β-lactams; the hydrophilic β -lactams with more than one charged group did not cross the cytoplasmic membrane readily, yet one such compound, Ceftriaxone, appeared to be extruded from the cells of more-resistant strains. They postulated that these strains of *P. aeruginosa* pumped out such hydrophilic β-lactams either from the periplasm or from the outer leaflet of the lipid bilayer of the cytoplasmic membrane.

Three mechanisms of resistance are known to cause quinolone resistance in *P. aeruginosa* (Cambau and Gutmann, 1993): alteration in DNA gyrase by mutations in *gyrA* or *gyrB* genes; decreased drug accumulation by decreased permeability of the cell wall, and enhanced efflux *gyrA* mutations appear to be the most prominent cause of resistance in clinical strains (Yoshida *et al*. 1994). The *gyrA* (*nfxA*, *nalA*, or *cfxA*) mutation causes an alteration in the subunit A of DNA gyrase (Robillard and Scarpa, 1988). The *nalB* (*cfxB*) (Masuda and Ohya, 1992), *nfxB* (Hirai *et al*. 1997), and *nfxC* (Fukuda *et al*. 1990) mutations cause a decrease in the level of accumulation of

Norfloxacin, and strains with these mutations show cross-resistance to structurally unrelated antimicrobial agents.

In our study, out of three *Proteus mirabilis* isolates, one was resistant to Amoxycillin and one to Co-trimoxazole, while both were susceptible to Ciprofloxacin, Nitrofurantoin, Norfloxacin, Ofloxacin, Cefotaxime and Cefixime. Bret *et al*. in 1998 had reported a chromosomally encoded class C β-lactamase produced by a clinical strain of *P. mirabilis* resistant to all penicillins and Cephalosporins, including Cephamycins and Aztreonam in France. In a recent study also in France conducted by Neuwirth *et al*. in 2001, a clinical strain of *Proteus mirabilis* (strain Pm 631) was reported to have produced a complex mutant β-lactamase: TEM-89 (CMT-3), which is an Inhibitor-resistant TEM (IRT) β-lactamase.

Among Gram positive isolates, the most effective drug was found to be Co-trimoxazole and Cloxacillin 70.0% each followed by Amoxicillin (60%). Cephalexin was found to be the least effective with the susceptibility of 40.0%. In our study, 3 isolates of *Enterococcus faecalis* were found to be MDR-strains. Vancomycin was 100% effective against the isolates while Amoxycillin and Erythromycin had a susceptibility of 33.33% and 0.00 %. Two mechanisms of β-lactam resistance in *E. faecalis*, the production of β-lactamase and the overproduction of penicillin-binding proteins (PBPs), have been reported. A recent study in 2005 by Ono *et al*. in Japan, suggested that development of high-level resistance to Penicillins and Imipenem in *Enterococcus faecalis* depends on point mutations (amino acid substitutions) of PBP4 at positions 520 and 605. Three different mechanisms account for the acquired resistance to Macrolide-lincosamide-streptogramin (MLS) antibiotics in Gram-positive bacteria: modification of the drug target (23S rRNA); inactivation of the drug, and active efflux of the antibiotic. Erythromycin resistance by *erm* methylases of the *ermB-ermAM* hybridization class has been described in *Enterococcus* isolates (Berryman and Rood**,** 1995).

Though no Vancomycin-resistant enterococci (VRE) were isolated in our study, the prevalence of VRE has been increasing in the United States in the past 15 years. According to the Centers for Disease Control and Prevention, the percentage of enterococcal isolates that were resistant to Vancomycin reported by U.S. intensive care units (ICUs) increased from 0.3% in 1989 to 25.2% in 1999 (CDC, 2000). Approximately 70% of all vancomycin-resistant isolates of *E. faecium* and *E. faecalis* in the United States exhibit the *vanA* phenotype, which is characterized by resistance to Vancomycin and Teicoplanin and is frequently associated with a multidrug resistance phenotype (French, 1998). Of the remaining 30% of Vancomycin-resistant isolates, most exhibit a *vanB* phenotype, which is characterized by resistance to Vancomycin and susceptibility to Teicoplanin (Moellering, 1998). In 2001, Zhanel *et al*. in Canada showed that Nitrofurantoin may provide effective treatment of UTIs caused by Vancomycin-resistant enterococci.

In our study, *S. aureus* was resistant to all the antibiotics evaluated except Vancomycin. Erythromycin resistance in *S. aureus* is part of the macrolide-lincosamide-streptogramin B resistance phenotype. This phenotype was first described by Chabbert shortly after the introduction of Erythromycin in clinical practice (Chabbert, 1956). This phenotype in *S. aureus* has been shown to be due to an Erythromycin resistance methylase (*erm* gene product) which renders newly synthesized ribosomes resistant to macrolide-lincosamide-streptogramin B antibiotics by methylating a specific adenosine residue of the 23S rRNA (Lai and Weisblum, 1971). In *S. aureus*, the genes encoding the methylase have been designated *ermA*, *ermB*, and *ermC* (Westh *et al.* 1995). Erythromycin resistance may also be due to active efflux of the antimicrobial agent by an ATP-dependent pump mediated by *msrA* (Ross *et al*. 1990).

Two different mechanisms of resistance of *S. aureus* to β-lactam antibiotics have been described since the introduction of Penicillin into clinical medicine. The first mechanism is the production of penicillinase, which accounted for the clinical failures

of Penicillin G reported in the early 1940s. Four variants of *S. aureus* β-lactamase can be distinguished by serotype (Rosdahl, 1973) and kinetic attributes (Kernodle *et al.* 1989). Although the genes (*bla*Z) encoding the type A, type C, and type D Staphylococcal β-lactamases are usually located on a plasmid, the gene for the type B β -lactamase is believed to reside on the chromosome of phage group II isolates (Meijers and Stobberingh**,** 1980).

The second mechanism, methicillin resistance, is associated with the production of an additional penicillin-binding protein (PBP 2' or 2a) with low affinity for β-lactam antibiotics (Hartman and Tomasz**,** 1986). Unfortunately, most MRSA are also resistant to aminoglycosides, macrolides, Tetracyclines, fluoroquinolones, and Rifampin in many countries (Mapple *et al*. 1989). Therefore, MRSA strains are multiple-resistant strains with therapeutic options that are largely limited to Vancomycin. However, the observation that Vancomycin resistance may be transferred from *Enterococcus faecalis* to *S. aureus* in vitro (Noble *et al*. 1993), together with the detection of Vancomycin resistance in clinical isolates of coagulase- negative Staphylococci, including *S. epidermidis* (Sanyal *et al*. 1991), raised the worrisome possibility that resistance to Vancomycin may emerge in *S. aureus*. In 1996, the first clinical isolate of *S. aureus* with reduced susceptibility to Vancomycin was reported from Japan (Hiramatsu *et al*. 1997). The Vancomycin MIC result reported for this isolate was in the intermediate range (Vancomycin MIC=8 µg/ml) using interpretive criteria defined by the National Committee for Clinical Laboratory Standards.

In June 2002, a clinical isolate of Vancomycin-resistant *S. aureus* (VRSA) (MIC, >32 µg/ml) was isolated from a dialysis patient in Michigan (Chang *et al*. 2003). For this highly resistant strain, which contained the *van*A determinant which mediates resistance to vancomycin in enterococci, the Vancomycin MIC was 1,024 µg/ml. The isolate was also resistant to Oxacillin, Levofloxacin, and Rifampin.

