CHAPTER-I

1. INTRODUCTION

Urinary tract infection is one of the commonest domiciliary and nosocomial bacterial infections, comprising of a variety of clinical conditions caused by microbial invasion of tissue lining the urinary tract which extends from renal cortex to urethral meatus. Infection of adjacent structures such as prostate and epididymis is also included in this entity. It also refers to the presence of bacteria undergoing multiplication in urine within the urinary drainage system and presence of more than 10^5 organisms/ml in the mid-stream sample of urine (Jha and Bapat, 2005; Leigh, 1990).

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). Infection may be expressed predominantly at a single site, kidney i.e. pelvis and cortex (pyelonephritis), pelvis and ureter (pyelitis), ureter (ureteritis), bladder (cystitis), prostate (prostatitis) and urethra (urethritis) but the entire urinary tract is always at a risk of invasion of bacteria, once any one of its part is infected (Brooks *et al*, 2004).

Infections of the urinary tract are the second most common type of infection in the body. Women are especially prone to UTIs for reasons that are not yet well understood. One factor may be that a woman's urethra is short, allowing bacteria quick access to the bladder. Also, a woman's urethral opening is near sources of bacteria from the anus and vagina. For many women, sexual intercourse seems to trigger an infection, although the reasons for this linkage are unclear. One woman in five develops a UTI during her lifetime. The prevalence of UTI in males varies according to age. Young men rarely develop a UTI, and the prevalence of bacteriuria is 0.1% or less. In contrast to UTI, prostatitis affects men of all ages and from 1990-1994, accounted for almost 2 million office visits per year in the United States (US). Only 5.0% of them have bacterial prostatitis, 64.0% have nonbacterial prostatitis and 31.0% have prostodynia (Cunha, 2006).

The pathogens causing UTIs are almost always predictable, with *Escherichia coli* the primary etiologic agent among both outpatients and inpatients (Sahm *et al*, 2001). Other pathogens include *Proteus mirabilis*, *Klebsiella* spp., other Enterobacteriaceae and *Staphylococcus saprophyticus*. In more complicated UTIs, particularly in recurrent infections, the relative frequency of infection caused by *Proteus, Pseudomonas, Klebsiella* and *Enterobacter* spp. increases. Hospitalized patients are most likely to be infected by *E. coli, Klebsiella* spp., *Proteus mirabilis*, staphylococci and other Enterobacteriaceae, *Pseudomonas aeruginosa* and enterococci. In addition, UTIs are leading cause of Gram negative sepsis in hospitalized patients and are the origin for about half of all nosocomial infections caused by urinary catheters (Forbes *et al*, 2002).

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). According to the annual report published by Department of Health Services (2059/60), morbidity of UTI in Nepal is 1, 25,058. Geographical distribution of UTI in Mountain, Hill and Terai regions of Nepal are 13, 518, 68,858 and 42,682 respectively.

Nepal, being a developing country, has about 61.4% illiterate people, who do not have any concept of hygiene and so are always vulnerable to infections. In many parts of Nepal, the facilities for urine culture and antimicrobial susceptibility testing are not available thus leading to incorrect diagnosis and management of UTI (Sharma, 1983). Most of the Nepalese people are not financially sound to have a routine check-up of their health status and even those who are financially capable do not care to do so. People generally seek for medical services only when the symptoms of the disease begin to become evident or aggravate. This negligence to asymptomatic diseases

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

The antimicrobial resistance is a serious emerging problem throughout the world. Multiple drug resistant (MDR) bacterial isolates have been frequently reported from different parts of the world as an emergence of treatment problem. The MDR strain is defined as the strain that showed resistance to three or more antibiotics among six commonly prescribed drugs (Tuladhar, 2001).

Antimicrobial resistance is an evolving and growing problem in UTI. 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 (TMP/SMX) and beta-lactam resistance rates is problematic. Of more concern, however, are the emerging issues of fluoroquinolone resistance and 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).

The present study was conducted with a broad objective to isolate the bacteria causing UTI and determine the trend of their antimicrobial resistance. The next objective of this study was to define the current status of multidrug-resistant strains among bacterial isolates causing UTI. The defining criterion for MDR in this study was resistance to ≥3 of the antibiotics belonging to different structural classes (Tuladhar, 2001).

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

- To isolate the bacteria causing UTI and determine their antimicrobial resistance trend at National Public Health Laboratory.

2.2 SPECIFIC OBJECTIVES

- To determine the prevalence of UTI among different age group and gender of patients visiting NPHL.
- To isolate and identify the pathogenic bacterial isolates from urine specimens.
- To determine the efficiency of screening test used in routine examination of urine specimens to detect UTI.
- To evaluate the antibiotic susceptibility pattern of the bacterial isolates.
- To find out the prevalence of multidrug resistant strains among the total isolates from UTI cases.

CHAPTER-III

3. LITERATURE REVIEW

3.1 URINARY TRACT INFECTION

Urinary tract infection 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. 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).

UTI is defined as the detection of both bacteriuria 10^5CFU/ml and pyuria >10 leucocytes/HPF (Goya *et al*, 1997). The term UTI refers to the invasion of the urinary tract by a non resident infectious organism. UTI encompasses a wide variety of clinical entities whose common denominator is microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus. 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.

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

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

Low count significant bacteriuria is subjected to the following conditions:

- Urine was collected before the organisms reached to the phase of growth after the entry of bacteria into the urinary tract.
- Patient under treatment.
- Sometimes in younger females, the count is low as honey moon cystitis.
- Patient with certain endocrine disorders e.g. diabetes.
- Chronic infection where concentration power of kidney is low.
- Obstruction of the ureter.
- Infection with relatively slow growing organisms. e.g.*Staphylococcus saprophyticus,* streptococci other than enterococci, *Haemophilus influenzae* (Pokharel, 2004).

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 (Forbes *et al*, 2002; Leigh, 1990).

3.3 PREDISPOSING FACTORS TO UTI

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

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

Bacterial Virulence Factors: Not all strains of *E. coli* are equally capable of infecting the intact urinary tract. Bacterial virulence factors markedly influence the likelihood that a given strain once introduced into the bladder, will cause UTI. Most *E. coli* strains that cause symptomatic UTIs in noncatheterized patients belong to a small number of specific O, K, and H serogroups (Stamm, 2003).

Numerous investigations suggest that the strains of *E. coli* that cause UTIs possess certain virulence factors that enhance their ability to colonize and invade the urinary tract. Some of these virulence factors include increased adherence to vaginal and uroepithelial cells by bacterial surface structures (adhesions, in particular, pili), 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 fimbria**. UPEC usually produce **siderophores** that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce **hemolysins** which are cytotoxic due to formation of

transmembranous pores in host cells. Another factor thought to be involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement-dependent bactericidal effect of serum (Todar, 2002).

The adherence property has also been demonstrated with other species of bacteria. *Proteus* 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 *S. saprophyticus*. Motility may be important for organisms to ascend to the upper urinary tract against the flow of urine and cause pyelonephritis (Forbes *et al*, 2002).

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

Kidney Stones: Kidney stones, in some cases, can cause obstruction followed by infection, particularly pyelonephritis. Symptoms of severe UTI in people with a history of kidney stones may indicate obstruction of the urinary tract, which is a serious

condition. Formation of infectious urinary calculi is the most common complication accompanying UTI by members of the genus *Proteus* supported by other studies (Li *et al*, 2002; Torzewska *et al*, 2003). Recent studies have shown that men have higher risk of forming renal stone than women (Curhan *et al*, 1998; Yagisawa *et al*, 1999).

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

Diabetes: UTI is an important clinical problem for people with diabetes. UTI is 2-3 times more common in adult diabetic patients than in non-diabetics (Leigh, 1996). There is an increased prevalence of asymptomatic bacteriuria in diabetic women, but not in diabetic men (Zhanel *et al*, 1991). On a population basis, diabetic women, depending on age, are 6-24 times more likely than non-diabetic women to be admitted for acute pyelonephritis; and diabetic men are 3.4-17 times more likely than their non-diabetic counterparts to be admitted for the same condition (Nicolle *et al*, 1996).

The risk for symptomatic complicated UTIs may also be higher in people with diabetes. In fact, certain UTI-related abscesses are reported only in patients with diabetes. These patients are also at higher risk for fungal-related UTIs. The suggested mechanisms of an increased susceptibility to UTI are decreased antibacterial activity due to ‘sweet urine’, defects in neutrophil function and increased adherence to uroepithelial cells (Todar, 2002).

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

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

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).

3.4 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).

Gram negative	**Gram positive**
Escherichia coli	*Staphylococcus aureus*
Klebsiella spp.	*Staphylococcus saprophyticus*
Proteus mirabilis	*Enterococcus faecalis*
Proteus vulgaris	Group B streptococci
Enterobacter spp.	
Citrobacter spp.	
Serratia spp.	
Morganella morganii	
Pseudomonas aeruginosa	

Other pathogens

Chlamydia trachomatis

Mycoplasma (*Ureaplasma urealyticum)*

Candida spp.
Mycobacterium tuberculosis
(Source: Cheesbrough, 2000; Fowler and Mariano, 1990)

Other less frequently isolated agents are other Gram negative bacilli, such as *Acinetobacter* and *Alcaligenes* spp., other *Pseudomonas* spp., *Citrobacter* spp. and beta-hemolytic streptococci. Gram positive pathogens such as *E. faecalis*, *S. saprophyticus* and group B streptococci can also infect the urinary tract. UTIs due to *E. 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 (Forbes *et al*, 2002, Leigh, 1990).

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).

3.5 CATEGORIZATION OF UTI

Different classifications have been devised to help physicians choose treatments and determine the causes of UTIs.

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

3.5.2 Uncomplicated and Complicated UTIs: They are also sometimes further defined as either being uncomplicated or complicated depending on the factors that trigger the infections.

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

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

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

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

Complicated UTIs: Complicated UTIs, which occur nearly as often in men as women, are also caused by bacteria but they occur as a result of some anatomical or structural abnormality, such as catheter use in the hospital setting, bladder and kidney dysfunction or kidney transplant. The common feature in most complicated UTIs is the inability of

the urinary tract to clear out bacteria because a physical obstruction to urine flow hinders treatment success.

