CHAPTER-I

1. INTRODUCTION

Urinary tract infection (UTI) is the infection of the urinary tract which is viewed as a single anatomical unit that is united by a continuous column of urine extending from the urethra to the kidney. UTI encompasses a wide variety of clinical entities whose common denominator is the microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus. Infection of prostate and epididymis is also included in the definition. Urethritis caused by Chlamydia and Gonococci is not included in the definition because of their unique characters and strict localization to the urethra and genital system (Pokhrel, 2004)). UTI is associated with multiplication of organisms in the urinary tract and is defined by the presence of more than 100,000 organisms per ml in the midstream sample of urine (Cheesbrough, 2000).

The anatomy of the female urethra is of particular importance to the pathogenesis of the UTI than male urethra. The female urethra is relatively short compared with the male urethra and also lies in close proximity to the perirectal region which is teeming with microorganisms, because of the short urethra the bacteria can reach the urethra and the bladder more easily in the female host (Forbes *et al*, 2002).

Most urinary tract infections in most occasions arise from one type of bacteria, *Escherichia coli* which is normally live in the colon, though this study is trying to find other organisms of importance causing UTI in a small Nepalese population as well. Usually the most recent infection stems from a strain or type of bacteria that is different from the infection before it, indicating a separate infection. National Institute for Health (NIH) funded research suggests that one factor behind recurrent UTIs may be the ability of bacteria to attach to cells lining of the urinary tract. UTIs caused by *Pseudomonas spp, Proteus spp, Klebsiella spp* and *Staphylococcus aureus* are generally associated with nosocomial infection often following catheterization and gynecological surgery. Infection due to *Proteus spp* is associated with renal stones. *Staphylococcus saprophyticus* infection is usually found in sexually active women.

In about 10%of patients with UTI, two organisms may be present and both may contribute to the disease process, the presence of 3 or more different organisms in a urine culture is strong presumptive evidence of improper collection or handling of the urine sample. However multiple organisms are often seen in UTI in patients with indwelling bladder catheters.

Kass (1955-1957) has reported that quantitative bacterial counting in both unselected and selected groups of population showed that when the urine contained over 10^5 CFU/ml this could be regarded as true or significant bacteriuria.

Bacteriological culture of urine is the only accurate way of diagnosing bacteriuria. Most laboratories use semi-quantitative methods. Although urine is supposed to be typically sterile, non-invasive methods for collecting urine must rely on a specimen that has been passed through the contaminated milieu. Therefore quantitative and semi-quantitative cultures for the diagnosis of UTI have been used to discriminate between contamination, colonization and infection (Leigh, 1990)).

Today antibiotics remain the front line therapy for conquering bacterial infection (Sharma, 2004). UTIs are treated with antibacterial drugs. The choice of drug and length of treatment depend on the patient's history and the urine tests that identify the offending bacteria.

Multiple drug resistance (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 commonly six prescribed drugs. An antibiotic resistance is defined as the microbe which is sensitive to certain antibiotic start gaining resistance against it. Infections caused by MDR strains often lead to death (Tuladhar *et al*, 2003).

UTI is a serious health problem affecting millions of people each year. It is the most important cause of mortality and morbidity in the world affecting all age groups across the life span. Infections of the urinary tract are common - only respiratory infections occur more often. Each year UTIs account for about seven million office visits and

another one million emergency department visits, resulting in about 100,000 hospitalizations. One woman in five develops a UTI during her lifetime; UTIs in men are less common. Nearly 20% of women who have a UTI will have another and 30% of those will have yet another episode of UTI (Urology channel, 2006).

Nepal being a developing country has about 61.4% people illiterate who are not aware of health and good hygienic living habit. Most of the diseases are due to poor sanitational knowledge and practices so there are always threats to infection caused by different types of organism. UTI is the commonest disease among Nepalese population. 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 Nepal in Mountain, Hill and Terai region of Nepal are 13, 518, 68,858 and 42,682 respectively.

As this introductory section has pointed out the urinary tract infection affects a sizable proportion of the population. The challenge it poses is exciting because there is evidence that early detection and eradication of bacteriuria and prevention of recurrence will reduce the incidence of later occurring life threatening consequences of persistent or repetitive urinary tract infection such as chronic pyelonephritis and chronic renal failure etc.

The purpose of this study is to isolate and identify the etiological agents of UTI and determine their antibiotic susceptibility pattern of that group of patients having different age and sex structures and who have recently visited one of the tertiary hospitals, TUTH situated at Katmandu, Nepal.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To study the bacteriology of urinary tract infection among the patients visiting TUTH.

2.2 SPECIFIC OBJECTIVES

1. To determine the number and types of bacteria causing urinary tract infection in different age and sex groups.
2. To establish the relationship between pyuria and bacteriuria.
3. To determine antibiotic susceptibility profile of the bacteria isolated from urine samples.
4. To analyze the MDR strains.

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).The presence of bacteria in urine is called bacteriuria (Cheesbrough, 1984).

UTI encompasses a wide variety of clinical entities whose common denominater is microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus. Infection of the prostrate and epididymis is also included in the definition. Urethritis caused by Gonococci and Chlamydia are not included in the definition because of their unique characters and strict localization to the urethra and genital system (Pokhrel, 2004).

UTI is defined as the detection of both bacteriuria (10^5 CFU/ml) and pyuria(>10 leucocytes per HPF)(Goya *et al,*1997). The term urinary tract infection refers to the invasion of the urinary tract by a non resident infectious organism. UTI is a bacterial infection that affects any part of the urinary tract. This includes the urethra, bladder and kidneys. Usually UTI is treated with increased fluid intake and antibiotics. In those few cases where physical obstruction is present, special medications or surgery may be required. In hospital patients, UTI is the most common source of bacteraemia (McCabe and Jackson, 1962).

Kass's (1956) definition of true or significant bacteriuria based on the presence of 10^5CFU bacteria/ml in a carefully collected sample of clean-voided or midstream urine made possible a clear distinction between infection and contamination. In order to confirm UTI with reasonable confidence, the following criteria must be met.

- Clinical features
- Significant bacteriuria
- Pyuria

Significant bacteriuria

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.

Kass, Marple and Sandford criteria to interpret significant bacteriuria

Less than 10,000 CFU/ml =contaminants
Equal to/ or more than 100000CFU/ml= significant bacteriuria
10,000 –100000 = low count significant bacteriuria, subject 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.
- Obstuction of the ureter.
- Infection with relatively slow growing organisms e.g.: *Staphylococcus saprophyticus,* Streptococci other than Enterococci*, Haemophilus influenzae (*Pokhrel, 2004).