Multiple drug resistance (MDR) was defined as resistance to three or more of the antimicrobial agents evaluated in the study (Kurutepe *et al*., 2005). Among the 219 isolates that were evaluated against 8 antimicrobials, MDR isolates accounted for 41.55% (91/219). Out of total 209 Gram negative isolates, 40.19% (84/209) were found to be multidrug-resistant and that of Gram positive isolates was 70% (7/10). The lower prevalence of MDR, 13.9 % was found in the study done by Oteo *et al*. (2001) when MDR criterion was resistance to 3 or more antibiotics. In a study done by Tuladhar *et al.* (2003) at TUTH, MDR bacterial strains were detected in 35.2% cases in which the most predominant was *E. coli* (22.2%) followed by *Klebsiella* spp. (6.1%) and *S. aureus* (2.2%). But in this study, MDR in *E. coli* were found to be 38.2% (68/178).

Outcome of prevalence of MDR depends on various factors, MDR criterion being the chief one followed by the types of antibiotics used in antibiogram and study population. The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used (Levy, 1997).

Higher rate of MDR was found in males 58.69% (27/46) than in females 36.99% (64/173). However, the association of MDR and non-MDR strains in males and females was found statistically significant ($p<0.05$). This finding does not agree with the study findings of other researchers: Sahm *et al*. (2001), Oteo *et al.* (2001) and Manandhar *et al.* (2005). This trend likely reflects the tendency for males to present more often with complicated UTIs, which may be associated with more antimicrobial-resistant pathogens (Sahm *et al.*, 2001). We found the antibiotic resistance was 1.56 times higher in patients who already had been exposed to different antibiotics during the medication compared to those who had not taken any antibiotics in the last one month though the response rate was very low to this particular question “Whether they took any antibiotics in the last one month”? This also might indicate the poor knowledge of people regarding the antibiotics.

MDR of *E. coli* was analyzed and 38.20% of isolates were found MDR. Among the MDR *E. coli* isolates, 94.11% (64/68) were resistant to Amoxicillin, 95.58% (65/68) to Norfloxacin, 86.76% (59/68) to Co-trimoxazole, 92.64% (63/68) to Ofloxacin and Ciprofloxacin, 17.64% (12/68) to Nitrofurantoin, 79.41% (54/68) to Cefotaxime and 77.94% (53/68) to Cefixime. The consistent and high-level susceptibility of *E. coli* to Nitrofurantoin may be influenced by Nitrofurantoin's narrow spectrum of activity, limited indication (treatment of acute cystitis), narrow tissue distribution (low or undetectable serum concentrations) and limited contact with bacteria outside the urinary tract (Hooper, 2000). In a study done by Kurutepe *et al.* (2005), 100.0% of MDR *E. coli* isolates were resistant to Ampicillin whereas 80.6%, 74.1% and 33.3% of them were resistant to Ciprofloxacin, Gentamicin and Nitrofurantoin respectively. High resistance rate of MDR *E. coli* isolates to Norfloxacin found in the present study is of great concern. A gradual decrease in the susceptibility of *E. coli* to fluoroquinolones (approximately 1.0% per annum) has also been reported by the US arm of the SENTRY surveillance program, with no change in susceptibility to Nitrofurantoin (Jones *et al*., 1999; Mathai *et al*., 2001). Increasing fluoroquinolone resistance among urinary *E. coli* has also been documented in studies conducted outside the US (Goettsch *et al*., 2000).

Previous reports have indicated that the high resistance of uropathogenic bacteria to antimicrobial agents in developing countries (Lester *et al*., 1990) is often due to self-medication, the suboptimal quality of antimicrobial drugs, and poor community and patient hygiene (Walson *et al*., 2001). Second, inappropriate use of antimicrobial agents is widespread as many people can easily buy antibiotics from some pharmacy stores and patent medicine stores, with or without prescriptions. This widespread and inappropriate use of antibiotics is recognized as a significant contributing factor to the spread of bacterial resistance and the development of resistance to antimicrobial agents (Mincey and Parkulo, 2001). Third, there is evidence that for most bacteria, increased usage of a particular antimicrobial agent correlates with increased levels of bacterial resistance to that agent (Granizo *et al*, 2000).

Effective management of UTIs in both the inpatient and outpatient settings has been complicated by the fact that many uropathogenic strains have developed resistance to antimicrobials, including Co-trimoxazole, the current first-line treatment for uncomplicated UTIs in the US and many other countries (Blondeau, 2004).

The plasmid profiling showed the largest plasmid was 51 Kb which was followed by 38 Kb, and 32.5 Kb. This result is in agreemetnt with the findings of Shehabi *et al*. (2002), Mandal *et al.* (2004), Wang *et al.* (2004) and Enabulele *et al.* (2006). We did not find very large plasmids as was mentioned by different researchers as Olukoya and Olasupo (1997) and Aibinu *et al*. (2004). In our study the plasmids having smaller size (2, 2.6, 3.2, 3.7, 4.5, 4.7, 6.5, 7.5, 10 Kb) were also found. Other researchers (Shakya, 2001; Ghimire, 2004; Paneru, 2002) also mentioned the occurrence of the small plasmids. The number of plasmid bands per isolate did not reflect the nature of resistant markers.

The resistant transfer study showed successful transfer of resistance to the recipients. This result is further supported by the findings of previous researchers (Mandal *et al.*, 2004; Aibinu *et al.*, 2004). The transfer of comparably small plasmids along with large plasmids were also encountered in our study as some researcher (Bartoloni *et al*., 2006) mentioned resistance traits could be transferred by conjugation, often en bloc, suggesting a linkage of the corresponding resistance genes in self-transferable or mobilizable plasmids.

6.2 CONCLUSION

The study was carried out to determine the prevalence of MDR bacterial isolates causing UTI and study of their plasmid and their role in transferring antibiotic resistance. The main findings of this study are that there is significant difference of positive growth between male and female patients ($P<0.05$). Similarly the statistical analysis show significant difference of MDR strains between genders ($P<0.05$). The highest susceptibility was shown by Nitrofurantoin (82.77%) against Gram negative isolates and Cloxacillin and Co-trimoxazole were the drug of choice against Gram

positive isolates. Vancomycin was drug of choice for enterococci and MRSA. The overall prevalence rate of MDR was found to be 41.55%. Among MDR bacterial isolates, there are high resistance rates to almost all antimicrobial agents evaluated except few and alarmingly high resistance rates to fluoroquinolones. This necessitates a re-evaluation of the first and second line therapies for the treatment of community acquired-UTI and regular monitoring of the usage of antimicrobials in order to make reliable information available for optimal empirical therapy for outpatients with UTIs. Plasmid profiling showed transferable conjugative plasmids transferring resistance to various antibiotics.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. Out of 710 mid-stream urine samples, 219 (30.85%) were growth positive with significant number of bacteria and 491 samples (69.15 %) showed either insignificant or no growth.
2. The infection rate was found to be higher in females (33.52%) than in males (23.71%). Association of significant bacteriuria and gender of patients was found to be statistically significant ($P<0.05$).
3. Altogether 16 different bacteria were isolated from growth positive urine samples. *E. coli* (81.28%) was found the most predominant organisms followed by *Citrobacter* spp. (5.02%), *Klebsiella* spp. and CONS (2.74%), *Enterobacter* spp. (1.83%), *Proteus mirabilis* and *Streptococcus faecalis* (1.37%), *Acinetobacter* spp. *P. aeruginosa* and *M. morganii* (0.91%), *S. aureus* and *Salmonella typhi* (0.46%).
4. Microscopy of pyuria showed the sensitivity of 91.66% and the specificity of 94.32%. The positive and negative predictive values were found to be 84.61% and 97.08% respectively.
5. The most effective antibiotic against Gram negative bacteria was found out to be Nitrofurantoin (82.77%) followed by Cefotaxime (68.42%) whereas Ampicillin was found out to be the least effective drug. Amikacin was found most effective against Gram negative bacteria used secondarily. Co-trimoxazole and Cloxacillin were found to be the most effective antibiotic against Gram positive bacteria with a susceptibility of rates of 70%. Multidrug resistance (MDR) was observed in 41.55% (91/219) of bacterial isolates. Multidrug resistance was found to be 38.20% (68/178) in *E. coli*.