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

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

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

Hospital acquired UTI (catheter associated or nosocomial UTI): Hospital acquired UTIs are those developing in patients after admission to the hospital, which were neither present nor in incubation at the time of hospitalization. As many as 20.0% of all hospitalized patients who receive short-term catheterization develop a UTI. Hospitalized patients are most likely to be infected by *E. coli, Klebsiella* spp, *Proteus mirabilis,* staphylococci, other Enterobacteriaceace, *Pseudomonas aeruginosa* and enterococci (Forbes *et al,* 2002). Studies have demonstrated the importance of the attachment and growth of bacteria on the surfaces of the catheter in the pathogenesis of catheter-

associated UTI. The encrustations formed on the catheter surface provide a refuge for bacteria and may protect them from antimicrobial agents and phagocytes (Stamm, 2003).

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

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

Acute Urethral Syndrome: Patients with this syndrome are primarily young, sexually active women, who experience dysuria, frequency, and urgency but yields fewer organisms than 10^5 CFU/ml urine on culture. This condition is usually caused by *E. coli* or other bacteria that cause cystitis, but in lower numbers, or by a sexually transmitted disease such as Chlamydia or gonorrhoea (Forbes *et al*, 2002).

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3.6 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).

3.6.1 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, suprapubic 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, suprapubic 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).

3.6.2 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 urinary tract infection 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).

3.6.3 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 nonhospitalized 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; Steward *et al,* 1985; 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).

3.6.4 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.

3.6.5 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 0.004 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).

3.6.6 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 (2000), since therapy of infection begins before laboratory

results are available, antibiotic susceptibility testing primarily plays a supplementary role in confirming that the organism is susceptible to the agent that is being used.

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).

3.7 BACTERIAL RESISTANCE TO ANTIBIOTICS

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 *Pseudomonas 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* (Smith, 2004; Todar, 2002).

Genetic resistance may be **chromosomal** or **transferable on plasmids or transposons**. Chromosomal Resistance develops as a result of spontaneous mutation in a locus that

controls susceptibility to a given antimicrobial drug serves as a selecting mechanism to suppress susceptible organisms favor the growth of drug-resistant mutants. Spontaneous mutation occurs with a frequency of 10^{-12} to 10^{-7} and thus is an infrequent cause of the emergence of drug resistance in the clinical practice.

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.

1. 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.
2. 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.
3. 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).
4. 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.
5. 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 (O'Brien, 2002; Fluit *et al*, 1999).

Chromosomal multiple-antibiotic resistance (Mar) locus: The multiple-antibiotic resistance (*mar*) locus was first described in *Escherichia 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).

3.8 GLOBAL SCENARIO

An estimated 11.0 % of women in the US report at least one physician-diagnosed urinary tract infection per year, and the lifetime probability that a woman will have a urinary tract infection is 60.0 % (Foxman *et al*, 2000). The clinical management of urinary tract infection is complicated by the increasing incidence of infections caused by strains of *Escherichia coli* that are resistant to commonly used antimicrobial agents. In recent studies in the US, the rates of resistance to trimethoprim–sulfamethoxazole among *E. coli* isolates from women with urinary tract infections ranged from 15.0-18.0 % (Gupta *et al*, 2000; Kahlmeter, 2000; Talan *et al*, 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 urinary tract infection in Barcelona, Spain (Prats *et al*, 2000).

Like death and taxes, resistance in UPEC appears to be inevitable, or nearly so. 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).

The ECO.SENS Project was the first international survey to investigate the prevalence and susceptibility of pathogens causing community acquired uncomplicated UTIs in women at 240 centres in 17 countries. *E. coli* accounted for the majority (80.0%) of uropathogens isolated in all 17 countries. The rates of resistance among *E. coli* strains were: ampicillin and sulphamethoxazole (30.0%), trimethoprim alone or with sulphamethoxazole (15.0%), nalidixic acid (6.0%), ciprofloxacin (3.0%), amoxicillin-

clavulanic acid, mecillinam, cefadroxil, nitrofurantoin and fosfomycin (≤2.0%) (Kahlmeter, 2000).

In a 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 *Klebsiella pneumoniae* and 7.7% for *Proteus mirabilis*. For *E. coli* and *Klebsiella 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).

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. Multiresistant, defined as resistance to norfloxacin and at least two of the other three antibiotics, increased from 0.5% in 1989 to 4.0% in 1998 (Goetsch *et al*, 2000).

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), cotrimoxazole (range, 14.8 to 17.0%), ciprofloxacin (range, 0.7 to 2.5%), and nitrofurantoin (range, 0.4 to 0.8%) 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 (James *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).

A study conducted in the United Kingdom (UK) during 1999-2000 showed that *E. coli* was the predominant pathogen causing UTI followed by *Enterococcus faecalis, Klebsiella pneumoniae* and *Proteus mirabilis*. Nitrofurantoin was very active against isolates of *E. coli* (96.3% susceptible) and *E. faecalis* but not against *K. pneumoniae, P. mirabilis* or *Pseudomonas aeruginosa*. Overall susceptibility to trimethoprim ranged from 58.1% to 84.5% for the most prevalent pathogens. Ciprofloxacin was highly active against the uropathogenic bacteria examined in this study with susceptibilities of between 88.6% and 97.7% for the most prevalent pathogens (Farrell *et al*, 2003).

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 nalidixic acid (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%). Other Gram negative bacteria (*P. aeruginosa* and *Acinetobacter* spp) accounted for 3.5% of the isolates. Only 10.2% of the isolates were Gram positive: *S. aureus* (4.5%), *Streptococcus agalactiae* (3.8%) and *Enterococcus faecalis* (0.6%). 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 most frequent pathogen found was *E. coli* (73.0%) followed by *Proteus* spp. (7.4%), *Klebsiella* spp. (6.6%) and *Enterococcus* spp. (4.8%). 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 the most common organisms were *E. coli* (57.5%), *K. pneumoniae* (12.4%), *Enterococcus* spp. (6.6%), *P. mirabilis* (5.4%), *P. aeruginosa* (2.9%), *Citrobacter* spp. (2.7%), *S. aureus* (2.2%), *Enterobacter cloacae* (1.9%), Coagulase-negative staphylococci (1.3%), *S. saprophyticus* (1.2%), *Klebsiella* spp. (1.2%), *Enterobacter aerogenes* (1.1%) and *Streptococcus agalactiae* (1.0%). Among all 1990 isolates, 45.9% were resistant to amp, 20.4% to cotrimoxazole, 14.3% to nitrofurantoin, 9.7% to ciprofloxacin and 8.1% to levofloxacin. Fluoroquinalone resistnce was highest in patients ≥65 years of age. For the 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).

In a study done 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).

3.9 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. And 94.5%, 60.0%, 38.0%, 44.0% and 0% of the isolated *Klebsiella* spp. were found to be resistant to same antibiotics respectively (Ghimire *et al*, 1994).

E. coli was found as the most predominant pathogen (57.0%) followed by *Klebsiella pneumoniae* (24.0%), *Proteus* spp (10.0%), *Pseudomonas 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%), nalidixic acid (69.0%) and chloramphenicol (60.0%) whereas cotrimoxazole

and amoxicillin were least effective antibiotics against these bacterial isolates (Gautam *et al*, 1997).

E. coli (47.4%) was the most predominant bacteria followed by *Klebsiella* spp. (13.2%), *S. aureus* (10.5%) and *Pseudomonas 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 *et al*, 1999).

E. coli was the most predominant pathogen (78.0%) followed by *K. pneumoniae* (9.0%), *Proteus* mirabilis (2.0%), *Pseudomonas 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 *et al*, 2000).

Rai *et al* (2000) found that *E. coli* (61.8%) was the most predominant pathogen followed by *Klebsiella 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 (2000) reported that in 1947 urine specimens, culture positive were found in 517 (26.6%) of which MDR bacterial strains were detected in 122 (23.6%) cases in which *E. coli* 72 (13.1%), *Klebsiella* spp. 20 (3.9%) and *S. aureus* 13 (2.1%) were the predominants. Out of 1479 urine specimens of hospitalized patients, there were 230 culture positive cases of which MDR bacterial strains were detected in 81 (35.2%) cases

in which the most predominants were *E. coli* 51 (22.2%), *Klebsiella* spp. 14 (6.1%) and *S. aureus* 5 (2.2%).

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

It was found that urine sample of diabetic patients showed 19.4% positive growth while those of non-diabetic patients showed 5.3%. Among 19.4% growth positive cases, bacterial isolates were found in 89.5% and fungal isolates were found in 10.5% (Gautam *et al*, 2004).

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 *Morganella 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 *et al*, 2005).

In a study done at TUTH, *E. coli* was the most common isolate accounting for 61.2% of all bacterial isolates followed by *Klebsiella* spp. (9.2%), *Pseudomonas aeruginosa* (7.1%), *Enterococcus faecalis* (6.1%), *Staphylococcus 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 *et al*, 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 amoxycillin 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).

CHAPTER-IV

4. MATERIALS AND METHODS

The present study was conducted at National Public Health Laboratory, Teku. The study was carried out from May to September 2006. During this period, a total of 352 urine samples from patients suspected of UTI were collected and processed according to the standard laboratory methods.

4.1 MATERIALS

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

4.2 METHODS

4.2.1 Data Collection

Each patient requested for urine culture was directly interviewed for his or her clinical history during sample collection. The gathered history of patients includes name, age, sex, immunosuppressive disease present or not, signs and symptoms (dysuria, frequency, urgency, fever, stomach pain etc.), date of onset, antibiotics used or not and the macroscopic examination (color, appearance) of urine was also performed before processing.

4.2.2 Specimen 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.

4.2.3 Specimen evaluation

Before proceeding, the urine specimens were evaluated in terms of their acceptability. Considerations included proper labeling, visible signs of contamination and any

transportation delays in getting the specimen to the laboratory. A properly labeled specimen contained patient's full name, date and time of collection. Single urine specimen 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

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 Chemical examination

The detection of albumin in urine was performed by using uristix. The uristix was dipped into the urine specimen for few seconds and the change in color in test area was noted after 30 seconds. The results were interpreted according to the color change of the test area, comparing with that of the given standard color for detection of albumin.

4.2.7 Culture of specimen

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:

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

4.2.8 Identification of isolates

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, biochemical properties and serotyping if required in specific cases. 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 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, 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.

4.2.9 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 National Committee for Clinical Laboratory Standards (NCCLS) using Mueller Hinton agar (MHA).

- Mueller Hinton Agar was prepared and sterilizd 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 wire loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into Mueller Hinton broth tube and was incubated at 37ºC for 2-4 hrs.
- After incubation, the turbidity of the suspension was matched with the turbidity standard of Mc Farland 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 IX respectively.