Many workers have reported that in women with acute infection of the lower urinary tract is characterized by frequency, dysuria and urgency. 30-50% will have count les than 10^5 organism /ml (Gallacher *et al,* 1965). Stamm *et al*, (1982) suggested that a low count of coliform bacteria (>100organism/ml) should always be considered significant in women with symptoms, particularly when there was pyuria. Similarly Stamey *et al*, (1965) had suggested that in men with urinary symptoms, counts of less than 10^5 organism/ml should not be considered insignificant, true infection may be found with counts as low as 10^5 organisms/ml (Leigh, 1990).

Significant bacteriuria may sometimes occur in the absence of symptoms and pyuria in patients who subsequently develop symptoms of UTI e.g. in pregnancy. The detection of

such asymptomatic bacteriuria is of value for there is good evidence of its association with the development of pyelonephritis in some patients.

3.2 TYPES OF URINARY TRACT INFECTION

UTI encompasses a broad range of clinical entities that differ in terms of clinical presentation, degree of tissue invasion, epidemiologic settings and requirements for antibiotic therapy (Forbes, 2002).

3.2.1 On the basis of symptoms, severity and inflammatory changes UTI can be classified as:

a Uncomplicated UTI

Uncomplicated UTIs occur in patients with urinary tracts that are normal from both a structural and functional perspective. In uncomplicated UTI microbial invasion of the urinary tract occurs but no any inflammatory changes are seen i.e. shows no structural and neurological lesions in the tract. The first episode in this type of UTI shows *E .cloi* and has been found particular in female. Most common organisms encountered in uncomplicated UTI are *E.coli, Klebsiella spp, Proteus spp, Citrobacter spp, Enterobacter spp etc*.

b Complicated UTI

Microbial invasion of the tract occurs along with the structural and neurological lesions. The main surgical problem occurring is due to the obstruction in the ureter by stone formation in kidney and passage of it in ureter and tumor formation in the tract due to which patient can not pass urine as normally. Complicated urinary infection occurs in the setting of functional or structural abnormalities of the genitourinary tract (Rubin *et al*, 1992).

3.2.2 UTI is classified as primary or recurrent depending on whether the infection is initial or whether they are repeated sequels.

3.2.3 On the basis of source of infection UTI can be classified as:

a 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 the most frequent cause of uncomplicated community acquired UTI. Other bacteria isolated from patients with UTI includes *Klebsiella spp*, other Enterobacteriaceae and *Staphylococcus saprophyticus* (Forbes *et al,* 2002).

b Hospital acquired UTI (catheter associated or nosocomial UTI)

The hospital environment plays an important role in determining the organisms involved in the UTIs. Hospital acquired UTI 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% of all hospitalized patients who receive short-term catheterization develop a UTI (Forbes *et al*, 2002).Hospital patients are most likely to be infected by *E. coli, Klebsiella spp, Proteus mirabilis,* Staphylococci, other Enterobacteriaceace, *Pseudomonas aeruginosa* and Enterococci.

The study by Tuladhar *et al*, 1990 in Tribhuvan University Teaching Hospital shows that UTI is the most prevalent nosocomial infection which accounted (62.7%) of total nosocomial infection . Similarly *E.coli* was the dominant etiological agent (48%) followed by *Pseudomonas spp* (25.8%), *Klebsiella spp* (22.5%) and *Staphylococcus aureus* (3.2%) to cause nosocomial UTI.

3.2.4 On the basis of anatomic site of infection and clinical forms UTI can be classified as:

The clinical presentation of UTIs may vary from asymptomatic infection to full blown pyelonephritis (infection of kidney and its pelvis).

a Urethritis

Urethritis is a common infection because *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Trichomonas vaginalis* are common causes of urethritis and are considered to be sexually transmitted. Colonization of the urethra with gram-negative aerobic bacilli commonly occurs in catheterized patients; in men they also may be acquired by sexual intercourse (Stamey, 1980).

b Cystitis

Infection of urinary bladder is called cystitis. According to Norrby (1990), bacteriuria limited to bladder is called cystitis. Typically patients with cystitis complain of dysuria, frequency and urgency. Often there is tenderness and pain over the area of the bladder. In some individuals, urine is grossly bloody with a bad odor. Because cystitis is a localized infection, fever and other signs of systemic illness are usually not present.

c Acute urethral syndrome

Patients with this syndrome are primarily young , sexually active women who experience dysuria, frequency and urgency but yield fewer organisms than 10^5 CFU/ml urine on culture (Hamilton ,1994; Kunin ,1993; Stamm,1992).Almost 50% of all women who seek medical attention for complaints of symptoms of acute cystitis fall into this group. *Chlamydia trachomatis* and *N. gonorrhoeae* urethritis, anaerobic infection, genital herpes and vaginitis account for some of the causes of acute urethral syndrome, most of these women are infected with organisms identical to cystitis but in numbers less than 10^5 CFU /ml urine. Approximately 90% of these women have pyuria, an important discriminating feature of infection.

d Pyelonephritis

Pyelonephritis refers to inflammation of the kidney parenchyma, calices and pelvis, usually caused by bacterial infection. The typical clinical presentation of an upper UTI includes fever, flank pain and frequency and lower tract symptoms include frequency, urgency and dysuria. Patients can also exhibit systemic signs of infection such as

vomiting, diarrhoea, chills, increased heart rate and lower abdominal pain. Of significance, 40% of patients with acute pyelonephritis will be bacteremic.

e Renal infection other than pyelonephritis

Renal carbuncle due to *Staphylococcus aureus* usually follows a skin infection such as a boil or carbuncle or an infection of an intravenous line; the organism reaches the kidney through the blood stream. Other infection are perinephric abscess and pyonephrosis, it results from bacterial infection arising in an obstructed ureter and is usually unilateral.

f Bacterial prostatitis

The term prostatitis has been used for various inflammatory conditions affecting the prostate including acute and chronic infections with specific bacteria and more commonly instances in which signs symptoms of prostatic inflammation are present but no specific organisms can be detected. Bacterial prostatitis is bacterial infection of the prostate gland (Nickel, 1996).

Acute bacterial prostatitis affects young men however it may also be associated with an indwelling urethral catheter and is characterized by fever, chills, dysuria and a tense or boggy, extremely tender prostate. Nonbacterial prostatitis is a recognized inflammatory condition of unknown etiology which may be caused by chemical disturbances in the gland or due to Chlamydia or *Ureaplasma spp.*

Antimicrobial therapy will usually ameliorate acute symptoms but is seldom successful in permanent eradication of bacteria from the prostate (Leigh, 1993).

3.3 RESIDENT MICROORGANISMS OF THE URINARY TRACT

In a healthy person, the kidney, urinary bladder and ureters are free of microorganisms. However bacteria are commonly found in the lower portion of the urethra of both males and females and colonize its epithelium in the distal portion. Some of these organisms are listed below (Forbes *et al*, 2002).