6. Higher status of MDR was found in males (58.69%, 27/46) than in females (36.99%, 64/173). Among the MDR *E. coli* isolates, 95.58% to Norfloxacin 94.11% were resistant to Ampicillin and 92.64% to Ciprofloxacin and ofloxacin. Most of the MDR *E.coli* were found to contain 32.5 Kb plasmid.

7.2 RECOMMENDATIONS

1. Microscopic examination of urine prior to culture is useful for correlating pyuria and bacteriuria. Thus, it should be done routinely.
2. As this study was confined to KMH, it does not necessarily reveal the picture of the country, therefore systematic prospective surveillance should be carried out throughout the year covering wide geographical region in order to obtain information on seasonal, geographical and ethnic variation of pathogens and their antibiotic susceptibility profile from the scientific and practical point of view.
3. All diagnostic laboratories should make records on multidrug resistance of strains during the long time intervals. These data when statistically evaluated can serve as criteria for consumption planning of antimicrobial drugs in a given region.
4. Infection control programmes should be established with responsibility for effective management of antimicrobial resistance in hospitals and it should be ensured that all hospitals have access to such a programme. Further study on etiology especially the organisms that cannot grow on the media used and the provided cultural conditions should be carried out as an extension of this study.
5. Together with the surveillance of R factors, however, well founded genetic and molecular biological studies of these factors and other bacterial plasmids are urgently needed. Purely basic studies must also be encouraged in parallel with the molecular studies.

6. Strict rules and regulations of antibiotic policy should be implemented in our country to check selling of antibiotics without prescription, and thus check the development of resistance to some extent.

CHAPTER VIII

8. REFERENCES

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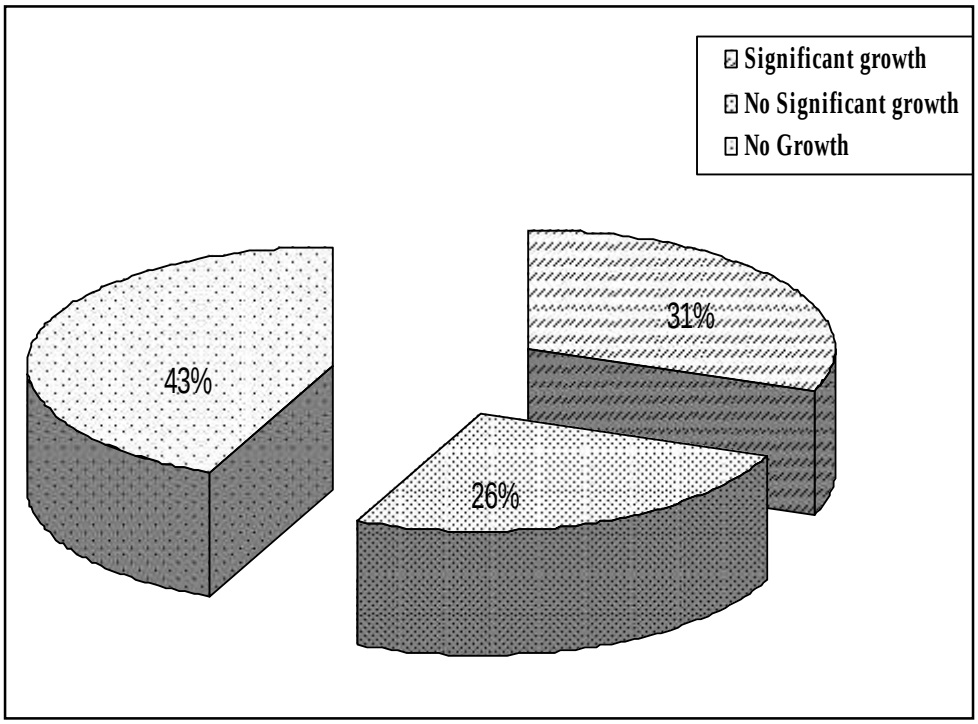
Significant growth
No Significant growth
No Growth
43%
31%
26%

APPENDIX-I

Questionnaire

Date :

Name : Sex :

Age : Lab. No. :

Inpatient / Outpatient

J Do you currently have any of the following symptoms?

Dysuria	
Frequency	
Urgency (compelling need to urinate)	
Tenderness and pain above bladder	
Flank pain	
Fever	
Vomiting	
Diarrhea	
Chills	
Lower abdominal pain	

---

1. Did you suffer from UTI before?
   Yes No

2. If yes, then mention
   Did you take any antibiotics last 1 month?
   Yes No

3. If yes, did you take full course?
   Yes No

4. Are you taking any other drug for the disease?
   Yes No

Day-1

Lab tests

a. Appearance of specimen

Clear

Cloudy

b.Microscopic examination of specimen

crystals Bacteria

Casts

Pus cells

Red cells

Yeast cells

Epithelial cells

b. Gram smear

Bacteria

- Gram positive cocci / rod
- Gram negative cocci / rod

Culture on Blood agar and Mac-conkey agar by semi- quantitative method

Day-2

a. Examine the culture on

- BA Haemolytic /Non-haemolytic
- MA LF / NLF

b. Observe for growth

Recommendation for reporting (Cheesbrough, 2000)

S.N.	Number of organisms/ml	result	Remarks
1.	$<10^4$ CFU/ml	Contaminants	
2.	Equal to or more than 10^5 CFU/ml	Significant bacteriuria	
3.	10^4-10^5 CFU/ml	Doubtful significant bacteriuria (Repeat sample)	

Tests

a. Gram staining

b. Oxidase test

c. Catalase test
d. Coagulase test

Day-3

a. Observe and identify the organism

S.N.	Tests	Results
1.	Sulphide	
2.	Indole	
3.	Motility	
4.	MR	
5.	VP	
6.	Citrate utilization	
7.	TSI	
8.	O/F	
9.	Urease	
10.	Nitrate reduction	
11.	MSA	

Organism identified as……

b. Antibiotic susceptibility test

S.N.	Gram positive bacteria	Sensitive	Resistant	Gram negative bacteria	Sensitive	Resistant
1.	Amoxycillin			Amoxicillin		
2.	Cephalexin			Cefixime		
3.	Ciprofloxacin			Cefotaxime		
4.	Cloxacillin			Ciprofloxacin		
5.	Co-trimoxazole			Co-trimoxazole		
6.	Erythromycin			Nalidixic acid		
7.	Norfloxacin			Nitrofurantoin		
8.				Norfloxacin		
9.				Ofloxacin		
10.						