4.2.10 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 *Staphylococcus 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.

4.2.11 Data analysis

Chi-square test was used to determine significant association of bacteriuria between genders/asymptomatic and symptomatic patients as well as MDR strains and gender. Test of present work are shown in Appendix- XI.

CHAPTER-V

5. RESULTS

This study was conducted among patients suspected of urinary tract infection visiting National Public Health Laboratory (NPHL), Kathmandu, Nepal. Three hundred and fifty two mid-stream urine samples were collected from patients for urine culture and the samples were processed at bacteriology laboratory of NPHL.

5.1 CLINICAL PATTERN OF RESULTS

Table 1: Age and gender wise distribution of patients requested for urine culture

Age group	Male		Female		Total	
	No.	Percentage	No.	Percentage	No.	Percentage
0-10	13	3.7	10	2.8	23	6.5
11-20	10	2.8	16	4.5	26	7.4
21-30	26	7.4	43	12.2	69	19.6
31-40	36	10.2	37	10.5	73	20.7
41-50	29	8.2	36	10.2	65	18.5
51-60	28	8.0	17	4.2	45	12.8
61-70	18	5.1	17	4.8	35	9.9
71-80	10	2.8	3	0.9	13	3.7
81-90	1	0.3	2	0.6	3	0.9
Total	**171**		**181**		**352**	

In this study, the age of the patient ranged from 5 months to 87 years. The highest number of patients 73 (20.7%) belonged to the age group 31-40 followed by 69 (19.6%) belonged to 21-30, 65 (18.5%) belonged to 41-50 and 45 (12.8%) belonged to 51-60 age group. Larger number of female patients 43 (12.2%) were found in the age group 21-30 followed by 37 (10.5%) in age group 31-40. Similarly, higher number of male

patients 36 (10.2%) were found in age group 31-40 followed by 29 (8.2%) in age group 41-50. The results are shown in table 1 and figure 2.

Table 2: Gender wise distribution of types of patients requested for urine culture

Type of patients	Male		Female		Total	
	No.	Percentage	No.	Percentage	No.	Percentage
Asymptomatic	103	60.2	53	29.3	156	44.3
Symptomatic	68	39.8	128	70.7	196	55.7
Total	**171**	**48.6**	**181**	**51.4**	**352**	**100.0**

As shown in above table, out of 156 asymptomatic patients, 103 were males while 53 were females. Similarly, out of 196 symptomatic patients, 68 were males while 128 were females. Asymptomatic patients include kidney transplant patients, diabetic patients, pregnant patients etc who visited for routine test only. Symptomatic patients include patients with symptoms like dysuria, frequency, urgency, stomach pain, fever etc.The detail about asymptomatic patients is mentioned in Appendix- XII.

5.2 MICROBIAL PATTERN OF RESULTS

Table 3: Pattern of urine culture results

S.N.	Growth	No. of samples	Percentage of samples
1.	Significant bacteriuria	80	22.7
2.	No growth	272	77.3
	Total	**352**	**100.0**

Out of total 352 MSU samples, 80 (22.7%) showed significant bacteriuria (i.e.$\geq 10^5$ CFU/ml) and 272 (77.3%) samples showed no growth. In this study, 13 mixed growth samples were obtained which were requested to repeat. After repetition, 9 samples showed no growth and the rest showed significant bacteriuria. The results are shown in table 3 and figure 3.

Table 4: Gender wise distribution of urine culture results

Gender	Significant bacteriuria		No growth	
	No.	%	No.	%
Male N=171	26	15.2	145	84.8
Female N=181	54	29.8	127	70.2
Total N=352	**80**	**22.7**	**272**	**77.3**

Of the total 80 cases of significant bacteriuria, 26 (15.2%) were males and 54 (29.8%) were females. Similarly, out of 272 sterile MSU samples, 145 (84.8%) were males and 127 (70.2%) were females. The association of significant bacteriuria in male and female patients was found to be statistically significant ($p<0.05$). The results are shown in table 4 and figure 4.

Table 5: Age and gender wise distribution of significant bacteriuria

S.N.	Age group	Male		Female		Total	
		No.	%	No.	%	No.	%
1.	0-10	6	7.5	4	5.0	10	12.5
2.	11-20	1	1.3	5	6.3	6	7.5
3.	21-30	3	3.8	14	17.5	17	21.3
4.	31-40	4	5.0	11	13.8	15	18.8
5.	41-50	3	3.8	9	11.3	12	15.0
6.	51-60	2	2.5	3	3.8	5	6.3
7.	61-70	4	5.0	4	5.0	8	10.0
8.	71-80	2	2.5	2	2.5	4	5.0
9.	81-90	1	1.3	2	2.5	3	3.8
	Total	**26**	**32.7**	**54**	**67.5**	**80**	**100.0**

Among the 80 significant bacteriuria cases, the highest number of significant bacteriuria was obtained from age group 21-30 years (21.3% of total growth positive), followed by age group 31-40 (18.8%). The least number of significant bacteriuria was obtained from age group 81-90 (3.8%).However, higher rate was found in the age group 0-10 (7.5%) in male patients as shown in table 5.

Table 6: Pattern of urine culture results according to types of patients

Type of patients	Culture positive		Culture negative	
	No.	%	No.	%
Asymptomatic N=156	19	12.2	137	87.8
Symptomatic N=196	61	31.1	135	68.9
Total N=352	**80**	**22.7**	**272**	**77.3**

Among the 352 patients, 44.3% (156/352) were asymptomatic patients, among whom 12.2% (19/156) showed positive culture results. Similarly, 196 (55.7%) were symptomatic patients, among whom 31.1% (61/196) showed positive culture results. The association of significant bacteriuria in asymptomatic and symptomatic patients was found to be statistically significant ($p<0.05$). The results are shown in table 6 and figure 5.

Table 7: Gender wise distribution of bacterial isolates

S.N.	Organisms isolated	Male		Female		Total	
		No.	%	No.	%	No.	%
Gram negative bacteria							

1.	*E. coli*	12	30.8	27	69.2	39	48.8
2.	*Klebsiella pneumoniae*	4	26.7	11	73.3	15	18.8
3.	*Klebsiella oxytoca*	1	33.3	2	66.7	3	3.8
4.	*Proteus mirabilis*	3	50.0	3	50.0	6	7.5
5.	*Proteus vulgaris*	4	80.0	1	20.0	5	6.3
6.	*Enterobacter spp.*	0	0	3	100.0	3	3.8
7.	*Citrobacter freundii*	0	0	2	100.0	2	2.5
8.	*Acinetobacter spp.*	1	100.0	0	0	1	1.3
9.	*Alcaligenes spp.*	0	0	1	100.0	1	1.3
Gram positive bacteria							
1.	*Staphylococcus aureus*	0	0	1	100.0	1	1.3
2.	CoNS	1	25.0	3	75.0	4	5.0
	Total	**26**	**32.5**	**54**	**67.5**	**80**	**100.0**

A total of 80 bacteria belonging to 11 different species were isolated from significant bacteriuria urine samples, which are tabulated in table 7 and figure 6. Among the isolates, *E. coli* (48.8%) was found to be the most predominant organism followed by *Klebsiella pneumoniae* (18.8%), *Proteus mirabilis* (7.5%), *Proteus vulgaris* (6.3%), CoNS (5.0%), *Klebsiella oxytoca* (3.8%), *Enterobacter* spp. (3.8%), *Citrobacter freundii* (2.5%), *Acinetobacter* spp (1.3%), *Alcaligenes* spp. (1.3%) and *Staphylococcus aureus* (1.3%).

Out of 80 growth positive patients, 54 (67.5%) were females and 26 (32.5%) were males. Among the different bacterial isolates, *E. coli* was the most predominant in female 27 (69.2%) as well as in male 12 (30.8%) as shown in table 7 and figure 7.

Table 8: Correlation of pyuria with culture result

Pyuria	Culture positive (%)	Culture negative (%)	Total (%)
Significant (≥5WBC/HPF)	77 (77.8)	22 (22.3)	**99 (28.1)**
Insignificant (<5WBC/HPF)	3 (1.2)	250 (98.8)	**253 (71.9)**
Total	**80 (22.7)**	**272 (77.3)**	**352 (100.0)**

Among the 352 samples, 71.9% (253/352) of samples showed insignificant pyuria, however among these, 1.2% (3/253) of samples gave positive culture results. Similarly, 99 (28.1%) of total samples showed significant pyuria, and among these 77.8% (77/99) samples gave positive culture results. The results are shown in table 8 and figure 8.

Table 9: Correlation of haematuria with culture result

Haematuria	Culture positive (%)	Culture negative (%)	Total (%)
Significant (≥3RBC/HPF)	19 (39.6)	29 (60.4)	**48 (13.6)**
Insignificant (<3RBC/HPF)	61 (20.1)	243 (79.9)	**304 (86.4)**
Total	**80 (22.7)**	**272 (77.3)**	**352 (100.0)**

Among the 352 samples, 86.4% (304/352) of samples showed insignificant haematuria, however among these, 20.1% (61/304) of samples showed positive culture results. Similarly, 48 (13.6%) of total samples showed significant haematuria, and among these 39.6% (19/48) samples showed positive culture results. The results are shown in table 9 and figure 9.

Table 10: Correlation of albuminuria with culture result

Albumin test	Culture positive (%)	Culture negative (%)	Total (%)
Positive (≥1+)	24 (52.2)	22 (47.8)	**46 (13.1)**
Negative (<1+)	56 (18.3)	250 (81.7)	**306 (86.9)**
Total	**80 (22.7)**	**272 (77.3)**	**352 (100.0)**

Out of total samples, 86.9% (306/352) of samples were negative for albumin test; however 18.3% (56/306) showed significant bacteriuria on culture. While 13.1% of samples showed positive albumin test out of which 52.2% (24/46) showed significant bacteriuria. The results are shown in table 10 and figure 10.