CONS (excluding *S. saprophyticus*)

Viridans and nonhemolytic Streptococci

Lactobacilli

Diptheroids(*Corynebacterium spp)*

Nonpathogenic (saprobic) *Neisseria spp*

Anaerobic cocci

Propionibacterium spp

Anaerobic gram-negative bacilli

Commensal *Mycobacterium spp*

Commensal *Mycoplasma spp*

Potential pathogens including gram-negative aerobic bacilli (Primarily Enterobacteriaceae) and occasional yeasts are also present as transient colonizers. Potential source of contamination of the bladder urine specimen are the urethra, vaginal vestibule, vagina, labia and pubic hair in the female (Pfau and Sacks, 1970).

The common bacterial flora of the female urethra as well as that of the vaginal vestibule and vagina consists of Lactobacilli (10^{5} or more colonies per ml of transport broth). Staphylococci (ranging usually between 10 and 10^3 colonies per ml) are rare and transitory in nature despite close proximity to anus (Fair *et al*, 1971).

3.4 HOST DEFENSE MECHANISM

Urine being a good culture medium, multiplication of organism may occur within the urinary tract after gaining access to the tract. But certain factors (defense mechanism) normally play an important role in limiting their multiplication (Chakraborty, 1995).That as many as 33% of patients with chronic bacteriuria undergoes spontaneous cure attests to the efficiency of this mechanism.

Some of the host defense factors are:

a Protection of the urinary tract against infection is strongly related to the constant flow of urine and regular emptying of the bladder.

b In normal fertile women, the vagina is colonized by Lactobacilli that maintains highly acidic environment. Acid is hostile to other bacteria. Lactobacilli also produce H_2o_2 which helps to eliminate bacteria and reduces the ability of *E. coli* to adhere to the vaginal cells (Shrestha, 2004).

c The urinary tract is full of valvular structures.It prevents the ascent of bacteria by reflux of urine. Ureterovesicular valve permit a constant flow of urine from ureter to bladder (Stephens and Lenagham, 1962) but prevent when bladder is full or during micturation. The role of ureterovesicular valves in protection of urinary tract against infection is not of significant importance.

d The actual role of mucosal defense mechanism is unclear but mucosal defense against infection may result either from the phagocytic properties of inflammatory cells or from the secretion by mucosal cells of substances that inhibit the adhesion of bacteria or kill them (Vivaldi *et al*, 1965).

e Prostatic secretions have been reported to have antibacterial activity in men(Stamey *et al*,1965).A zinc containing polypeptide referred to as prostatic antibacterial factor or PAF appears to be the most significant antimicrobial constituents of prostatic fluid (Flair and Wehner, 1971).In addition to bactericidal activity against gram- negative bacilli, the substance inhibits the growth of viruses(Fridlender *et al*, 1978), Yeast (Gip and Molin, 1970), Trichomonads(Krieger and Rein, 1982) and *Chlamydia trachomatis* (Mardh *et al*,1980).

f The urine contains many types of antibodies (immuno-globulins) and these are considerably increased in amount when infection occurs in the tract. They may have a protective role in the renal tissue but in the urine no antibacterial activity has been established (Kaye, 1968).Bacterial-specific urinary antibody of the IgG and Ig A classes also appear to inhibit bacterial adherence (Fowler and Mariano, 1990).

3.5 FACTORS PREDISPOSING TO UTI

a Gender and sexual activity

Females are more frequently affected by UTI (particularly cystitis) due to colonization of urethra with colonic gram-negative bacilli because of its proximity to anus, short length of urethra and sexual intercourse.

b Pregnancy

Pregnancy predisposes to upper tract infection due to dilation of ureters and renal pelvis, stasis in right ureter, atony-reduced tone in uretic musculature and temporary incompetence of the vesicoureteral valves. Bladder catheterization during or after delivery causes additional infections. Increased incidences of low birth-weight infants, premature delivery and newborn mortality result from UTIs during pregnancy, particularly those infections involving the upper tract.

c Other factors

- Obstruction to flow of urine (by tumor, stricture, stone or prostatic hypertrophy)
- Use of catheters, probes and swabs
- Neurogenic bladder dysfunction(interference with the nerve supply to the bladder , as in spinal cord injury, tabes dorsalis, multiple sclerosis, diabetes and other disease may be associated with UTI)
- Vesicoureteral reflux (reflux of urine from bladder up into ureters and sometimes in the renal pelvis)
- Bacterial virulence factors
- Genetic factors (genetically determined receptors on uroepithelial cells)

Many different factors predispose or have been said to predispose to urinary tract infection (Leigh, 1990) shown in table 1.

Table 1: Precipitating factors in the development of urinary tract infection

Anatomical	Congenital abnormalities, prostatic hypertrophy, cystox cystocele, uterine prolapse

Pathological	Surgical operations on the urogenital tract, tumors on the bladder and prostate, urethral catheterization, atrophic vaginitis, neurological disorders of bladder
Infective	Vulvovaginitis, vaginal discharge
Social	Sexual intercourse, masturbation, menstruation, sanitary pads, intrauterine coli, diaphragm, contraceptive pills
Environmental	Exposure to cold, swimming, nylon underwear, tight clothes, sedentary occupation, long-distance driving

3.6 MULTIPLICATION OF BACTERIA IN URINE

As the bacteria enter into the urinary tract system, they multiply so rapidly that within an hour, the numbers of organisms exceed one million, since the urine is good culture media for the growth of bacteria.

Infection of urinary tract occurs when bacteria capable of proliferating in urine get access into the tract because human urine contains no hormonal or cellular defenses against bacterial growth (Fowler and Mariano, 1990). Normal urine does not contain significant amounts of immuno-globulins while the infected adult and children contain high amount of IgA and IgG proteinuria. The absence of opsonins and wide range of urinary osmolality impairs the phagocytosis of bacteria. The urine does not contain any antibacterial activity. Thus growth of urinary pathogens is higher (Jha and Yadav, 1992).

The bacterial population will be controlled by its growth rate and the balance between the speed of urinary flow and the volume of the system (O'Grady and Cattell, 1966).The growth rate or mean generation time will vary widely according to bacterial species and the composition of the urine (Asscher and Sussman, 1966; Roberts and Beard, 1965). The urinary flow may be affected by diuresis or dehydration and the total volume of the urinary drainage system may be greatly increased in physiological conditions such as pregnancy.

The ability of the urine to support bacterial growth is related to urinary pH, osmolality and chemical constituents such as glucose, amino acids and organic acids. The number of bacteria in the urine of diabetic patients was significantly higher than in that of non-diabetic controls due to high level of glucose (O'Sullivan and Fitzgerald, 1961).