Approved by: ……

Signature:
Date:

APPENDIX –II

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

A. EQUIPMENTS

Hot air oven	Universal (India)
Microwave oven	LG
Incubator	Memmert (Germany)
Autoclave	
Refrigerator	Sanyo (Japan)
Microscope	Olympus (Japan)
Centrifuge	Remi (India), Eppendorf (Germany)
Weighing Machine	Scaltec instruments (Germany)
Water bath shaker	Grant, OLS 200 (UK)
UV transilluminator	Alpha Innotech Corporation (Germany)
Thermal printer	Alpha Innotech Corporation (Germany
Electrophoretic apparatus	International Biotechnologies Inc, USA
Voltguard	Fisher Scientific,USA
AC automatic voltage regulator	Matsunaga ,Japan
Spectrophotometer	Alpha Innotech Corporation (Germany)

B. MICROBIOLOGICAL MEDIA

Blood agar	Mueller Hinton broth
MacConkey agar	Simmons Citrate agar
Nitrate broth	Hugh Leiffson media
MR-VP medium	Mueller Hinton agar
Sulphur Indole Motility agar	Nutrient agar
Triple Sugar Iron agar	Luria Berteni agar / broth
Urea agar	Bile esculin agar

C. CHEMICALS AND REAGENTS

3%Hydrogen peroxide	Barritt's reagent	Barium chloride
Crystal violet	Absolute (95%) alcohol	Kovac's reagent
Gram's iodine	Phenol (equilibrated)	Sulphuric acid
Safranine	Tris base	Chloroform
Sodium Chloride	Ethylene diamine tetra acetate (EDTA)	
Tris buffer	Sodium hydroxide	Potassium acetate
Glacial acetic acid	Ethidium bromide	SDS
Glucose	Glycerol	Agarose
Bromophenol blue	Sucrose	α-Naphthylamine
Acetic acid	Sulfanilic acid	

D. ANTIBIOTIC DISCS

All the antibiotics discs used for the susceptibility tests were from Hi-Media Laboratories Pvt. Limited, Bombay, India. The antibiotics used were as follows

Ampicillin (10mcg)	Gentamicin (10mcg)
Ceftriazone(30mcg)	Nitrofurantoin (300mcg)
Ciprofloxacin (5mcg)	Norfloxacin (10mcg)
Cloxacillin (1mcg)	Ofloxacin (5mcg)
Cotrimoxazole (1.25/23.75mcg)	Oxacillin (1mcg)
Erythromycin (15mcg)	Ceftazidime (30mcg)
Chloramphenicol (30mcg)	Amikacin (30mcg)

E. MISCELLANEOUS

Conical flasks, Cotton, Distilled water, Droppers, Forceps, Glass slides and cover slips, Immersion oil, Inoculating loop, Inoculating wire, Lysol, Measuring cylinder,Erlenmeyer flask, Petri dishes, Pipettes, Plastic containers, Spatula, Test tubes, Wooden applicator sticks**,** Antibiotic powders

APPENDIX-III

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. MacConkey Agar (MA)

(With sodium taurocholate, with salt and crystal violet)

Ingredients	gm/liter
Peptone	20.0
Lactose	10.0
Sodium taurocholate	5.0
Crystal violet	0.015
Sodium chloride	5.0
Neutral Red	0.04
Agar	20.0

Final pH (at 25^0C) 7.4±0.2

55 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.

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 121^0C for 15 minutes.

6. Bile Esculin agar

Ingredients	gm/litre
Peptone	5.0
Beef extract	3.0
Oxgall (bile)	40.0
Esculin	3.0
Ferric citrate	0.5
Agar	15.0

Final pH (at 25^0C) 6.6±0.2

64.5 grams of the medium was dissolved in 1000 ml distilled water and autoclaved at 121^0C for 15 minutes.

7. Trypticase Soy Broth

Ingredients	gm/litre
Trypticase (animal peptone)	15.0
Phytone (Soy peptone)	5.0
Sodium chloride	5.0
Final pH (at 25^0C)	7.3

25 grams of the medium was dissolved in 1000 ml distilled water and autoclaved at 121^0C for 15 minutes.

8. Lauria- Bertani Broth/ Agar

Ingredients	gm/litre
Tryptone	10.0
Yeast extracts	5.0
Nacl	10.0
*[Agar	15.0 (for agar plates)]

* 15 grams agar added in the case of preparation of LB agar

9. LB broth

All the ingredients were added to a clean 2 L flask that has been rinsed with deionised or distilled water. 1l deionized or distilled water was added to the flask. 0.5 ml of 4-M NaOH was added. The dry ingredients were stirred to dissolve; preferably using a magnetic stir bar. The preparation was autoclaved for 20 minutes at 121^0C.

10. LB broth + antibiotic (can be stored at 4^0C for 3 months)

1 ml of 10 mg/ml antibiotic was sterilely added to 100 ml of cooled LB broth.The preparation was swirled to mix.

11. LB agar + antibiotic (can be stored at 4^0C for 2 months) (1000 ml)

LB agar solution was made from the ingredients listed above and autoclaved. When the agar flask was cool enough to hold, 10ml of 10mg /ml antibiotic solution was added sterilely. The flask was swirled to mix the antibiotic. The media solution was poured on the sterile Petri-plates.

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 and autoclaved for 15 minutes at 15 lbs pressure (121^0C).

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 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 and sterilized by autoclaving at 121^{0}C for 15 minutes. After autoclaving tubes containing medium were tilted to form slant.

5. Nitrate Broth

Ingredients	gm/litre
Beef extract	3.0
Peptone	5.0
Potassium Nitrate	1.0

Final pH (at 25^{0}C) 6.8±0.2

9 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.

6. 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^{0}C) 7.4±0.2

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

7. 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^{0}C) 7.4±0.2

24 grams of the medium was suspended in 950 ml distilled water and sterilized by autoclaving at 121^0C 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.

2. Normal saline

Sodium Cholride	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.

3. 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 - Napthol	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. Nitrate Reduction Test

Solution A

A-Naphthylamine	0.1g
Acetic acid (5M/l)	20 ml

Preparation: Dissolve 0.1g α-Naphthylamine in 20ml acetic acid.

Solution B

Sulfanilic acid	0.16g
Acetic acid (5M/l)	20 ml

Preparation: Dissolve 0.16g Sulfanilic acid in 20 ml acetic acid. Label it corrosive.

5. Chemicals of plasmid DNA analysis

A. Tris- acetate EDTA buffer (50X) (Electrophoresis buffer)

Tris base	242g
Glacial acetic acid	57.1ml
0.5 M EDTA	100 ml (p^H 8.0)
Working solution	1X

B. 6X gel loading buffer (100 ml)

Bromophenol blue	0.25%
Sucrose in water	40% (w/v)

C. 0.5 M Ethylene Diamine Tetra acetic acid (EDTA) (p^H 8.0) 100ml (can be stored at room temperature indefinitely).

18.6 g of EDTA (disodium salt, m. w. 372.24) was added to 80 ml of deionized or distilled water. The p^H was adjusted to 8.0 by slowly adding the sodium hydroxide. The preparation was mixed vigorously using a magnetic stirrer.

D. 5 M potassium acetate (200 ml, store at room temperature.)

98.1 g potassium acetate was added to 160 ml of distilled water. Distilled water was added to make final volume of 200ml.

E. 10 % Sodium Dodecyl Sulfate (100ml, store at room temperature.)

10 g of electrophoresis grade SDS (m.w. 288.37) was dissolved in 80 ml of distilled water.the final volume was made 100 ml.

F. Solution I (Glucose / Tris / EDTA (GTE) (100 ml) store at room temperature)

Glucose	0.9g (m.w. 180.16)
Tris	2.5 ml of 1 M (p^H 8.0)
EDTA	2 ml of 0.5M
Water	94.5 ml

G. Solution II (1% SDS/ 0.2 N NaOH 10ml)

SDS	1 ml (10%)
NaOH	0.5ml (4N)
Water	8.5 ml

H. Solution III (Potassium acetate / acetic acid 100ml, store at room temperature)

11.5 ml of glacial acetic acid was mixed with 60 ml of 5M potassium acetate and 28.5 ml distilled water.