5.3 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES CAUSING UTI

Table 11: Antibiotic Susceptibility Pattern of Gram negative bacteria

S.N.	Antibiotics used	Total no. of isolates	Susceptibility Pattern					
			Resistant		Moderate		Susceptible	
			No.	%	No.	%	No.	%
1.	Ampicillin	75	71	94.7	0	0	4	5.3
2.	Ceftriaxone	75	16	21.3	2	2.7	57	76.0
3.	Ciprofloxacin	75	22	29.3	2	2.7	51	68.0
4.	Cotrimoxazole	75	33	44.0	0	0	42	56.0
5.	Gentamicin	75	13	17.3	2	2.7	60	80.0
6.	Nitrofurantoin	75	13	17.3	7	9.3	55	73.0
7.	Norfloxacin	75	33	44.0	1	1.3	41	54.7
8.	Ofloxacin	75	22	29.3	3	4.0	50	66.7

Among the common antibiotics used against all Gram negative bacteria, the most effective antibiotic was found to be gentamicin (80.0%) followed by ceftriaxone (76.0%). Most of the Gram negative bacteria i.e.71 (94.7%) was resistant to ampicillin as shown in table 11 and figure 11.

Table 12: Antibiotic Susceptibility Pattern of Gram positive bacteria

S.N.	Antibiotics used	Total no. of isolates	Susceptibility Pattern					
			Resistant		Moderate		Susceptible	
			No.	%	No.	%	No.	%
1.	Ampicillin	5	1	20.0	0	0	4	80.0
2.	Ceftriaxone	5	1	20.0	0	0	4	80.0
3.	Ciprofloxacin	5	0	0	1	20.0	4	80.0
4.	Cotrimoxazole	5	1	20.0	0	0	4	80.0
5.	Cloxacillin	5	2	40.0	0	0	3	60.0
6.	Erythromycin	5	1	20.0	0	0	4	80.0
7.	Nitrofurantoin	5	0	0	0	0	5	100.0

8.	Oxacillin	5	2	40.0	0	0	3	60.0

Among the 5 Gram positive bacteria, 100.0% of them were susceptible to nitrofurantoin. Erythromycin, ceftriaxone, ciprofloxacin, cotrimoxazole and ampicillin showed a common susceptibility of 80.0%. Oxacillin and cloxacillin (60.0%) were found to be the least effective as shown in table 12 and figure 12.

Table13: Antibiotic Susceptibility Pattern of bacterial isolates

S.N.	**Organisms isolated**	**Antibiotics used**	**Antibiotic Susceptibility Pattern**					
			Resistant		**Moderate**		**Susceptible**	
			No.	**%**	**No.**	**%**	**No.**	**%**
1.	*Escherichia coli* N=39	Ampicillin	38	97.4	0	0	1	2.6
		Ceftriaxone	8	20.5	1	2.6	30	77.0
		Ciprofloxacin	12	30.8	1	2.6	26	66.7
		Cotrimoxazole	18	46.2	0	0	21	53.9
		Gentamicin	7	18.0	2	5.1	30	77.0
		Nitrofurantoin	4	10.3	2	5.1	33	84.6
		Norfloxacin	21	53.9	0	0	18	46.2
		Ofloxacin	13	33.3	2	5.1	24	61.5
2.	*Klebsiella pneumoniae* N=15	Ampicillin	13	86.7	0	0	2	13.3
		Ceftriaxone	4	26.7	0	0	11	73.3
		Ciprofloxacin	4	26.7	1	6.7	10	66.7
		Cotrimoxazole	6	40.0	0	0	9	60.0
		Gentamicin	3	20.0	0	0	12	80.0
		Nitrofurantoin	4	26.7	1	6.7	10	66.7

		Norfloxacin	5	33.3	1	6.7	9	60.0
		Ofloxacin	4	26.7	1	6.7	10	66.7
3.	*Klebsiella oxytoca* N=3	Ampicillin	3	100.0	0	0	0	0
		Ceftriaxone	1	33.3	0	0	2	66.7
		Ciprofloxacin	2	66.7	0	0	1	33.3
		Cotrimoxazole	2	66.7	0	0	1	33.3
		Gentamicin	1	33.3	0	0	2	66.7
		Nitrofurantoin	0	0	0	0	3	100.0
		Norfloxacin	3	100.0	0	0	0	0
		Ofloxacin	2	66.7	0	0	1	33.3
4.	*Proteus mirabilis* N=6	Ampicillin	6	100.0	0	0	0	0
		Ceftriaxone	1	16.7	1	16.7	4	66.7
		Ciprofloxacin	0	0	0	0	6	100.0
		Cotrimoxazole	2	33.3	0	0	4	66.7
		Gentamicin	0	0	0	0	6	100.0
		Nitrofurantoin	0	0	3	50.0	3	50.0
		Norfloxacin	0	0	0	0	6	100.0
		Ofloxacin	0	0	0	0	6	100.0
5.	*Proteus vulgaris* N=5	Ampicillin	4	80.0	0	0	1	20.0
		Ceftriaxone	1	20.0	0	0	4	80.0
		Ciprofloxacin	1	20.0	0	0	4	80.0
		Cotrimoxazole	2	40.0	0	0	3	60.0
		Gentamicin	1	20.0	0	0	4	80.0
		Nitrofurantoin	1	20.0	1	20.0	3	60.0
		Norfloxacin	1	20.0	0	0	4	80.0
		Ofloxacin	0	0	0	0	5	100.0
6.	*Enterobacter*	Ampicillin	3	100.0	0	0	0	0
		Ceftriaxone	0	0	0	0	3	100.0

	spp. N=3	Ciprofloxacin	1	33.3	0	0	2	66.7
		Cotrimoxazole	1	33.3	0	0	2	66.7
		Gentamicin	0	0	0	0	3	100.0
		Nitrofurantoin	1	33.3	0	0	2	66.7
		Norfloxacin	1	33.3	0	0	2	66.7
		Ofloxacin	1	33.3	0	0	2	66.7
7.	*Citrobacter freundii* N=2	Ampicillin	2	100.0	0	0	0	0
		Ceftriaxone	0	0	0	0	2	100.0
		Ciprofloxacin	1	50.0	0	0	1	50.0
		Cotrimoxazole	1	50.0	0	0	1	50.0
		Gentamicin	0	0	0	0	2	100.0
		Nitrofurantoin	1	50.0	0	0	1	50.0
		Norfloxacin	1	50.0	0	0	1	50.0
		Ofloxacin	1	50.0	0	0	1	50.0
8.	*Acinetobacter* spp. N=1	Ampicillin	1	100.0	0	0	0	0
		Ceftriaxone	1	100.0	0	0	0	0
		Ciprofloxacin	1	100.0	0	0	0	0
		Cotrimoxazole	1	100.0	0	0	0	0
		Gentamicin	1	100.0	0	0	0	0
		Nitrofurantoin	1	100.0	0	0	0	0
		Norfloxacin	1	100.0	0	0	0	0
		Ofloxacin	1	100.0	0	0	0	0
9.	*Alcaligenes* spp. N=1	Ampicillin	1	100.0	0	0	0	0
		Ceftriaxone	0	0	0	0	1	100.0
		Ciprofloxacin	0	0	0	0	1	100.0
		Cotrimoxazole	0	0	0	0	1	100.0
		Gentamicin	0	0	0	0	1	100.0
		Nitrofurantoin	1	100.0	0	0	0	0

		Norfloxacin	0	0	0	0	1	100.0
		Ofloxacin	0	0	0	0	1	100.0
10.	*S. aureus* N=1	Ampicillin	0	0	0	0	1	100.0
		Ceftriaxone	0	0	0	0	1	100.0
		Ciprofloxacin	0	0	0	0	1	100.0
		Cotrimoxazole	1	100.0	0	0	0	0
		Cloxacillin	1	100.0	0	0	0	0
		Erythromycin	0	0	0	0	1	100.0
		Nitrofurantoin	0	0	0	0	1	100.0
		Oxacillin	1	100.0	0	0	0	0
11.	CoNS. N=4	Ampicillin	1	25.0	0	0	3	75.0
		Ceftriaxone	1	25.0	0	0	3	75.0
		Ciprofloxacin	0	0	1	25.0	3	75.0
		Cotrimoxazole	0	0	0	0	4	100.0
		Cloxacillin	1	25.0	0	0	3	75.0
		Erythromycin	1	25.0	0	0	3	75.0
		Nitrofurantoin	0	0	0	0	4	100.0
		Oxacillin	1	25.0	0	0	3	75.0

Among the antibiotics evaluated, *E. coli* was found to be highly susceptible towards nitrofurantoin (84.6%) followed by gentamicin and ceftriaxone (77.0%). Most of the *E. coli* isolated was resistant to ampicillin (97.4%).

Gentamicin (80.0%) was found to be the most effective antibiotic for *Klebsiella pneumoniae* followed by ceftriaxone (73.3%). Ciprofloxacin, nitrofurantoin and ofloxacin (66.7%) were found to be equally susceptible towards *Klebsiella pneumoniae* whereas most of the isolates were resistant to ampicillin (86.7%). *Klebsiella oxytoca* was found to be 100.0% susceptible to nitrofurantoin followed by ceftriaxone (66.7%) whereas 100.0% resistant to ampicillin as well as norfloxacin.

Proteus mirabilis was found to be 100.0% susceptible towards gentamicin, ciprofloxacin, ofloxacin and norfloxacin whereas 100.0% resistant to ampicillin. Half of *Proteus mirabilis* isolates were susceptible to nitrofurantoin (50.0%) and half of them were moderate to nitrofurantoin (50.0%). *Proteus vulgaris* was found to be 100.0% susceptible to ofloxacin. Ceftriaxone, ciprofloxacin, gentamicin and norfloxacin showed a common susceptibility of 50.0%.

Nitrofurantoin (100.0%) was found to be the drug of choice against Gram positive bacteria (*Staphylococcus aureus* and CoNS) whereas oxacillin and cloxacillin (60.0%) were found to be the least effective. The results are shown in table 13.

5.4 ANALYSIS OF MDR ISOLATES

Table14: Resistance Pattern and distribution of MDR bacterial isolates

Organisms	**Total no. of isolates**	**Resistant to**				
		0 drug	**1 drug**	**2 drugs**	**MDR Strains**	
					≥3 drugs	**%**
E. coli	39	1	8	10	**20**	**51.3**
K.pneumoniae	15	3	4	3	**5**	**33.3**
K. oxytoca	3	0	0	0	**3**	**100.0**
Proteus mirabilis	6	0	5	0	**1**	**16.7**
Proteus vulgaris	5	1	1	1	**2**	**40.0**
Enterobacter spp.	3	0	1	1	**1**	**33.3**
Citrobacter freundii	2	0	0	1	**1**	**50.0**
Acinetobacer spp.	1	0	0	0	**1**	**100.0**
Alcaligenes spp.	1	0	0	1	**0**	**_**
S. aureus	1	0	0	1	**0**	**_**
CoNS	4	0	2	0	**2**	**50.0**
Total	**80**	**5**	**21**	**18**	**36**	**45.0**

Most of the bacteria isolated were found to be resistant to ≥3 drugs (45.0%) and were considered MDR. It was found that 5 (6.3%) were found to be susceptible to all antibiotics evaluated in the study and 21 (26.3%) isolates were resistant to 1 drug and 18 (22.5%) isolates were resistant to 2 drugs. Among the MDR strains, 51.3 % (20/39) of *E. coli* and

33.3 % (5/15) of *K. pneumoniae* were found to be MDR. The results are shown in table 14 and figure 13.