3.7 PATHOGENESIS

3.7.1 Source of infection

The urinary tract is a complex drainage system consisting of distinct anatomical and physiological areas. The great majority of bacterial infection, whether or not with symptoms, occurs in the bladder (cystitis) after the ascending migration of bacteria from the urethra or perineum. Infection of the kidney may follow the haematogenous spread of bacteria, but more often the organisms ascend from the bladder via the ureter and the renal pelvis and calyces. Infection of the renal cortex is called nephritis, between cortex and pelvis is pyelonephritis, between pelvis and ureter is called pyelitis. Infection may also occur in prostate gland (prostatitis) and seminal vesicles in men and in paraurethral glands in women.

3.7.2 Mode of infection

There are different routes by which bacteria can invade and cause a UTI .Bacteria may gain access to the tract by ascending route, haematogenous route and lymphatic route.

a Ascending route

The ascending route of infection is now believed to be the usual one by which bacteria from the faecal flora spread to the perineum before ascending into the bladder. The ascending route of infection (i.e. urethral organisms spreading to or invading the bladder) is the most important means by which the urinary tract becomes infected (Stamey, 1981).

Although the ascending route is the most common route of infection in females, ascent in association with instrumentation (e.g. urinary catheterization, cystoscopy) is the most common cause of hospital –acquired UTIs in both sexes.

b Haematogenous route

In rare cases, urinary tract infection may result from haematogenous spread. Haematogenous spread usually occurs as a result of bacteremia. Bacteremia is part of many infections; this may lead to the formation of an abscess in the renal parenchyma or merely to the excretion of the organism in the urine e.g *Salmonella, Listeria*. These organisms cannot be considered to be urinary pathogens in that there is no evidence of persistent bacterial multiplication in the urine. *Candida albicans*, *Mycobacterium tuberculosis, Salmonella spp, Leptospira spp* or *Staphylococcus aureus* in the urine often indicate pyelonephritis acquired via haematogenous spread, or the descending route (Baron and Finegold, 1990).

c Lymphatic route

Direct extension of bacteria from the adjacent organ via lymphatic may occur in unusual circumstances such as severe bowel infection or retroperitoneal abscess. Experimental evidence suggests that lymphatic connections between upper and lower tracts may play a role in UTI in animals. The role of lymphatic in human UTI however is unknown at this time (Valenti and Reese, 1993).

3.7.3 Causative agents of urinary tract infection

The bladder and urinary tract are normally sterile except urethra which may contain a few commensals (Cheesbrough, 2000) and also the perineum (wide variety of gram- positive and negative organism) which can contaminate urine when it is being collected.

Urine is sterile body fluid and excellent culture medium for growth of microorganisms. If a bacterium is contaminated in the urine it multiplies so rapidly that within an hour numbers of bacteria exceeded to million per ml.

Many different microorganisms can infect the urinary tract but by far the most common agents are the gram-negative bacilli. *E. coli* causes approximately 80% of acute infection in patients without catheters, urologic abnormalities or calculi (Baron and Finegold, 1990)). Other gram-negative rods, especially *Proteus* and *Klebsiella* and occasionally *Enterobacter* account for a smaller proportion of uncomplicated infections. *Serratia* and *Pseudomonas* assure increasing importance in recurrent infections and in infection associated with urologic manipulation, calculi or obstruction and play a major role in nosocomial infection.

Organism	Possible pathogens
Gram-positive bacteria	*Staphylococcus aureus*, Cogulase negative *Staphylococcus* (CONS) *Staphylococcus saprophyticus* and *Enterococcus faecalis*
Gram-negative bacteria	*Escherichia coli, Klebsiella pneumoniae ,Klebsiella oxytoca P. vulgaris, Proteus mirabilis, Enterobacter cloacae Enterobacter aerogenes* , *Providencia stuartii , Providencia rettgeri Morganella morganii, Citrobacter fruendii*, *Citrobacter diversus Serratia marcescens* and *Pseudomonas aeruginosa*
Occasionally	*Salmonella typhi, Salmonela paratyphi* and *Neisseria gonorrhoea*
Fungi	Yeast (*Candida albicans*)
Parasites	*Schistosoma haematobium and Trichomonas vaginalis*
Occasionally	*Enterobius vermicularis, Wuchereria bancrofti and Onchocera volvulus*

Source: (Cheesbrough, 1984)

Gram-positive cocci play a lesser role in UTIs. However *Staphylococcus saprophyticus*-Novobiocin resistant CONS accounts for 10-20% of urethritis and cystitis in sexually active but otherwise healthy young women. There have been numerous reports of the distribution of slime production among clinically important CONS isolates. These suggest that the production of slime facilitates adherence of CONS to biopolymers and may be responsible for poor therapeutic response and relapse in vitro (Freedman *et al*, 1965).

Among coagulase negative Staphylococci*, S. saprophyticus* and *S.epidermidis* are frequently isolated organism. In addition to these CONS, *S. hemolytic, S. hominis, S .simulans* and *S. cohnii* are also isolated from urine samples (Saini *et al*, 1983).

Among the bacteria most frequently isolated from patients with community acquired acute cystitis are *E.coli, Klebsiella spp*, other Enterobacteriaceae *and Staphylococcus saprophyticus.* Other less frequently isolated agents are as Acinetobacter, *Alkaligens spp. Pseudomonas spp, Citrobacter spp, Gardnerela vaginalis ,* Beta-haemolytic Streptococci*, Neisseria gonorrhea* and *Trichomonas vaginalis.*

In a study done by Gautam *et al,* in 1998 found *E.coli* as the most predominant pathogen (57%) followed by *Klebsiella pneumoniae* (24%), *Proteus spp* (10%) *Pseudomonas aeruginosa* (1.7%), *Salmonella typhimurium* (1.7%), *Shigella boydii (*1.7%), *Streptococcus faecalis* (1.7%) and *Staphylococcus aureus* (1.7%).

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* (Chhetri *et al*, 2001).

Similarly study done by Karki *et al,* in 2004 found five different bacterial species of *E coli (*33.3%), *Proteus spp* (27.7%), *Klebsiela spp* (16.6%), *Staphylococcus aureus* (8.8%) and *Pseudomonas aeruginosa* (1.1%).

Table 2: Microbial causes of infections of the urinary tract (Leigh, 1990)

Site of infection	Uncomplicated (domicillary)		Complicated(hospital)	
Bladder	*E. coli*	90%	*E. coli*	50%
	Proteus mirabilis	5-8%	*Proteus. spp*	10-12%
	Klebsiella spp	1-2%	*Klebsiella spp*	15-20%
			Enterobacter spp	2-5%
			P. aeruginosa	10-15%
			Citrobacter spp	< 1%

	 CONS 1-2% *Streptococcus spp* <1% *Streptococcus faecalis* <1%	*Acinetobacter spp* < 1% CONS 1-2% *S .aureus* <1% *S. faecalis* 10-12%
Prostate	*E. coli* *Corynebacterium spp* Anaerobic bacteria	
Urethra	*E. coli* CONS *Streptococcus spp* *Neisseria gonorrhoea* *Chlamydia trachomatis* *Ureaplasma urealyticum*	

3.7.4 Bacterial virulence factors

The mechanism in bacterial infections involves bacterial adhesion to specific molecules on cell surfaces followed by invasive disease. Initially bacterial adhesion occurs via bacterial fimbriae in the case of gram-negative bacteria while gram-positive bacteria adhere more frequently via extracellular polysaccharides (Roberts, 1996). The vast majority of urinary tract infections are caused by strains of uropathogenic *E. coli* that encode filamentous adhesive organelles called type 1 pili. These structures mediate both bacterial attachment to and invasion of bladder epithelial cells. However, the mechanism by which type 1 pilus-mediated bacterial invasion contributes to the pathogenesis of a urinary tract infection is unknown (Matthew *et al*, 2001).