I. Solution IV (phenol/chloroform (v/v))

Phenol	50 ml (equilibrated)
Chloroform	50 ml

J. Solution V (Tris EDTA buffer 100 ml store at room temperature)

Tris	1 ml of 1M (p^H 8.0)
EDTA	200µl of 0.5M
Water	99 ml

APPENDIX-IV

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.

APPENDIX-V

1. BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

A. Catalase test

This test is performed to demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide.During aerobic respiration, in the presence of oxygen, microorganisms produces hydrogen peroxide, which is lethal to the cell itself. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, the main exception being *Streptococcus* spp.

Procedure: A small amount of a culture from Nutrient Agar plate was taken in a clean glass slide and about 2-3 drops of 3% H_2O_2 was put on the surface of the slide. The positive test is indicated by the formation of active bubbling of the oxygen gas. A false positive reaction may be obtained if the culture medium contains catalase (e.g. Blood Agar) or if an iron wire loop is used.

B. Oxidase test

This test is performed for the detection of cytochrome oxidase in bacteria which catalyzes the transport of electrons between electron donors. In the presence of redox dye Tetramethyl-*p*-phenylene diamine dihydrochloride, the cytochrome oxidase oxidizes it into a deep purple colored end product Indophenol which is detected in the test. The test is used for screening species of *Neisseria, Alcaligenes, Aeromonas, Vibrio, Campylobacter* and *Pseudomonas* which give positive reactions and for excluding the Enterobacteriaceae, all species of which give negative reactions.

Procedure: A piece of filter paper was soaked with few drops of oxidase reagent (Whatman's No. 1 filter paper impregnated with 1% tetramethyl-p-phenylene diamine dihydrochloride). Then the colony of the test organism was smeared on the filter paper. The positive test is indicated by the appearance of blue-purple color within 10 seconds.

C. Indole Production test

This test detects the ability of the organism to produce an enzyme: 'tryptophanase' which oxidizes tryptophan to form indolic metabolites: indole, skatole (methyl indole) and indole acetic acid. The enzyme tryptophanase catalyses the deamination reaction attacking the tryptophan molecule in its side chain and leaving the aromatic ring intact in the form of indole.

Procedure: A smooth bacterial colony was stabbed on SIM (Sulphide Indole Motility) medium by a sterile stab wire and the inoculated media was incubated at 37°C for 24 hours. After 24 hours incubation, 2-3 drops of Kovac's reagent was added. Appearance of red color on the top of media indicates indole positive. Indole if present combines with the aldehyde present in the reagent to give a red color in the alcohol layer. The color reaction is based on the presence of the pyrrole structure present in indole.

D. Methyl Red test

This test is performed to test the ability of an organism to produce and maintain stable acid end product from the fermentation of glucose to give a red color with the indicator methyl red and to overcome the buffering capacity of the system. Medium used in the study was Clark and Lubs medium (MR/VP broth, pH 6.9). Methyl red is an indicator which is already acid and will denote changes in degree of acidity by color reactions over a pH range of 4.4- 6.0.

Procedure: A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of methyl red reagent was added and mixed well. The positive test was indicated by the development of bright red color, indicating acidity and negative with yellow color.

E. Voges-Proskauer (VP) test

The principle of this test is to determine the ability of some organisms to produce a acetyl methyl carbinol, a neutral end product (acetoin) or its reduction product 2, 3-butanidiol during fermentation of carbohydrates. An organism of the Enterobacteriaceae group is usually either methyl red positive and Voges- proskauer- negative or methyl red negative and Voges-Proskauer positive. The Voges proskauer test for acetoin is used primarily to separate *E. coli* from *Klebsiella* and *Enterobacter* species.

Procedure: A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of Barritt's reagent was added and shaken well for maximum aeration and kept for 15 minutes, positive test is indicated by the development of pink red color.

F. Citrate Utilization test

This test is performed to detect whether an organism utilizes citrate as a sole source of carbon for metabolism with resulting alkalinity. The medium used for citrate fermentation (Simmon’s Citrate medium) also contains inorganic ammonium salts. Organisms capable of utilizing citrate as its sole carbon source also utilizes the ammonium salts present in the medium as its sole nitrogen source, the ammonium salts are broken down to ammonia with resulting alkalinity.

Procedure: A loopful of test organism was streaked on the slant area of Simmon's Citrate Agar medium and incubated at 37°C for 24 hours. A positive test was indicated by the growth of organism and change of media by green to blue, due to alkaline reaction. The pH indicator bromothymol blue has a pH range of 6.0-7.6, i.e. above pH 7.6; a blue color develops due to alkalinity of the medium.

G. Motility test

This test is done to determine if an organism was motile or non-motile. Bacteria are motile by means of flagella. Flagella occur primarily among the bacilli; however a few cocci forms are motile .Motile bacteria may contain a single flagella. The motility media used for motility test are semisolid, making motility interpretations macroscopic.

Procedure: Motility of organism was tested by hanging drop and cultural method. In cultural method, the test organism was stabbed in the SIM medium and incubated at 37ºC for 48 hours. Motile organisms migrate from the stabline and diffuse into the medium causing turbidity. Whereas non-motile bacteria show the growth along the stabline, and the surrounding media remains colorless and clear.

H. Triple Sugar Iron (TSI) Agar Test

The TSI agar is used to determine the ability of an organism to utilize specific carbohydrate incorporated in the medium (glucose, sucrose and lactose in concentrations of 0.1%, 1.0% and 1.0% respectively), with or without the production of gas (indicated by cracks in the media as well as an air gap at the bottom of the tube) along with determination of possible hydrogen sulfide production (detected by production of black color in the medium). A pH indicator (phenol red) included in the medium can detect acid production from fermentation of these carbohydrates and it gives yellow reaction at acidic pH, and red reaction to indicate an alkaline surrounding.

Procedure: The test organism was streaked and stabbed on the surface of TSI and incubated at 37°C for 24 hours .Acid production limited only to the butt region of the tube is indicative of glucose utilization, while acid production in slant and butt indicates sucrose or lactose fermentation. The results are interpreted as follows:

a. Yellow (Acid)/ Yellow (Acid), Gas, H_2S → Lactose/ Sucrose fermenter, H_2S producer

b. Red (Alkaline) / Yellow (Acid), No Gas, No H_2S → Only Glucose, not lactose/ Sucrose fermenter, not aerogenic, No H_2S production

c. Red (Alkaline) / No Change → Glucose, Lactose and Sucrose non-fermenter

d. Yellow (Acid)/ No Change → Glucose- Oxidiser

e. No Change / No Change →Non-fermenter

I. Urea Hydrolysis test:

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia and carbon dioxide. Ammonia thus produced changes the color of indicator (phenol red) incorporated in the medium.

Procedure: The test organism was inoculated in a medium containing urea and the indicator phenol red. The inoculated medium was incubated at 37°C overnight. Positive organism shows pink red color due to the breakdown of urea to ammonia. With the release of ammonia the medium becomes alkaline as shown by a change in color of the indicator to pink.

J. Coagulase test

This test is used specifically to differentiate species within the genus *Staphylococcus*: *S. aureus* (usually positive) from *S. saprophyticus*, *S epidermidis* (negative). A positive coagulase test is usually the final diagnostic criterion for the identification of *S. aureus.* Free coagulase and bound coagulase are the two types of coagulase possessed by this organism; most strains possess both free and bound coagulase.

Slide Coagulase Test

Bound coagulase (Clumping Factor) is detected by slide test. The bound coagulase is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma.

Procedure: For slide coagulase test, a drop of physiological saline was placed on three places of a slide, and then a colony of the test organism was emulsified in two of the drops to make thick suspensions. Later a drop of plasma was added to one of the suspensions and mixed gently. Then a clumping was observed within 10 seconds for the positive coagulase test. No plasma was added in second suspension. This was used for the differentiation of any granular appearance of the organism from true coagulase clumping. The third drop of saline was used for a known strain of coagulase positive staphylococci.