Table 15: Gender wise distribution of MDR strains

Gender	Total no. of samples with significant bacteriuria	Total MDR	Percentage (%)
Male	26	15	57.7
Female	54	21	38.8
Total	**80**	**36**	**45**

Higher rate of MDR was found in male patients (57.7%, 15/26) compared to female patients (38.8%, 21/54). The association of MDR and non-MDR strains in males and females was found to be statistically insignificant ($p>0.05$). The results are shown in table 15 and figure 14.

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

S.N.	Antibiotics used	MDR *E. coli* isolates	Isolates resistant to antibiotics	
			No.	Percentage
1.	Ampicillin	20	20	100.0
2.	Ceftriaxone		8	40.0
3.	Ciprofloxacin		12	60.0
4.	Cotrimoxazole		13	65.0
5.	Gentamicin		6	30.0
6.	Nitrofurantoin		3	15.0
7.	Norfloxacin		18	90.0
8.	Ofloxacin		13	65.0

As shown in above table and figure 15, among the MDR *E. coli* isolates, 100.0% (20/20) were resistant to ampicillin, 90.0% (18/20) to norfloxacin, 65.0% (13/20) to cotrimoxazole and ofloxacin, 60.0% (12/20) to ciprofloxacin and 40.0% (8/20) to ceftriaxone.

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

Nepal is one of the developing countries of South East Asia having comparatively very poor health status due to illiteracy, lack of hygienic and sanitary knowledge, malnutrition, economic status and lack of proper techniques in using medical procedures. So, people are usually victimized by many infectious diseases. According to annual report published by Department of Health Services (1996/1997), the morbidity of UTI in Nepal is 0.42% 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 NPHL, Teku. Three hundred and fifty two mid-stream urine samples were collected from patients visiting NPHL 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 352 urine samples, 272 (77.3%) urine samples showed no growth and 80 (22.7%) samples showed significant growth. A similar study carried out by Chhetri *et al* (2001) showed growth positivity of 21.8%. The low growth positive rate observed in this study might be due to inclusion of kidney transplant patients and others for routine check up only. 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 from elsewhere (Talukder, 1987).

Females are more susceptible to UTI than males. Also in the present study, this fact was supported where the rate of growth positivity was found to be 29.8% (54/181) in females and 15.2% (26/171) 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 result confirms and expands the previous findings of Steenberg *et al* (1969) in Denmark, Jha and Yadav

(1992), Chhetri *et al* (2001), Jha and Bapat (2005) and Rajbhandari and Shrestha (2002) in Nepal.

In this study, age group of 21-30 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), Manandhar *et al* (1996), Rajbhandari and Shrestha (2002), Regmi *et al* (2003), Shrestha *et al* (2005) and Jha and Bapat (2005). 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. Kunin and McCamack (1968) had 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 0-10 years. This indicated that bacteriuria is quite common in males during the first year of life (Forbes *et al*, 2002). In this present study, all growth positive males were below 2 years of age.

Among the 352 patients, 44.3% (156/352) were asymptomatic patients, among whom 12.2% (19/156) showed positive culture results. Similarly, 55.7% (196/352) were symptomatic patients, among whom 31.1% (61/196) showed positive culture results. Among asymptomatic patients, 64.1% (100/156) were kidney transplant patients among which 73.0% were males and 27.0% were females who visited NPHL for their routine follow up tests and significant bacteriuria was seen only in 8.0% cases. The study done by Ghimire *et al* (2004) on prevalence of UTI among kidney transplant patients visiting NPHL included 73.0% males and 27.0% females and significant bacteriuria was seen only in 30 cases (15.0%).

Among the total 80 bacterial isolates, 75 (93.8%) were Gram negative bacilli and only 5 (6.3%) 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. The higher incidence of UTI by Gram negative bacteria was also accounted in the study done by Dhakal *et al* (1999), Manandhar *et al* (1996), Shrestha *et al* (2003) and Tuladhar *et al* (1987).

Altogether 11 different bacterial isolates were found in this study. Among the isolates, *E. coli* (48.8%) was found to be the most predominant organism followed by *Klebsiella*

pneumoniae (18.8%), *Proteus mirabilis* (7.5%), *Proteus vulgaris* (6.3%), CoNS (5.0%), *Klebsiella oxytoca* (3.8%), *Enterobacter* spp. (3.8%), *Citrobacter freundii* (2.5%), *Acinetobacter* spp (1.3%), *Alcaligenes* spp. (1.3%) and *Staphylococcus aureus* (1.3%).

Higher prevalence of *E. coli* seen in this study also resembled the study done by various other workers viz: Chhetri *et al* (2001), Sharma *et al* (1983), Tuladhar *et al* (1989), Jha and Yadav (1992), Manandhar *et al* (1996) and Dhakal *et al* 1999 in Nepal. The result is also in harmony with the study done at international context: Steenberg *et al* (1969), Kahlmeter (2000), Farrell *et al* (2003), Leigh (1990), Fowler (1990) and Kosakai (1990).

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. Strains of *E. coli* appear well adapted to invade urinary tract (serogroups 02, 04, 06, 07, 08, 09) which forms the majority of isolates of UTI (Chakraborty, 1995). 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 69.2% in females whereas 30.8 % in males (out of total *E. coli* isolates). Similar type of result was found by Dhakal *et al* (1999), Gautam *et al* (1998), Kosakai *et* al (1990) and Vorland *et al* (1985). Bacteriuria in ambulatory adult women is caused primarily by Gram negative bacilli derived from the faecal flora (Fowler, 1990). This statement of Fowler resembles with the present study as well as the finding of above workers.

Klebsiella pneumoniae was isolated as the second commonest pathogen in frequency causing UTI. *K. pneumoniae* accounted for 18.8 % (15/80). The finding of this study is in harmony with the study done by Astal *et al* (2002), Das *et al* (2006), Gautam *et a*l (1998), Ghimire *et al* (1995), Hadiza *et al* (2003), Kumari *et al* (2005), Manandhar *et al* (2005), Sharma (1983) and Zhanel *et al* (2005). In the present study, the incidence of *K. pneumoniae* was found to be 18.8% and that of *K. oxytoca* was 3.8%, which follows the statement of Fowler (1990)- *"K. pneumoniae* is the primary pathogen in the genus although *K. oxytoca* may also cause bacteriuria." 73.3% of *K. pneumoniae* was isolated from female patients only.

Proteus mirabilis accounted for 7.5% of total bacterial isolates and *Proteus vulgaris* accounted for 6.3%. *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 and Torzewska *et al*, 2003). Eighty percent of *Proteus vulgaris* was isolated from male patients in this study. *Proteus* spp is a common cause of UTI in boys and men and is associated with renal abnormalities, particularly calculi. In hospitalized patients, it may cause chronic UTI in association with obstruction or use of instrument (Leigh, 1990).

Three isolates of *Enterobacter* spp. were isolated during the study period. All the isolates belonged to female patients. Only one isolate of *Acinetobacter* spp. and *Alcaligenes* spp. (1.3%) each was isolated during the study period. This finding is in harmony with the study done by Modi and Erch (2006) in which *Alcaligenes faecalis* and *Acinetobacter calcoaceticus* accounted for 1.0% and 2.3% respectively. Only two isolates of *Citrobacter freundii* were isolated during the study period and belonged to the female patient.

Among Gram positive bacteria, CoNS was found to be the most predominant with 5.0% of the total isolates. The present finding agrees with the studies done by Dhakal *et al* (2002) and Shrestha *et al* (2005) in which CoNS accounted for 5.3% and 6.9% of total isolates respectively. 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 1.25% 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 urinary tract infection. But other many workers (Abyad *et al,* 1991; Chakraborty, 1995; Wargotz *et a*l, 1987 and Ziloski and Smucker, 1989) concluded that pyuria is significant if >5 leucocytes are seen per HPF.

In this study, significant pyuria was observed in 28.1% (99/352) of requests. In this study, out of 253 cases of insignificant pyuria, only 3 showed culture positive while remaining 250 showed culture negative results. Based on this result, the sensitivity and specificity of pyuria as a screening test for UTI were calculated as 96.3% and 91.9%. Positive predictive value of WBC count of ≥5/HPF for growth positive culture was found out to be 77.8%.

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 occured 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.

Similarly, out of 48 cases of significant haematuria (≥3 RBCs/ HPF), 19 showed culture positive while remaining 29 showed culture negative; while out of 304 cases of insignificant haematuria, 61 cases were culture positive and 243 were culture negative. Based on this, the sensitivity and specificity of haematuria were calculated as 23.8% and 89.3% respectively. The predictive value of positive test (PPV) was found to be 39.6% and the predictive value of negative test (NPV) was found to be 79.9%. Due to higher quantity of false negative results seen, using microscopy of RBCs as a screening test of UTI was not found to be reliable.

Schumann and Schweitzer (1991) suggested that the observation of 0-2 RBCs/HPF on the urinary sediment is normal both in males and females. But Wargotz *et al* (1987) and Steward *et al* (1995) reported that they are abnormal if the RBC count was ≥3/HPF. The mechanism through which RBC enter urine is not known yet but it is believed that increased number of erythrocytes are seen in renal disease, lower urinary tract disease, extra renal disease, toxic reaction due to drugs and sometimes in physiologic causes including exercise.