The epithelial cells of the human urinary tract contain glycoconjugate receptors for a pathogenic bacterium. Most *E. coli* isolated from the urine of patients with acute pyelonephritis recognize and bind to human uroepithelial cell membrane glycolipids with the sequence Gal 1| 4Gal (Block , 1990).

An interesting and potentially important aspect of this uroepithelial receptor is that the sequence Gal 1| 4Gal is recognized by the *E. coli* ligand regardless of whether it is present internally or terminally inside the chain. In contrast antibodies directed against the same glycolipid recognize primary terminal groups. Thus, binding to internal groups of glycoconjugate receptors may function to assure adhesion by avoiding differences that often appear in terminal sequences (Warner and Kim, 1989).

Uropathogenic strains of *E. coli* are believed to display a variety of virulence properties that assists in colonization of host mucosal surfaces. A limited number of virulence factors including adhesions, siderophores, toxins, capsules and a protease have implicated as important traits allowing uropathogenic *E. coli* to cause disease (Agrawal *et al*, 1989; Caprioli *et al*, 1987; Donnenberg and Welch, 1996; Lundrigan and Webb, 1992).

Many virulence factors of uropathogenic bacteria are encoded by foreign DNA stretches inserted into the core genome. These pathogenicity islands or islets were obviously acquired via horizontal gene transfer creating new path types more efficient in establishing infection (Oelschlaeger *et al*, 2002).

3.7.5 Clinical features

Clinical signs and symptoms cannot be relied upon to diagnose urinary tract infection correctly or to localize the site of infection. Many patients with significant bacteriuria (including some with upper tract infection) have no symptoms at all. Of those with significant bacteriuria and symptoms of cystitis, about one-half have lower tract infection and about one- half have clinically silent upper tract infection that is evident only upon performing localization studies.

1 Asymptomatic

Symptom less UTI or covert bacteriuria is not uncommon (about 5% of adult women, 1-3% of girls and 0.3% of boys).

2 Symptomatic

Symptoms include dysuria, frequent micturation, suprapubic pain along with loin pain and tenderness. There may be fever and rigors. Fever is associated with pyelonephritis(Chakraborty,1995). Symptoms that indicate a lower UTI are increased frequency, micturation, dysuria and urgency. The symptoms that indicate an upper UTI are fever, dysuria and nausea (Car and Sheikh, 2003).

3.8 LABORATORY DIAGNOSIS OF URINARY TRACT INFECTION

Pre-therapy culture of urine is necessary for identification of the organism, antimicrobial susceptibility test and assessment of the results of treatment.

3.8.1 Specimen collection

Prevention of contamination by normal vaginal, perineal and anterior urethral flora is the most important consideration for collection of a clinically relevant urine specimen. For the isolation and identification of bacteria in urine sample, the sample collection is very important. Generally there are four types of sample collection.

- Clean -Catch, Midstream Urine
- Catheter specimen
- Suprapubic aspiration
- Sterile urinary bag attached on the genital organs

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 4ºC until delivery to the laboratory and processed no longer than 18 hours after collection (Basic laboratory manual in clinical bacteriology, 1991).Transport medium that can be used for urine specimens are 1.8% boric acid, sodium chloride or polyvinylpyrolidine (Pokhrel, 2004).

Laboratory examination of urine generally includes:

i Description of the appearance of the urine

Color and turbidity of urine is noted in the very initial step through which preliminary test results for infection are identified. When left to stand, cloudiness may develop due to

precipitation of urates in acid urine or phosphates and carbonates in alkaline urine. Urates may give the pink orange color.

Table 3: Macroscopic observation of urine (Cheesbrough, 2000)

Appearance	Possible cause
Cloudy Urine usually has an unpleasant smell and Contains WBC	- Bacterial urinary infection
Red and Cloudy Due to red cells	- Urinary schistosomiasis - Bacterial infection
Brown and cloudy Due to haemoglobin	- Black water fever - Other conditions that cause intra vascular haemolysis
Yellow brown, or green brown Due to bilirubin	- Acute viral hepatitis - Obstructive jaundice
Yellow orange Due to urobilin, i.e.oxidised urobilinogen	- Haemolysis, Hepatocellular jaundice
Milky white Due to chyle	- Bancroftian filariasis

ii Microscopic examination of urine

Urine is examined microscopically as a wet preparation to detect:

- Significant pyuria i.e. WBC is in excess of 10 cells/µl (10^6/l) of urine
- red cells
- epithelial cells
- casts
- yeast cells
- *T. vaginalis* motile trophozoites
- *S. haematobium* eggs
- Bacteria(providing the urine is freshly collected)

Microscopic observation of urine is an indispensable tool for in the diagnosis of genitourinary disorders (Fowler and Mariano, 1983).

Pus cells: These are round 10-15 µm in diameter cells that contains granules. In urinary infections they are often found in clumps (Cheesbrough, 1984). The observation of pus cells in urine is indicative of bacteriuria but may result from any inflammatory disorder of the urinary tract. Increased numbers of leucocytes in the urine principally neutrophils seen in almost all renal diseases and diseases of urinary tract. Pyuria is considered significant if more than or equals to 5 pus cells are seen per HPF in urine sediments. The other criterion for significant pyuria is the leucocyte count of _10 leucocytes per HPF (Robins *et al,* 1975).

Sterile pyuria is defined as 5 to 10 white blood cells or pus cells per HPF in the absence of apparent UTI or urethral discharge (Gleckman and Esposito, 1978).The possible cause of sterile pyuria are undiagnosed bacterial urinary tract infection, renal tuberculosis, interstitial nephritis, kidney or bladder stones, renal papillary necrosis, chronic bacterial prostatitis, chlamydial infection and gonorrhea (Ziloski and Smucker, 1989).

Alongside bacteriuria, finding of significant pyuria is of great importance for UTI diagnosis and it strengthens further the microscopic diagnosis while erythrocytes and epithelial cells are of very poor significance for UTI diagnosis (Merila *et al,* 1987).