Tube Coagulase Test

This test is carried out to detect production of free coagulase. Plasma contains coagulase reacting factor (CRF) which activates free coagulase. The activated coagulase acts upon prothrombin thus converting it to thrombin. Thrombin converts fibrinogen into fibrin which is detected as a firm gel (clot) in the tube test. Tube test is performed when negative or doubtful results are obtained in slide coagulase test.

Procedure: In the tube coagulase test, plasma was diluted 1:10 in physiological saline. Four small tubes were taken, one for test organism, one for positive control, one for negative control, and one to observe self clotting of plasma. Then 0.5 ml of the diluted plasma was pipetted into

each tube and 0.5 ml of test organism, 0.5 ml of positive control (*S. aureus* culture), and 0.5 ml negative control (*S. epidermidis* culture) was added to three tubes, to the fourth tube, 0.5 ml sterile broth was added. After mixing gently, all tubes were incubated at 37^0C on a water bath for 6 hours and observed for gel formation in every 30 minutes. The clotting is observed by gently tilting the tube for positive coagulase test.

APPENDIX-VI

METHOD OF COLLECTION OF MID-STREAM 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. Mid-stream urine (MSU) for microbiological examination is collected as follows:

WOMEN

Women who are 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 the 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 to 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.

MEN

A man who is ambulatory should:

1. Wash his hands.
2. Pull back the foreskin (if not circumcised) and pass a small amount of urine into a sterile container.
3. Still holding back the foreskin, pass most of the remaining urine into a sterile container. This is a mid-stream urine specimen.
4. Place the cover on the container and hand to the nursing staff for prompt delivery to the laboratory.

For bedridden patients

1. If necessary, nursing personnel should pull back the foreskin, wash and dry the glans with soapy water and gauze pads.
2. With foreskin pulled back, the patient should pass a small amount of urine into a urinal.

3. The patient should then pass most of the remaining urine into the sterile container.

The cover should be placed on the container and the specimen transported to the laboratory.

INFANTS AND CHILDREN

Collection of a clean-catch urine specimen from infants and children who are ill in bed or uncooperative can be a problem. Give the child water or other liquid to drink. Clean the external genitalia. The child can be seated on the lap of the mother, nurse or ward attendant, who should then encourage the child to urinate and collect as much urine as possible in sterile container. The container should then be covered and delivered to the laboratory for immediate processing.

APPENDIX-VII

Table: Distinguishing reactions of the commoner and pathogenic 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

ZONE SIZE INTERPRETATIVE CHART

Antimicrobial Agents used	**Symbol**	**Disc Content**	**Resistant (mm or less)**	**Intermediate (mm)**	**Susceptible (mm or more)**
Amikacin	Ak	30 µg	14	15-16	17
Ampicillin When testing gram-			13 28	14-16 -	17 29

negative enteric organisms When testing Staphylococci	Amp	10 µg			
Ceftazidime	Ca	30 µg	14	15-17	18
Ceftriaxone	Ci	30 µg	13	14-20	21
Cephalexin	Cp	30 µg	14	15-17	18
Chloramphenicol	C	30 µg	12	13-17	18
Ciprofloxacin	Cf	5 µg	15	16-20	21
Cloxacillin	Cx	5 µg	12	12-13	14
Cotrimoxazole	Co	1.25/23.75µg	10	11-15	16
Erythromycin	E	15 µg	13	14-22	23
Gentamicin	G	10 µg	12	13-14	15
Nitrofurantoin	Nf	300µg	14	15-16	17
Norfloxacin	Nx	10 µg	12	13-16	17
Ofloxacin	Of	5 µg	12	13-15	16
Oxacillin	Ox	1 µg	10	11-12	13
Vancomycin When testing Staphylococci When testing Streptococci	Va	30 µg	- -	- -	15 17

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

APPENDIX-IX

PROTOCOLS

A. Extraction of plasmid DNA

1. 1.5 ml of MDR *E.coli* culture was transferred to a clean microfuge tube and centrifuged at 10000 rpm.

2. The supernatant was discarded and again the E. coli culture was transferred to the same tube and centrifuged to collect cells from 3 ml of culture.

3. The supernatant was discarded and any residual media was blotted away.

4. 100 µl of ice cold GTE (solution I) was added to the tube. The cells pellet was resuspeded by pipetting ins and pipetting out or by vortexing.

5. 200 µl of SDS/NaOH (solution II) was added. The contents were mixed by closing and rapidly inverting the tubes for five times. The tube was incubated on ice for 5 minutes.

6. 150 µl of ice cold Potassium acetate was added. The contents were mixed by closing and rapidly inverting the tubes for five times. White precipitate appeared.

7. The tube was incubated on ice for 5 minutes.

8. The tubes were centrifuged at 13000 rpm for 10 minutes at 4^0C.

9. 400 µl of supernatant was transferred to a clean microfuge tube avoiding the precipitate.

10. 400 µl of Phenol / chloroform (solution IV) was added and shaken vigorously. It was then centrifuged at 10000 rpm for 2-3 minutes.

11. The upper aqueous phase was transferred to a clean microfuge tube avoiding the white organic substances.

12. 1 ml absolute ethanol was added to the tube and placed at -20^0C for overnight and centrifuged at 13000 rpm for 10 – 15 minutes.

13. The supernatant was discarded and the pellet so formed was dissolved in 100 µl of TE buffer and stored in 4^0C.

B. Agarose gel electrophoresis:

I Preparation of agarose gel (0.5%)

1. 0.5g of agarose was weighed out into a 250mL conical flask. 100mL of 1X TAE was added and swirled to mix.
2. The preparation was microwaved for about 1 minute to dissolve the agarose.
3. It was left to cool on the bench for 5 minutes down to about 60°C.
4. 5µL of ethidium bromide was added (10mg/mL) and swirled to mix.

5. The gel was poured slowly into the tank. Any bubbles were pushed away to the side using a disposable tip. The comb was inserted and double checked that it is correctly positioned.
6. It was left to set for at least 30 minutes, preferably 1 hour, with the lid on if possible.
7. 1X TAE buffer was poured into the gel tank to submerge the gel to 2–5mm depth. This is the running buffer.

II. Preparation of sample

15 µL of each sample was transferred to a fresh microfuge tube.An appropriate amount (3 µL) of 6X gel loading buffer was added into each tube and left the tip in the tube.Similarly the DNA markers were prepared.

III. Loading of sample in the wells

The first well was loaded with 15 µL marker.The samples (15 µL) were loaded in the adjacent wells.

IV. Electrophoresis

1. The gel tank was closed, the power-source was switched on and the gel was run at 70V for 3 hours.
2. Current flowing was checked.

V. Monitoring

1. The progress was monitored on the gel by reference to the marker dye.
2. The gel was stopped when the bromophenol blue had run $3/4^{th}$ the length of the gel.
3. The power was switched off.

VI. Photodocumentation (Visualization)

1. The gel was carried (in its holder if possible) to the room to look at on the UV trans-illuminator.
2. The gel was illuminated at 300nm UV and the picture was saved for analysis.

VII. Decontamination of ethidium bromide

Sufficient water was added to reduce the concentration of EtBr to < 0.5 ug/ml.One volume of 0.5 M $KMnO_4$, one of volume of 2.5 N HCl was mixed and it was allowed to stand for several hours at room temperature.1 volume of 2.5 N xxvi as mixed and the solution was discarded.