In this study, out of total urine samples 306 (86.9%) were negative for albumin test while only 46 (13.1%) were positive for albumin test. Based on this, the sensitivity and specificity of albumin test were calculated as 30.0% and 91.9% respectively. The predictive value of positive test (PPV) was found to be 52.2% and the predictive value of negative test (NPV) was found to be 81.7%. There are various conditions in which protein (albumin) appear in urine, UTI is one of them. According to the North Thames Regional Guidelines for Diagnosis and Management of Urinary tract Infection, if dipstick proteinuria is consistently more than 1+, then this may indicate UTI and a MSU specimen for culture should be taken. So, it could be concluded that detection of protein (albumin) in urine is also important for diagnosis of UTI and MSU samples should be cultured in cases when there is absence of significant pyuria.

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, gentamicin (80.0%) was found to be the most effective antibiotis against Gram negative bacteria followed by ceftriaxone with a susceptibility of 76.0%. In a similar study carried out by Jha and Bapat (2005) at Sukhraraj Tropical Hospital, 92.5% of urinary isolates were susceptible to gentamicin.

On the other hand, ampicillin was found to be the least effective drug against Gram negative bacteria (94.7% resistant). Similarly, 97.4% of *E. coli* and 86.7% of *K. pneumoniae* were found to be resistant to ampicillin in this study. Resistant to ampicillin was also observed by various other researchers (Arosio *et al,* 1978 and Obi *et al,* 1996). Similar results were found by Sharma (1983) in which ampicillin resistance was present in more than 93.0% cases with *E. coli.* In this study, nitrofurantoin moderately inhibited the growth of *Proteus mirabilis* (50.0%). Similar type of result was found by Modi and Erch (2006).

About 44% of Gram negative bacteria were resistant to cotrimoxazole and norfloxacin each. Similarly, resistance of ciprofloxacin and ofloxacin against Gram negative bacteria was

found to be 29.3%. In contrast to this result, norfloxacin has been recommended as highly effective antimicrobial (Chattopadhyay and Mandal, 1993; Esko and Renkonen, 1985). It has been suggested that fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin) are a logical choice for empirical therapy of uncomplicated urinary tract infections (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). Resistance of *E. coli* to quinolones has remained rare until recently, until their use increased (Oteo *et al*, 2001).

Among Gram positive isolates, the most effective drug was found to be nitrofurantoin (100.0%) followed by ampicillin, ceftriaxone, cotrimoxazole, ciprofloxacin and erythromycin with the susceptibility of 80.0% for all five drugs. Cloxacillin and oxacillin were found to be the least effective with the susceptibility of 60.0%.

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 80 isolates that were evaluated against 8 antimicrobials, MDR isolates accounted for 45.0% (36/80). Out of total 75 Gram negative isolates, 45.3% (34/75) were found to be multidrug-resistant and that of Gram positive isolates was 40.0% (2/5). 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 *Staphylococcus aureus* (2.2%). But in this study, MDR in *E. coli* were found to be 51.3% (20/39).

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 57.7% (15/26) than in females 38.8% (21/54). However, the association of MDR and non-MDR strains in males and females was found statistically insignificant ($p>0.05$). This finding agrees 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).

MDR of *E. coli* was analyzed and 51.3% of isolates were found MDR. Among the MDR *E. coli* isolates, 100.0% were resistant to ampicillin, 90.0% to norfloxacin, 65.0% to cotrimoxazole and ofloxacin, 40.0% to ceftriaxone and 30.0% to gentamicin. But only 15.0% of MDR *E. coli* were resistant to nitrofurantoin. 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).

Similarly, 33.3% of *K. pneumoniae* were found MDR among which 100.0% of them were resistant to ampicillin, cotrimoxazole and norfloxacin and 80.0% were resistant to ceftriaxone, ciprofloxacin and ofloxacin. But resistance of nitrofurantoin was found to be 20.0% for *K. pneumoniae*. The only isolate of *Acinetobacter* spp. was found MDR which was resistant to all the antimicrobials evaluated. The evaluation of antimicrobial resistance among MDR isolates indicated a high resistance against all antimicrobial agents evaluated except nitrofurantoin. This may have been due to the fact that this antibiotic has not been widely used in treating UTI.

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 cotrimoxazole, the current first-line treatment for uncomplicated UTIs in the US and many other countries (Blondeau, 2004).

6.2 Conclusions

The study was carried out to determine the prevalence of MDR bacterial isolates causing UTI among patients visiting NPHL.

The main findings of this study are that there is significant difference of positive growth between male and female patients ($P<0.05$). But the statistical analysis failed to show significant difference of MDR strains between gender. The highest susceptibility was shown by gentamicin (80.0%) against Gram negative isolates and nitrofurantoin was the drug of choice against Gram positive isolates. The overall prevalence rate of MDR was found to be 45.0%. 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 reevaluation 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.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 Summary

1. Out of 352 mid-stream urine samples, 80 (22.7%) were growth positive with significant number of bacteria and 272 samples (77.3 %) showed no growth.
2. The infection rate was found to be higher in females (29.8%) than in males (15.2%). Association of significant bacteriuria and gender of patients was found to be statistically significant ($P<0.05$).
3. Altogether 11 different bacteria were isolated from growth positive urine samples. *Escherichia coli* (48.8%) was found the most predominant organisms followed by *Klebsiella pneumoniae* (18.8%), *Proteus mirabilis* (7.5%), *Proteus vulgaris* (6.3%), CoNS (5.0%), *K. oxytoca* (3.8%), *Enterobacter* spp. (3.8%), *Citrobacter freundii* (2.5%), *Acinetobacter* spp.(1.3%), *Alcaligenes* spp. (1.3%) and *Staphylococcus aureus* (1.3%).
4. Microscopy of pyuria showed the sensitivity of 96.3% and the specificity of 91.9%. The positive and negative predictive values were found to be 77.8% and 98.8% respectively.
5. Microscopy of haematuria showed the sensitivity of 23.8% and the specificity of 89.3%. The positive and negative predictive values were found to be 39.6% and 79.9% respectively.
6. The most effective antibiotic against Gram negative bacteria was found out to be gentamicin (80.0%) followed by ceftriazone (76.0%) whereas ampicillin was found out to be the least effective drug. Nitrofurantoin was found to be the most effective antibiotic against Gram positive bacteria with a susceptibility of 100.0%.
7. MDR was observed in 45.0% (36/80) of 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)
8. Higher status of MDR was found in males (57.7%, 15/26) than in females (38.8%, 21/54).
9. Among the MDR *E. coli* isolates, 100.0% were resistant to ampicillin, 90.0% to norfloxacin and 65.0% to cotrimoxazole and ofloxacin. Among the MDR *K. pneumoniae* isolates, 100.0% were resistant to ampicillin, cotrimoxazole and norfloxacin.

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 NPHL, 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.
3. All the hospitals and health institutions should have a provision of keeping the data of MDR strains causing UTI.
4. 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. Genotypic characterization of MDR strains should be done in order to ascertain the location of drug resistance genes and to characterize the mechanism of drug resistance.
6. There are some limitations in the present study. The organisms were tested against few panels of antibiotics of first line. Hence, it is recommended that the study should be carried out using more antibiotics including the second line drugs.
7. Strict rules and regulations of antibiotic policy should be established in our country to check selling of antibiotics without prescription, and thus check the development of resistance to some extent.

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APPENDIX-I

QUESTIONAIRE

CLINICAL AND MICROBIOLOGICAL PROFILE OF PATIENT

Clinical Profile:

Name of Patient: ……

Sex: …….. Lab No: …….

Age: ….

Address: …..

Date: …….

Clinical History:

Patient on Antibiotics: a. Yes b. No

Immunosuppressive disease present: a. Yes b. No

If yes, what: a. Kidney transplant patient b. Diabetes c. Others

Symptoms: a. Dysuria b. Frequency c. Fever d. Stomach pain e. Others

Microbiological Profile:

DAY 1

Time of sample collection: …….

Specimen: …….

Method of sample collection: ……..

Macroscopic Observation:

Color: ……. Appearance: ……

Others: ……..

Direct Microscopic Observation:

Wet mount Preparation of Centrifuged Urine

Observation	Number per HPF	Comments
Pus Cells		
RBCs		

Culture of specimen on: a.MacConkey Agar b. Blood Agar

Incubation: a. Aerobic b.Anaerobic c. Microaerophillic

Incubation temperature and period: …….

DAY 2:

Reading of Culture Plates

Colony Characteristics on MacConkey Agar/Blood Agar

Media used	Shape	Size	Color	Texture	Haemolysis on BA	Lactose fermentation	Growth
MacConkey							
Blood Agar							

Gram-staining test: ……..

Catalase test: …… Oxidase test: …….

Coagulase test: …….. Others: …….

Provisional Identification of Organism: …….

DAY 3:

Biochemical Tests:

Results:

a. TSI: ……. b. SIM: …….. c.Citrate: ……..

d. Urea Hydrolysis: …….. e. MR: …… f. VP: ……..

Organism Identified as: ……

Antibiotic Sensitivity Test (Kirby- Bauer Method)

Antibiotics used	Zone of inhibition (mm)	Interpretation

Comments on Drug Resistance Pattern: MDR/ NonMDR

Resistant to …….. Number of Antibiotics.

Performed by Checked by

…… ……..

APPENDIX –II

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

A. EQUIPMENTS

Hot air oven Memmert (Japan)

Incubator	Sakura (Japan)
Autoclave	Stermite (Japan)
Refrigerator	Sanyo (Japan)
Microscope	Olympus (Japan)
Centrifuge	Hitachi (Japan)
Weighing Machine	Chyo MP (Japan)
Water Distillation Plant	India
Water bath	Boekel 148003 (Japan)

B. MICROBIOLOGICAL MEDIA

Blood agar	Mueller Hinton broth
MacConkey agar	Simmons Citrate agar
MR-VP medium	Sulphur Indole Motility agar
Mueller Hinton agar	Triple Sugar Iron agar
Nutrient agar	Urea broth

C. CHEMICALS AND REAGENTS

3%Hydrogen peroxide	Barritt’s reagent
Crystal violet	Kovac’s reagent
Gram’s iodine	Barium chloride
Absolute (95%) alcohol	Sulphuric acid
Safranine	

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)	

E. MISCELLANEOUS

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

APPENDIX-III

A. COMPOSITION AND PREPARATION OF DIFFERENT CULTURE MEDIA

The culture media used were from two companies:

a. Hi-Media Laboratories Pvt. Limited, Bombay, India.
b. Oxoid Unipath Ltd. Basingstoke, Hampshire, England

(All compositions are given in grams per liter and at 25^0C temperature)

1. Blood agar base (Oxoid, England)

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

Ingredients	**gm/liter**

Protease peptone	15.0
Liver extract	2.5
Yeast extract	5.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25^0C) 7.4±0.2

Direction: 42.50 grams of the blood agar base medium was suspended in 1000 ml distilled water, dissolved by boiling and sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes. After cooling to 45-50^0C, 7% sterile defibrinated sheep blood was added aseptically, then mixed with gentle rotation and immediately poured in sterile petriplates.