Erythrocytes: Haematuria is generally used to derive findings of blood cells in urine. It may be found in urinary schistosomiasis, bacterial infection, acute glomerulonephritis, sickle cell disease, leptospirosis, infective endocarditis, calculi 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 (Pandey,2002). Finding of red cells in urine of females may also be due to presence of menstrual blood in urine.

Erythrocytes are found in small numbers in normal urine. In normal males and females occasional red cells (0 to 2 per HPF) may be seen on microscopic examination of the sediment. The finding of red blood count greater than or equal to three per high power

field is considered as abnormal (Froom *et al*, 1986; Steward *et al,* 1985; Wargotz *et al,* 1987).

Epithelial cells: It is normal to find a few epithelial cells in urine. When seen in large number however they usually indicate inflammation of the urinary tract or vaginal contamination of the specimen (Cheesbrough, 1984).

The finding of large numbers of squamous epithelial cells or approximately 1-2/ HPF in the voided specimen of a female is not uncommon if proper techniques for the collection of an uncontaminated specimen are not followed (Fowler and Mariano, 1990).

Wargotz *et al,* 1987 reported that greater than or equal to five squamous epithelial cells per high power field is considered as abnormal.

iii Biochemical test of urine

Biochemical test which are helpful in investigating UTI include

- Protein
- Nitrate reductase (Greiss) test
- Leucocyte esterase test
- Triphenyl tetrazolium chloride reduction
- Catalase test

iv Culture of urine

Known volume of urine specimen obtained is inoculated on MacConkey agar and Blood agar without any delay with the help of calibrated loop. The urine should be mixed thoroughly before plating. The calibrated loop method using quarter plates of culture media is recommended because it is inexpensive, simple to perform and provides individual colonies that are easier to identify and remove for antimicrobial susceptibility testing.

v Interpretation of urine culture results

Count the approximate number of colonies. Estimate the number of bacteria, i.e. colony forming units (CFU) per ml of urine.

UTI may be completely asymptomatic, produce mild symptoms or cause life-threatening infections. Of importance the criteria most useful for microbiologic assessment of urine specimens is dependent not only on the type of urine submitted (e.g., voided, straight catheterization) but the clinical history of the patient (e.g. age, sex, symptoms, antibiotic therapy). Report the bacterial count as:

- Less than 10,000 organisms/ml (10^4 /ml), not significant.
- (10000-100000/ml) 10^4-10^5/ml), doubtful significance (suggested repeat specimen)
- More than 100 000/ml (10^5/ml), significant bacteriuria (Cheesbrough, 2000).

vi Identification and antibiotic susceptibility testing of causative organism

The causative organism isolated was identified using various techniques. Gram staining and biochemical tests as required. If similar colonies are found in numbers suggesting significant bacteriuria, a separate colony or a portion of apparently pure growth should be sub-cultured for identification and testing of its susceptibility to antibiotics. The appearance of the primary growth on MacConkey medium will suggest the kind of organism that is present. Probably coliform bacilli should be differentiated into *E. coli, Klebsiella spp, Proteus spp* and *Staphylococcus aureus* should be distinguished from other Staphylococci and Enterococci should be distinguished from other Streptococci.

Modified Kirby-Bauer disk diffusion technique was followed for antibiotic susceptibility testing of causative organism.

3.9 ANTIBIOTIC SUSCEPTIBILITY TESTING

An antibiotic was originally defined as a substance produced by one microorganism which inhibited the growth of other microorganisms. The advent of synthetic methods has however resulted in a modification of this definition and an antibiotic now refers to a substance produced by a microorganism or to a similar substance (produced wholly or partly by chemical synthesis) which in low concentrations inhibit the growth of other microorganism (Hugo and Russel, 1983).

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. These tests are assays designed to determine the extent of an organism's acquired resistance.

For laboratory tests to accurately determine organism-based resistance the potential influence of environmental factors on antimicrobial activity must be minimized. To control the impact of environmental factors, the conditions for susceptibility testing are extensively standardized. The components of antimicrobial susceptibility testing that are standardized and controlled include the following:

- Bacterial inoculum size
- Growth medium: pH, cation concentration, blood and serum supplements and thymidine content
- Incubation temperature, atmosphere and duration
- Antimicrobial concentrations tested

However the use of standard conditions there are some limitations. Most notably the laboratory test conditions in no way mimic the *in vivo* environment at the infection site where the antimicrobial agent and bacteria will actually interact. Factors such as bacterial inoculum size, pH, cation concentration, and oxygen tension can differ substantially depending on the site of infection. Because of the lack of correlation between *in vitro* test conditions and the *in vivo* setting, antimicrobial susceptibility testing cannot and should not be used as predictors of therapeutic outcome use for the use of particular antimicrobial agents. Additionally several other important factors that play key roles in patient outcome are not taken into account by susceptibility testing. Some of these factors include:

- Antibiotic diffusion in tissues and host cells
- Serum protein binding of antimicrobial agents
- Drug interactions and interference
- Status of patient defense and immune system
- Multiple simultaneous illnesses
- Virulence and pathogenesis of infecting bacterium
- Site and severity of infection

Despite these limitations the goal of in vitro susceptibility testing to detect resistance provides valuable data that are used in conjunction with other diagnostic information to optimize therapy (Forbes *et al*, 2002).

Some general considerations which are to be made while undertaking susceptibility tests may include:

Inoculum preparation

The two important requirements for appropriate inoculum preparation include use of a pure culture and a standardized inoculum.

Interpretation of results obtained with a mixed inoculum is not reliable and failure to use a pure culture can substantially delay reporting of results. Pure inocula are obtained by selecting four or five colonies of the same morphology, inoculating them to a broth medium and allowing the culture to achieve good active growth.

Use of standard inoculum size is as important as culture purity and is accomplished by comparison of the turbidity of the organism suspension with a turbidity standard. The 0.5 McFarland standards provide an optical density comparable to the density of a bacterial suspension of 1.5×10^{8} CFU/ml.

The decision to perform susceptibility testing depends on the following criteria:

- Clinical significance of a bacterial isolate.
- Predictability of a bacterial isolates susceptibility to the antimicrobial agents most commonly used against them often referred to as the drugs of choice.
- Availability of reliable standardized methods for testing the isolate.

Susceptibility testing strategies focused on production of accurate results have two key components**:**

- Use of methods that produce accurate results.
- The application of real-time review of results before reporting.