C. Transformation

1. A single bacterial colony *(E.coli* TB1) was picked from a plate that had been incubated for 16-20 hours at 37^0C.The colony was transferred into 10 of LB broth. The culture was incubated for 3 hours at 37^oC with vigorous shaking monitoring the growth of the culture until OD_{600} ~0.4 was achieved.
2. The bacterial cells were transferred to sterile eppendorf tube. The cultures were cooled to 0^0C by storing the tubes on ice for 10 minutes.

I. Preparation of competent cells

1. 1.5 ml of the chilled bacterial culture was transferred to a sterile microfuge tube prechilled on ice.
2. The tube was centrifuged at 0^0C for 90 seconds at 10000 rpm.
3. The supernatant was poured off and, while the tube was inverted, any residual growth medium was quickly blotted away. The tube was immediately returned to the ice.
4. Steps 1-3 above were repeated using the same microfuge tube.
5. Cell pellet was re-suspended gently in 1000µl of ice cold competent cell buffer1 (20mM $CaCl_2$ and 80mM Mg Cl_2) and then centrifuged for 90 seconds at 10000 rpm.
6. The supernatant was poured off and the tube was returned immediately to the ice.
7. Cell pellet was re-suspended gently in 200µl of ice cold competent cell buffer 2 (100mM $CaCl_2$).
8. The cell suspension was returned to the ice bath until use in the transformation.

II. Transformation of plasmid DNA

1. 75µl of the competent cell preparation was transferred to the bottom of the sterile plastic tube that had been pre-chilled on ice. The tube was marked for identification.
2. 10µl of the plasmid DNA (0.5ug/ml) preparation was pipetted into the competent cell suspension in the bottom of the tube.
3. The tube was incubated on ice for 10 minutes (to permit binding of the DNA to the cells).
4. The tube was transferred to 42^0C water bath for 90 seconds and then immediately placed it on ice.
5. 900µl sterile Lauria Bertani broth was added to the tube and incubated with shaking at 37^0C for 30-40 minutes.
6. 100µl of the transformation mixture was pipetted onto the surface of L+AMP+S, L+CIP+S, L+TR+S agar. The inoculum was spread over the plate surface using a glass spreader sterilized with flaming ethanol.
7. The plates were incubated at 37^0C for 16-18 hours and the antibiotic resistant colonies were counted.

D. Conjugation

1. The resistant isolates were grown in 5 ml nutrient broth for $4^{1/2}$ with constant shaking (250 rpm).

2. At the same time the recipient strain was also grown in the nutrient broth at 37^0C for 41/2 hours with constant shaking.
3. Then the conjugation mixture was prepared having following composition:
   0.2 ml Donor culture
   2.0 ml Recipient culture
   2.5 ml Warm nutrient broth
4. Then the mixture was incubated at 37^0 C for 24 hours.
5. Transconjugants were selected by plating in suitable media containing appropriate antibiotics.

E. Determination of minimum inhibitory concentration (MIC)

1. Preparation of antibiotic stock solutions

- Informations regarding expiry date, potency, solubility, stability as a powder and in solution, storage conditions and any relevant COSHH (Control of Substances Hazardous to Health) information was obtained from the supplier.

- Appropriate solvent, diluents and storage conditions for different antibiotics used viz.Ampicillin,Chloramphenicol, Cefixime, Ciprofloxacin, Trimethoprim, Norfloxacin, Gentamycin and Streptomycin were chosen.

- Suitable range of antibiotic concentrations for the test organisms (Drug resistant donor strains, transconjugants and transformants) were made.

- **Stock solutions were prepared using the formula**

$$\frac{1000}{P} \times V \times C = W$$

Where, P = Potency given by the manufacturer (µg/mg), V = volume required (ml), C = final concentration of solution (multiples of 1000) (mg/L), and W = weight of antibiotic (mg) to be dissolved in volume V (ml).

II. Preparation of antibiotic dilution range

Dilution range: - 16 -1024 µg/ml

- Ten screw capped tubes were labeled according to antibiotic dilution. A parallel set of tubes containing dilution range for different antibiotics were taken to examine the potency of antibiotics using the reference strain *E. coli* ATCC 25922. Antibiotic ranges should be prepared one step higher than the final dilution range required for the compensation of the addition of an equal volume of inoculum.

- One (1ml) Mueller-Hinton broth was substituted for each tubes i.e. tube 1 to tube 10.

III. Preparation of inoculum

Bacterial suspensions that have been matched the turbidity of the 0.5 McFarland standard (i.e., 1.5 X 10^8 CFU/ml) served as the inoculum. Preparation of 0.5 McFarland standards is mentioned in appendix V.

1. Ten micro-liter (10 µl) inoculums were added to each tube so that final concentration of inocula used was made 5 X 10^5 CFU/ml.
2. All the tubes were incubated for 16-20 hrs at 35^0C. MICs end point for test organisms were determined by examining lowest concentration of antibiotic at which there is no visible growth. The MIC of the reference strain was found within one, two- fold dilution of expected MIC as given by CLSI.

APPENDIX-X

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1. Association of urine culture positive and negative among male and female patients.

	Culture	Culture	Total
Male	46	148	194
Female	173	343	516
Total	219	591	710

Test statistics is χ^2

H_0: There is no significant association of culture positive and culture negative male and female patients.

H_1: There is significant association of culture positive and culture negative among male and female patients.

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ we find from $\chi^2 = 5.27$

Thus χ^2_{cal} (5.27) > χ^2_{tab} at α =0.05(3.84). Hence, H_0 is rejected.

Result: There is significant association of culture Positive and negative among male and female patients i.e. the higher proportion of culture positive cases seen among female cases is statistically significant

2. Association of multi-drug resistance among out-patients and in-patients among uropathogens.

	MDR strains	Non-MDR strains	Total
Out-patients	70	42	112
In-patients	21	4	25
Total	91	46	137

Test statistics is χ^2

H_0: There is no significant association of MDR strains from out-patients and in-patients among uropathogens

H_1: There is significant association of MDR strains from out-patients and in-patients among uropathogens.

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ we find from $\chi^2 = 4.23$

Thus χ^2_{cal} (4.23) > χ^2_{tab} at α =0.05(3.84). Hence, H_0 is rejected.

Result: There is no reason to say that there is no significant association multi-drug resistance among types of patients suffering of UTI i.e. r percentage of MDR strains found in in-patients is statistically significant. xxx

3. Association of MDR and Non-MDR strains among Male and Female patients.

	MDR strains	Non-MDR strains	Total
Male	27	6	33
Female	64	40	104
Total	91	46	137

Test statistics is χ^2

H_0: There is no significant association of MDR and non-MDR strains among male and female patients.

H_1: There is significant association of MDR and non-MDR strains among male and female patients.

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ we find from $\chi^2 = 4.61$

Thus χ^2_{cal} (4.61) < χ^2_{tab} α =0.05(3.84). Hence, H_0 is rejected.

Result: There is significant association of MDR and non-MDR strains among male and female patient i.e. higher possibility of multidrug resistance among male patient is statistically significant.

4. Association of Culture Isolates and MDR strains among Gender.

	No. of Isolates	MDR strains	Total
Male	46	27	73
Female	173	64	237
Total	219	91	310

Test statistics is χ^2

H_0: There is no significant association of Culture Isolates and MDR strains among Gender.

H_1: There is significant association of Culture Isolates and MDR strains among Gender.

From $\chi^2 = \Sigma \frac{(O-E)^2}{E}$ we find from $\chi^2 = 6.68$

Thus χ^2_{cal} (6.68) < χ^2_{tab} α =0.05(3.8 xxxi H_0 is rejected.

Result: There is significant association of Culture Isolates and MDR strains among gender i.e. higher proportion of MDR strains found on male patients is not statistically significant.