2. MacConkey Agar (Oxoid, England)

Ingredients	**gm/liter**
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral Red	0.075
Agar	12.0
Final pH (at 25^0C)	7.4±0.2

Direction: 52 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. The medium was poured into sterile petriplates.

3. Mueller Hinton Agar (Oxoid, England)

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

Direction: 38 grams of the medium was suspended in 1000 ml distilled water and the medium was warmed to dissolve completely. It was sterilized by autoclaving at 121ºC (15 lbs pressure) for 15 minutes and poured into sterile petriplates.

4. Nutrient Agar (Oxoid, England)

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

Direction: 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. Mueller Hinton Broth (Oxoid, England)

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

Direction: 21 grams of the medium was dissolved in 1000 ml of distilled water, boiled and dispensed into small containers. It was then sterilized by autoclaving at 121˚C for 15 minutes.

B. COMPOSITION AND PREPARATION OF DIFFERENT BIOCHEMICAL TESTS MEDIA

1. MR-VP Medium (Hi-Media laboratories)

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

Direction: 15 gm powder was dissolved in 1000 ml of distilled water & mixed well. 3 ml of medium was distributed in each test tube and autoclaved at 121^0C for 15 minutes.

2. Sulphide Indole Motility (SIM) medium (Oxoid, England)

Ingredients	gm/litre
Tryptone	20.0
Peptone	6.1
Ferrous ammonium sulphate	0.2
Sodium Thiosulphate	0.2
Agar	3.5
Final pH (at 25^0C)	7.3±0.2

Direction: 30 grams of the medium was suspended in 1000 ml of distilled water and dissolved completely. Then it was distributed in tubes to a depth of about 3 inches and sterilized by autoclaving at 121ºC for 15 minutes.

3. Simmon's Citrate Agar (Oxoid, England)

Ingredients	gm/litre
Magnesium sulfate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	1.0
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Agar	15.0
Bromothymol Blue	0.08
Final pH (at 25^0C)	6.8±0.2

Direction: 23 grams of the medium was dissolved in 1000ml of distilled water. 3ml medium was distributed in test tubes and sterilized by autoclaving at 121^0C for 15 minutes. After autoclaving, tubes containing medium were tilted to form slant.

4. Triple Sugar Iron Agar (TSI) (Oxoid, England)

Ingredients	gm/litre
Lab-lemco powder	3.0
Yeast Extract	3.0

Peptone	20.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium chloride	5.0
Sodium thiosulphate	0.3
Phenol red	0.025
Agar	12.0
Final pH (at 25^0C)	7.4±0.2

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

5. Urea Broth Base

Ingredients	**gm/litre**
Monopotassium phosphate	9.1
Dipotassium phosphate	9.5
Yeast extract	0.1
Phenol red	0.01
Sterile 40% urea solution	5ml

Direction: As directed by manufacturing company, 1.87 grams of the medium was suspended in 95 ml of distilled water and sterilized by autoclaving at 121^0C for 15 minutes. After cooling to about 55^0C, 5 ml of sterile urea solution was added aseptically, mixed well and distributed 5 ml amount in sterile test tubes.

C. COMPOSITIN AND PREPARATION OF DIFFERENT STAINING AND TESTS 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

Direction: 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. Final 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

Direction: 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. Final volume was made 1 litre by adding D/W.

(c) Acetone-Alcohol Decoloriser

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

Direction: 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

Direction: 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 Chloride	0.85 g
Distilled Water	100 ml

Direction: 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. Biochemical Test Reagents

(a) Catalase Reagent (For Catalase test)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

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

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

(For Oxidase Test)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD) 1 gm

Distilled Water 100 ml

Direction: 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) Kovac's Indole Reagent (For Indole Test)

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

Direction: 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) Methyl Red Solution (For Methyl Red Test)

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

Direction: 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) Barritt's Reagent (For Voges-Proskauer Test)

Solution A

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

Direction: To 25 ml ethanol, 5 g of *α*-naphthol 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

Direction: 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.

4. McFarland tube (No. 0.5)

0.5 ml of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2.H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% w/v) with constant stirring. The McFarland standard was thoroughly mixed to ensure that it is evenly suspended. Using matched cuvettes with a 1 cm light path and water as a blank standard, the absorbance was measured in a spectrophotometer at a wavelength of 625 nm. The acceptable range for the turbidity standard is 0.08-0.13. The standard was distributed into screw-cap tubes of the same size and volume as those used to prepare the test inoculum. The tubes were sealed tightly to prevent loss by evaporation and stored protected from light at room temperature. The turbidity standard was then vigorously agitated on a vortex mixer before use. Standards may be stored for up to 6 months, after which time they should be discarded.

APPENDIX-IV

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 1 minute.
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 (safranine) for 1 minute 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 100X.

APPENDIX-V

METHODOLOGY OF BIOCHEMICAL TESTS USED 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

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM URINE SAMPLE

Bacteria	**Morphological Characteristics**	**Cultural Characteristics**
Escherichia coli	Gram negative rod of 1-3µm×0.4-0.7µm size, aerobic and anaerobic, nonsporing, motile, noncapsulated	On BA: Large 1-4 mm in diameter, grayish white, moist, smooth, convex and opaque. The colonies may appear mucoid and some strains are haemolytic. On MA: Bright pink colonies due to lactose fermentation, smooth, glossy and translucent.
Klebsiella spp.	Gram negative, short and thick rods of 1-2µm × 0.8µm size, nonsporing, nonmotile and capsulated.	Large dome shaped moist and usually viscid or mucoid colonies when cultured on BA and MA. Most Klebsiella species are lactose fermenting.
Proteus spp.	Gram negative rods of 1-3 µm × 0.4-0.6µm size, non capsulated, nonsporing motile rods.	On BA: when cultured aerobically, most strains are swarming type and have a characteristic fishy odour. On MA: *Proteus* species produce individual non-lactose fermenting colonies after overnight incubation at 35ºC to 37ºC. Swarming is prevented on MA because this media contains bile salts.
Enterobacter spp.	Gram negative rods, non sporing, noncapsulated.	About 2 to 3 mm in diameter, moist, yellowish coloured, LF, motile organism.
Citrobacter freundii	Gram negative, non sporing, noncapsulated, motile rods.	Smooth, convex 2-4 mm colonies, sometimes rougher mucoid forms occur

		On BA: Non-haemolytic On MA: Non-lactose fermenting colonies.
Acinetobacter spp.	Gram negative, short, stout, non- motile rods that become almost coccoid, frequently capsulated, strict aerobes.	They grow well on ordinary media and form white of cream, glistening smooth and often rather viscid colonies about 1mm in diameter.
Alcaligenes spp.	Gram negative rod actively motile by peritrichate flagella, non-capsulated, strict aerobes.	Colonies on NA are grayish white. On BA: Non-haemolytic
Staphylococcus aureus	Gram positive, spherical cocci, 0.8-1 µm in diameter, non sporing, facultative anaerobe, non-motile, except for rare strains, non capsulated. They are arranged in characteristics grape like clusters or in small groups, pairs, singles and short chain(less than five cocci in line).	On BA: Large, 2-4 mm diameter. Circular, smooth with glistening surface, entire edge, soft butyrous consistency and opaque. The pigmentation is golden yellow to cream coloured.Some strains are beta-haemolytic when grown aerobically. On MA: Small (pin head size), 0.1-0.5mm, pink or pink orange due to lactose fermentation. Some strains are non-lactose fermenting.
Coagulase Negative *Staphylococcus* species	Morphology of CoNS is similar to *Staphylococcus aureus*.	Colonies on media are similar to that of *Staphylococcus aureus* although often smaller and are grey or white in color, though some may be pigmented usually cream to yellow.

APPENDIX-VIII

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-IX

ZONE SIZE INTERPRETATIVE CHART

Antimicrobial Agents used	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Susceptible (mm or more)
Ampicillin When testing gram-negative enteric organisms When testing Staphylococci	Amp	10 µg	13 28	14-16 -	17 29
Ceftriaxone	Ci	30 µg	13	14-20	21
Ciprofloxacin	Cf	5 µg	15	16-20	21
Cloxacillin	Ob	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

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

APPENDIX-X

CALCULATION OF SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUE AND EFFICIENCY

Tests	True positive (a)	False positive (b)	False negative (c)	True negative (d)

WBC counts	77	22	3	250
RBC counts	19	29	61	243
Albumin tests	24	22	56	250

Calculation of sensitivity

Sensitivity can be calculated as:

Sensitivity = a/ (a+c) ×100%

Sensitivity of WBC count = 77/ (77+3) ×100%=96.3%

Sensitivity of RBC count = 19/ (19+61) ×100%=23.8%

Sensitivity of Albumin test= 24/ (24+56) ×100%=30.0%

Calculation of Specificity

Specificity can be calculated as

Specificity=d/ (b+d) ×100%

Specificity of WBC count=250/ (22+250) ×100%=91.9%

Specificity of RBC count=243/ (243+29) ×100=89.3%

Specificity of Albumin test= 250/ (250+22) ×100%=91.9%

Calculation of Positive Predictive Value (PPV)

PVP can be calculated as

PVP=a/ (a+b) ×100%

PVP of WBC count =77/ (77+22) ×100%=77.8%

PVP of RBC count=19/ (19+29) ×100%=39.6%

PVP of Albumin test=24/ (24+22) ×100%=52.2%

Calculation of Negative Predictive Value (NPV)

PVN can be calculated as

PVN=d/(c+d) ×100%

PVN of WBC count =250/ (250+3) ×100=98.8%

PVN of RBC count=243/ (243+61) ×100=79.9%

PVN of Albumin test =250/ (56+250) ×100=81.7%

Calculation of Efficiency

Efficiency can be calculated as

Efficiency= a+d/ (a+b+c+d) ×100%

Efficiency of WBC count=77+250/ (77+22+3+250) ×100%=92.9%

Efficiency of RBC count=19+243/ (19+29+61+243) ×100%=74.4%

Efficiency of Albumin test=24+250/ (24+22+56+250) ×100%=77.8%

APPENDIX-XI

DATA ANALYSIS (CHI-SQUARE TEST)

1. Association of significant bacteriuria between genders

Gender	Culture positive	Culture negative	Total
Male	26	145	**171**
Female	54	127	**181**
Total	**80**	**272**	**352**

Test statistic is χ^2

Ho: There is no significant association of significant bacteriuria in male and female patients.