Classification of antibiotics

Over 6000 antibiotics have been described by now (Dhungel *et al,* 2001) and these antibiotics are classified in several ways.

a By their antibacterial potency

i) Bactericidal: A bactericidal agent kills bacteria in usual dosages e.g. Penicillin, Aminoglycosides.

ii) Bacteriostatic: A bacteriostatic agent does not kill bacteria but stop the active multiplication of bacteria in usual dosages e.g, Chloramphenicol, Tetracyclines and Erythromycin.

b By the spectrum of their biological action

i) Broad spectrum: Antibiotics of broad spectrum activity are active against both gram-positive and gram-negative bacteria. The broad spectrum antibiotics are Tetracyclines, Chlroramphenicol.

ii) Narrow spectrum: Antibiotics of narrow spectrum are active against gram-positive or gram-negative bacteria e.g. Penicillin, Bacitracin, Cloxacillin against gram-positive bacteria.

c By their chemical structures

- -lactam antibiotics
- Tetracyclines
- Rifamycins
- Amino glycosides (Streptomycin, Neomycin, Tobramycin, Amikacin)
- Macrolides (Erythromycin, Oleandomycin , Azerythromycin)
- Polypeptides (Bacitracin, Polymyxin)
- Miscellaneous (Chloramphenicol, Vancomycin, Novobiocin, D-cycloserine)
- Antifungal antibiotics (Greseofulvin, Polyenes)
- Synthetic antibiotics (Sulphonamides, Antitubercular drugs, Nitrofurantoin, Quinilones as Nalidixic acid, Ciprofloxacin)

3.9.1 Measurement of antimicrobial activity

Determination of the susceptibility of a bacterial pathogen to antimicrobial drugs can be done by one of two principle methods: dilution or diffusion.

Using an appropriate standard test organism and a known sample of drug for comparison, these methods can be employed to estimate either the potency of antibiotic in the sample or the susceptibility of the microorganism.

a Dilution method

For quantitative estimates of antibiotic activity, dilutions of the antibiotic may be incorporated into broth or solid bacteriologic media (agar medium) which is then inoculated with the test organism. The lowest concentration that prevents growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent. The test can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacteria. The MBC is usually taken as the lowest concentration able to reduce the original inoculum by a factor of a thousand, e.g. from 10^5 CFU/ml to 10^2 CFU/ml or below (Greenwood *et al*, 2001).

This MIC or MBC value is then compared with known concentration of the drug obtainable in the serum and in other body fluids to assess the likely clinical response.

b Diffusion method

The disk diffusion test was developed by Bauer, Kirby, Sherris and Turck in 1966. A filter paper disk containing a measured quantity of a drug is placed on the surface of a solid medium that has been inoculated on the surface with the test organism. After incubation, the diameter of the clear zone of inhibition surrounding the disk is taken as a measure of the inhibitory power of the drug against the particular test organism .This method is subjected to many physical and chemical factors in addition to the simple interaction of drug and organisms (e.g. the nature of the medium and diffusibility, molecular size and the stability of the drug). The antibiotic-impregnated disc absorbs moisture from the agar and antibiotic diffuses into the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases, there is a logarithmic reduction in the antibiotic concentration. The extent of antimicrobial diffusion is affected by the depth of the agar.

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 (Collee *et al*, 1999). Following incubation, the diameter of the zone of inhibition around each disk is measured in millimeters.

The Kirby-Bauer method (Bauer *et al,* 1966) and its modifications recognize three categories of susceptibility: susceptible, intermediate and resistant.

Susceptible: An organism is called "susceptible" to drug when the infection caused by it is likely to respond to treatment with this drug, at the recommended dosage.

Intermediate: It covers two situations. It is applicable to strains that are moderately susceptible to an antibiotic that can be used for treatment at a higher dosage because of its low toxicity or because the antibiotic is concentrated in the focus of infection (e.g. urine). The classification also applies to strains that show intermediate susceptibility to a more toxic antibiotic that cannot be used at a higher dosage.

Resistant: This term implies that the organism is expected not to respond to a given drug, irrespective of the dosage and of the location of the infection.

There are various types of disc diffusion sensitivity tests which vary in their methods of standardization, reading and control:

- Kirby-Bauer method
- Stokes disc diffusion method
- Ericssion method
- The comparative method

Modified Kirby-Bauer Method:

For clinical and surveillance purposes and to promote reproducibility and comparability of results between laboratories, WHO recommends the NCCLS (National Committee for

Clinical Laboratory Standards) modified Kirby-Bauer disc diffusion technique (Cheesbrough, 2000).

In this technique, the broth culture of test organism (comparable to McFarland tube no.0.5, inoculum density 1.5×10^8 organism per ml) is uniformly carpeted on the surface of Mueller Hinton agar. Then antibiotic discs were seeded over the lawn culture of test organism. The inoculated and seeded MHA plate is then incubated overnight at 37 ºC. After incubation diameter of zone of inhibition of each antibiotic is interpreted using the interpretive chart and the organism reported resistant, Intermediate and sensitive. The control strain is inoculated on a separate plate.

Control strains used to test the performance of this method are:

Staphylococcus aureus (ATCC 25922), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa (*ATCC 27853).

3.9.2 Choice of antibiotics for susceptibility tests in urinary tract infection

A number of considerations are involved in selecting appropriate antimicrobial agents to treat an infection (Lenette *et al*, 1985). The purpose of antimicrobial susceptibility testing in urinary infection is to provide *in vitro* information to assist the clinician in selecting the antimicrobials that would be most reliable in inhibiting growth of infecting organisms in urine. Susceptibility testing of certain antimicrobials including Nitrofurantoin, Nalidixic acid and Norfloxacin is relevant only for urinary infection as these agents achieve concentrations sufficient to be reliably inhibitory to bacterial growth only in urine (Nicolle, 1991). NCCLS currently recommends the routine testing of urinary isolates include Cinoxacin or Nalidixic acid, Ciprofloxacin, Nitrofurantoin , Norfloxacin , Sulphamithozaxole and Ceftriazone (Sahm *et al*, 1998). Nitrofurantoin is active against many *Enterobacter in vitro* but has no value in the treatment of urinary infections caused by *Proteus spp* and other urease positive organisms which render the urine alkaline and reduces the activity of the antibiotics.

All the gram-positive and gram-negative organisms are tested against more than three group antimicrobials: - lactam antibiotic, Aminoglycosides, Quinolone and other antimicrobial agents such as Amoxicillin/Ampicillin, Cephalexin, Cotrimoxazole, Chloramphenicol, Nitrofurantoin, Ceftriazone.

Gram-positive bacteria are additionally tested with antimicrobial agents specific for gram-positive organisms such as Penicillin, Methicillin, Erythromycin and Vancomycin.

Gram-negative bacteria are also tested against specific antimicrobial agents such as Gentamycin, Nalidixic acid and Amikacin.

The second and 3rd sets of antimicrobial agents are only tested for multiple drug resistant isolates (MDR).

3.10 ANTIMICROBIAL DRUG RESISTANCE

Resistance can be defined as the temporary or permanent ability of an organism and its progeny to remain viable or multiply under environmental conditions that would destroy or inhibit other cells (Hugo and Russell, 1983).

An antibiotic resistance is defined as the microbe which is sensitive to certain antibiotic starts gaining resistance against it. The MDR strain is defined as the strain that showed resistance to three or more antibiotics among the six commonly prescribed drugs (Tuladhar *et al,* 2003).