5. Evaluation of urine culture test to detect UTI on the basis of pyuria.

Screening test results	Culture positive	Culture negative	Total
(≥5WBC/HPF)(Cases)	44	8	**52**
(<5WBC/HPF) (Control)	4	133	**137**
Total	**48**	**141**	**189**

a. Sensitivity = (44/48) x 100 = 91.66 %

b. Specificity = (133/141) x 100 = 94.32%

c. Positive predictive value = (44/52) x 100 = 84.61%

d. Negative predictive value = (133/137) x 100 = 97.08 %

6. Association between previous antibiotic exposure and drug resistance

		Resistance to antibiotic		**Total**
		Present	**Absent**	
Exposure to antibiotic	**Yes**	14	4	**18**
	No	18	8	**26**
Total		**32**	**12**	**44**

Odds ratio $= \frac{14 \times 8}{18 \times 4} = 1.56$

This means the cases were 1.56 times more likely than the controls to have drug resistance.

APPENDIX-XI

Susceptibility of Donors and their Transconjugants

Organisms	**A**	**C**	**Cf**	**Cfx**	**G**	**Nx**	**Tr**	**S**
U6	>1024		>1024	>1024		1024	1024	
U6T	>1024		128	**>1024**		1024	1024	
U12	>1024		>1024	>1024		>1024	>1024	
U12T	>1024		**1024**	**>1024**		128	1024	
U14	1024		>1024	1024		>1024	>1024	
U14T	1024		**>1024**	1024		1024	1024	
U27	>1024		>1024	>1024		>1024	>1024	
U27T	>1024		**>1024**	**>1024**		1024	1024	
U24	1024		>1024	>1024		**>1024**	**>1024**	
U24T	1024		**>1024**	1024		1024	1024	
U21	>1024		>1024	>1024	>1024	>1024	>1024	
U10	1024		>1024	1024		512	1024	
U10T	256							
U29	1024		1024	>1024		1024	1024	
U29T	256							
U26	>1024		>1024	>1024		>1024	>1024	
U26T	>1024		128	**>1024**		1024	**>1024**	

U25	1024		1024	>1024		1024	>1024	
U25T	1024		1024	**>1024**		1024	**>1024**	
U9	>1024	1024	>1024	>1024		>1024	>1024	
U9T	>1024	1024	**>1024**	1024	>1024	**>1024**	**>1024**	
Transformants								
U9	256				256		128	
U10	128						64	
U21	128		32		32		32	
E.coli TB1								512
E.coli HB101								512

APPENDIX-XII

Plasmid profiles of selected MDR *E.coli* xxxiii

Organism	Antibiotic Resistance pattern	No.of plasmids	Size of plasmid
U1	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	3	4.5, 4.7, 2
U2	Am,Cf,Ce,Cfx,Co,Of,Nx,C,Nf,Ca,G,Ci	3	32.5, 4.7, 2
U3	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	1	7.5
U4	Am,Cf,Ce,Cfx,Co,Of,Nx,Ak,C,Ca,G,Ci	3	32.5, 7, 2
U5	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	3	32.5, 6.5, 4.7
U6	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,Ci	1	32.5
U7	Am,Cf,Ce,Cfx,Co,Of,Nx,C,Nf,Ca,G,Ci	4	32.5, 4.7,2.6, 2
U8	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,Ci	7	38, 10, 7.5, 6.5, 5.6, 4.7, 3.9
U9	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,C,G,Ci	4	32.5, 4.7, 2.6, 2
U10	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	1	51
U11	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	4	32.5,7.5,4.7, 3.7
U12	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,Ci	5	51,32.5, 10, 4.7, 3.9
U13	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	1	32.5
U14	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,C,G,Ci	1	32.5
U15	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,C,G,Ci	4	38, 4.7, 3.2, 2.6

U16	Am,Cf,Co,Of,Nx,Nf	1	38
U17	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	1	32.5
U18	Am,Cf,Ce,Cfx,Co,Of,Nx,Nf,C,Ca,G,Ci	3	32.5, 4.7, 2.6
U19	Am,Cf,Co,Of,Nx	1	51
U20	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	2	32.5, 4.7
U21	Am,Cf,Ce,Cfx,Co,Of,Nx, Nf,Ca,G,Ci	1	32.5
U22	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,Ci	1	32.5
U23	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	4	32.5, 7.5, 4.7, 3.2
U24	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,G,Ci	4	32.5,5.4, 2.6, 2
U25	Am,Cf,Ce,Cfx,Co,Of,Nx,C,Ca,G,Ci	3	32.5,7.5, 4.7
U26	Am,Cf,Ce,Cfx,Co,Of,Nx,Ak,Ca,G,Ci	2	32.5, 7.5
U27	Am,Cf,Co,Nx,Of,Ca,Ci	1	38
U28	Am,Cf,Ce,Cfx,Co,Of,Nx,Ca,C,Ci	2	32.5,7.5
U29	Am,Cf,Co,Nx,Ca,Ci	1	38

APPENDIX-XIII

Causative organisms of UTI

Gram negative

E. coli, Klebsiella spp., *Proteus mirabilis, Proteus vulgaris, Enterococcus faecalis, Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., *Morganella morganii, Pseudomonas aeruginosa*

Gram positive

S. aureus, S. saprophyticus, Staphylococcus epidermidis, Group B streptococci

Other pathogens

Chlamydia trachomatis, Mycoplasma (*Ureaplasma urealyticum), Candida* spp. *Mycobacterium tuberculosis* (Cheesbrough, 2000; Fowler and Mariano, 1990).

APPENDIX-XIV

1. Age and gender wise distribution of growth positive culture

Age group	Male		Female		Total	
	No.	%	No.	%	No.	%
0-9	3	1.37	4	1.83	7	3.19
10-19	3	1.37	11	5.02	14	6.39
20-29	12	5.48	63	28.77	75	34.25
30-39	8	3.65	31	14.15	39	17.82
40-49	3	1.37	14	6.39	17	7.76
50-59	4	1.83	14	6.39	18	8.22
60-69	6	2.74	25	11.42	31	14.15
70-79	4	1.83	8	3.65	12	5.48
80-89	2	0.91	2	0.91	4	1.83
>90	1	0.46	1	0.46	2	0.91
Total	**46**	**21.01**	**173**	**78.98**	**219**	**100**

2. Age wise distribution of bacterial isolates

Organisms isolated	Age of the patient									
	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	>90
Acinetobacter spp.	0	0	1	0	0	0	0	1	0	0
C. freundii	0	1	2	2	1	0	0	0	1	1
C. diversus	0	1	0	0	0	0	0	2	0	0
Enterobacter cloacae	0	0	1	0	0	0	0	0	0	0
E. aerogens	0	1	1	0	1	0	0	0	0	0
E. coli	6	8	57	26	20	16	27	14	3	1
K. oxytoca	0	0	0	0	1	1	0	0	0	0
K. pneumoniae	0	0	1	3	0	0	0	0	0	0
M. morganii	0	0	0	0	1	0	1	0	0	0
Proteus mirabilis	0	0	1	2	0	0	0	0	0	0
P. aeruginosa	0	0	0	1	1	0	0	0	0	0
Salmonella typhi	0	0	1	0	0	0	0	0	0	0
S. aureus	0	0	1	0	0	0	0	0	0	0
S. epidermidis	1	0	1	2	0	0	0	0	0	0
S. saprophyticus	0	0	1	1	0	0	0	0	0	0
Streptococcus	0	0	1	0	0	0	2	0	0	0
Total	**7**	**11**	**69**	**37**	**25**	**17**	**30**	**17**	**4**	**2**