H1: There is significant association of significant bacteriuria in male and female patients.

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=10.7$

Thus χ^2_{cal} (10.7) > χ^2_{tab} at $\Im$=0.05 and d.f. = 1 i.e.3.841

Hence, Ho is rejected i.e. the higher proportion of positive cases seen among female patients is statistically significant.

2. Association of significant bacteriuria in asymptomatic and symptomatic patients

	Culture positive	**Culture negative**	**Total**
Asymptomatic patients	19	137	**156**
Symptomatic patients	61	135	**196**
Total	**80**	**272**	**352**

Test statistic is $\chi 2$

Ho: There is no significant association of significant bacteriuria in asymptomatic and symptomatic patients.

H1: There is significant association of significant bacteriuria in asymptomatic and symptomatic patients.

From $\chi^2 = \Sigma \frac{(O-E)^2}{E}$ we find $\chi^2 = 17.9$

Thus χ^2_{cal} (17.9) > χ^2_{tab} at $\Im = 0.05$ and d.f. =1 i.e 3.841

Hence, Ho is rejected i.e. the higher proportion of positive cases seen among symptomatic patients is statistically significant.

3. Association of MDR and Non-MDR strains between genders

	MDR strains	Non-MDR strains	Total
Male	15	11	**26**
Female	21	33	**54**
Total	**36**	**44**	**80**

Test statistic is $\chi 2$

Ho: There is no significant association of MDR and non-MDR strains between genders.

H1: There is significant association of MDR and non-MDR strains between genders.

From $\chi^2 = \Sigma \frac{(O-E)^2}{E}$ we find $\chi^2 = 2.51$

Thus $\chi^2_{cal\ (}2.51) < \chi^2_{tab}$ at $\Im = 0.05$ and d.f. =1 i.e 3.841

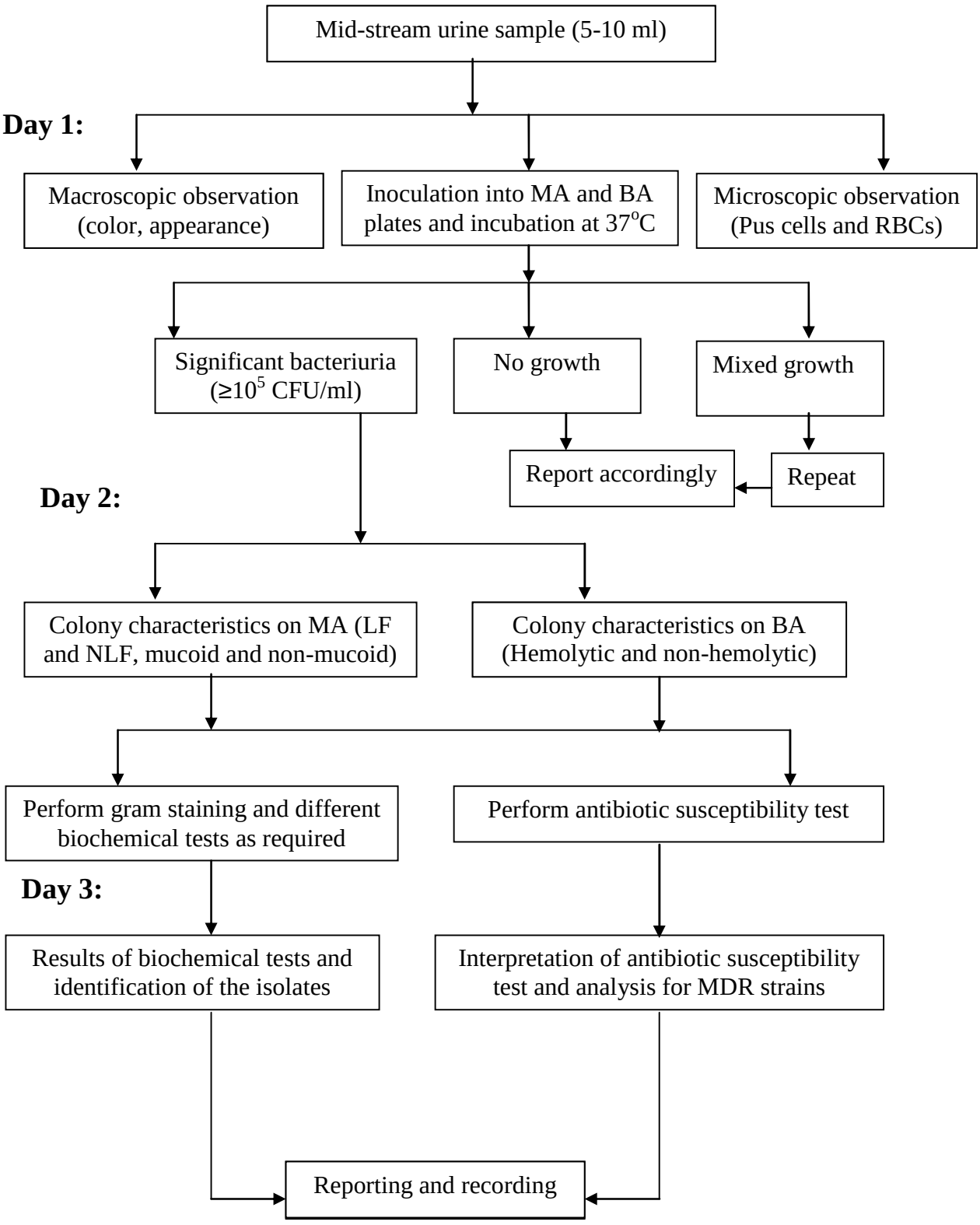
Hence, Ho is accepted i.e. there is no significant association of MDR and non-MDR strains between genders.

APPENDIX-XII

LIST OF ASYMPTOMATIC PATIENTS INCLUDED IN THE STUDY

S.N.	Asymptomatic patients	Total cases		Culture positive	
		No.	%	No.	%
1.	Kidney transplant patients	100	64.1	8	42.1
2.	Diabetic patients	15	9.6	5	26.3
3.	Pregnant patients	8	5.1	1	5.3
4.	Patient with catheter	1	0.6	1	5.3
5.	Patient with prostatitis	2	1.2	1	5.3
6.	Patient with kidney problem	12	7.7	3	15.8
7.	Patient with previous UTI	3	1.9	0	0
8.	Others	15	9.6	0	0
	Total	**156**	**44.3**	**19**	**23.8**

Figure 1: Flow chart showing processing of urine sample



(Source: Cheesbrough, 2000)

Photograph 1: Pure culture of *Escherichia coli* isolated on MacConkey agar plate
(Isolate no. 511 NPHL 14/4/063)

Photograph 2: Pure culture of *Klebsiella pneumoniae* isolated on MacConkey agar plate (Isolate no. 281 NPHL 1/3/063)

Photograph 3: Biochemical tests of *Klebsiella pneumoniae*
Legend: From left
A- A/A gas, H_2S negative B- H_2S negative, Indole negative, Non-motile

C- Citrate positive D- Urease positive E- MR negative F-VP positive
(Isolate no. 281 NPHL 1/3/063)

Photograph 4: Biochemical tests of *Proteus mirabilis*
Legend: From left
A- Alk/A, H_2S positive B- H_2S positive, Indole negative, Motile

C- Citrate positive D- Urease positive E- MR positive F-VP negative
(Isolate no. 336 NPHL 16/3/063)

Photograph 5: Antibiotic susceptibility test of *Escherichia coli*: MDR strain
A, B, D and F – Resistant and C and E – Susceptible
(Isolate no. 525 NPHL 16/4/063)

Photograph 6: Antibiotic susceptibility test of *Klebsiella pneumoniae*: MDR strain A, B, C, D, E and F – All Resistant
(Isolate no. 281 NPHL 1/3/063)

Photograph 7: Investigator culturing urine specimen

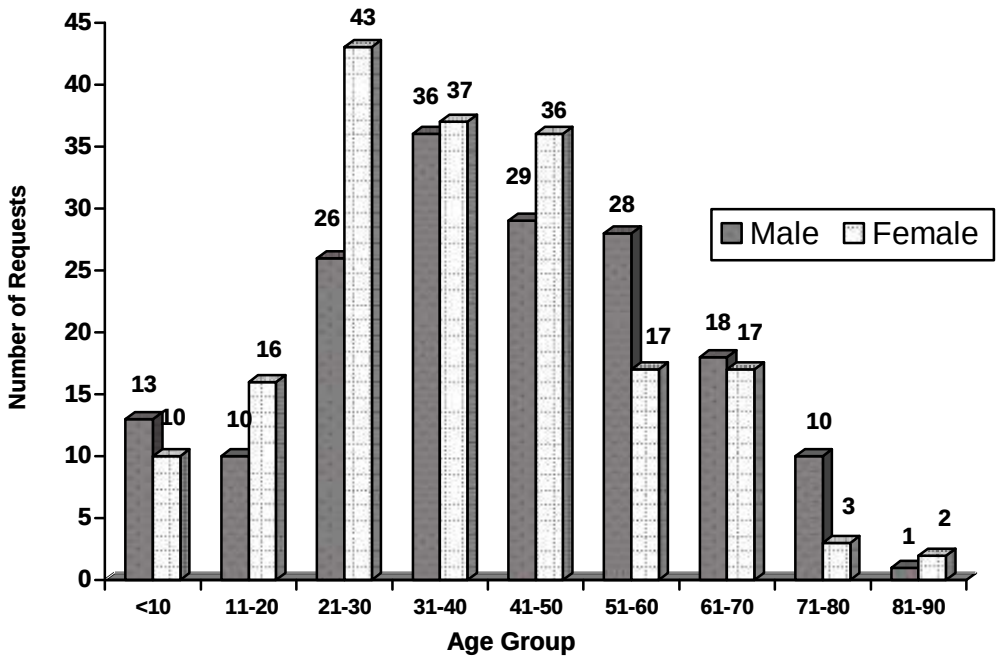


Figure 2: Age and Gender-wise Distribution of Patients Requested for Urine Culture

Figure 3: Pattern of Urine Culture Results