MDR isolates are defined as those isolates resistant to three or more group of antimicrobial agents (Rijal *et al,* 2004).

Bacteria like all other life forms are subject to the driving force of evolution of natural selection. On repeated use of a particular drug, bacteria are subjected to a so-called '*selective pressure'*. Such selective pressure on higher forms of life can take thousands of years to have an effect but with bacteria it happens much more quickly. Consequently, drug resistance in bacterial colonies can develop very quickly.

3.10.1 Antibiotic resistance mechanisms

An antibiotic is said to be resistant when that antibiotic in prescribed amount and concentration is unable to kill / suppress the growth of the pathogens. The emergence and spread of antimicrobial resistance determinants is a problem of increasing importance worldwide, particularly among nosocomial bacterial pathogens. Bacterial strains are now emerging that is resistance to all currently available antimicrobial drugs. The past two decades have also witnessed a significant increase in clinically important resistance in a variety of bacterial species as well as the emergence of significant pathogens of intrinsically resistant strains previously considered to be of low pathogenicity (Hugo and Russel, 1983).

There are several factors influencing these disturbing increases in bacterial resistance. The increased use of a variety of antimicrobials and the clinical introduction of numerous closely related compounds are clearly related to the emergence and dissemination of resistant strains .The flourishing of intensive care units with the prolonged stays and close proximity of many patients whose physical and immunological defenses are compromised have created settings in which the emergence and spread of resistant bacteria have accelerated.

Environmentally mediated antimicrobial resistance

Antimicrobial resistance is the result of nearly inseparable interactions among the drug, the microorganism and the environment in which they are brought together. Environmentally mediated resistance is defined as resistance that directly results from physical or chemical characteristics of the environment that either directly alter the antimicrobial agent or alter the microorganism’s normal physiologic response to the drug. Examples of environmental factors include pH, anaerobic atmosphere, cat ion concentrations and thymine-thymidine content.

- Antibacterial activities of Erythromycin and Aminoglycosides diminish with decreasing pH while the activity of tetracycline decreases with increasing pH.

- Aminoglycoside activity requires intracellular uptake across the cell membrane, much of which is driven by oxidative processes so that in the absence of oxygen, uptake and hence activity is substantially diminished.

Microorganism mediated antimicrobial resistance

Microorganism mediated resistance refers to antimicrobial resistance that is due to genetically encoded traits of the microorganism and is the type of resistance that *in vitro* susceptibility testing methods are targeted to detect. Organism-based resistance can be divided into two subcategories: intrinsic or inherent resistance and acquired resistance.

A Intrinsic resistance

Antimicrobial resistance resulting from the normal genetic, structural or physiologic state of a microorganism is referred to as intrinsic resistance. Intrinsic resistance profiles are useful for determining which antimicrobial agents should be included in the battery of drugs that will be tested against specific types of organisms.

For example, Aminoglycosides are ineffective against Enterococci due to lack of sufficient oxidative metabolism to drive uptake of Aminoglycosides. Similarly Sulphonamides, Trimethoprim, Tetracycline or Chloramphenicol are futile against *P. aeruginosa* due to lack of uptake resulting from inability of antibiotics to achieve effective intracellular concentrations (Forbes *et al*, 2002).

B Acquired resistance

Antibiotic resistance that results from altered cellular physiology and structure caused by change in a microorganism's usual genetic makeup is known as acquired resistance. Unlike intrinsic resistance, acquired resistance may be a trait associated with only some strains of a particular organism group or species but not others. *Streptococcus haemolyticus* has remained sensitive to benzyl penicillin after more than 40 years of exposure to the drug.

The genetic basis of antimicrobial resistance

The genetic change from drug sensitivity to resistance may come about in bacteria by following modes:

1Spontaneous mutation

The appearance of resistant cells can be explained by the relatively infrequent occurrence (approximately 1 per 10^7 cells per cell division) of spontaneous gene mutations which confer drug resistance.

Transfer of genetic information

i Conjugation

In conjugation, there is physical contact between two genetically different bacterial cells of the same or closely related species. There is no exchange of genetic material during conjugation, only unilateral transfer (Pelczar *et al,* 1993).

In addition to R factors, the transfer of genes via F factor located together with the resistance genes on the plasmids creates a highly efficient mechanism for dissemination of antimicrobial resistance (Dhungel, 2001).

Transposons: These are linear pieces of DNA, often including genes for antibiotic resistance that are mobile. Almost certainly it is the activity of such transposons which have resulted in R-plasmids with resistance markers to many antibiotics (Madigan *et al*, 2003).

ii Transduction

Transduction is the transfer of `genetic information between bacteria by bacteriophages (virus that invades bacteria). In the clinical setting, transduction may be more important in spreading resistance among gram-positive bacteria than gram-negative cells. Transduction of drug-resistance plasmids in *Staphylococcus aureus* has been observed in infected mice and on human skin (Hugo and Russel, 1983).

iii Transformation

It is a process in which a free DNA molecule is transferred from a donor to a recipient bacterium. The DNA released from the donor cell upon cell lysis may be absorbed by ‘competent’ cells and integrated into their genomes. However it is perhaps unlikely that the clinical problem of resistance among gram-positive pathogens owes much to transformation.

Biochemical basis of antimicrobial resistance

The following are the important possible mechanisms by which cells might resist the toxic effects of a growth-inhibiting drug (Hugo and Russel, 1983).

1 Conversion of active drug to an inert derivative by enzyme produced by the resistant cells.

- Inactivation of ß-lactam antibiotics
- Inactivation of Chloramphenicol by acetylation
- Inactivation of Aminoglycoside antibiotics
- Changes in the antibiotic target site resulting in drug resistance
- Reduction in cellular permeability to antibiotics

In order to suppress the growth of bacteria, a drug must achieve an inhibitory concentration at its target site. Therefore a decrease in cellular permeability to the drug may depress the drug concentration at the target site below the inhibitory level.

- Permeability of the outer membrane
- Antagonism of antibiotic transport process
- Development of an altered metabolic pathway

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 STUDY POPULATION

The study of Microbiology of UTI was conducted among patients suspected of UTI attending at Tribhuvan University Teaching Hospital, Kathmandu, Nepal. The period for this research work was from June 2006 to August 2006.

During this research work, 185 midstream urine specimens were collected from patients clinically suspected of UTI and processed at bacteriology laboratory of microbiology department. The age of the patients ranged from 2 to 90 years. The history of all the patients including age, gender, symptoms were recorded in the data collection form from the requisition form obtained along with the mid stream urine for culture.

The materials required for present work are mentioned in the appendix II**.**

4.2 SPECIMEN COLLECTION

The patients attending at TUTH with clinical features of UTI were given a clean, dry sterile and leak proof container and requested for 5 to 10 ml midstream urine sample and examined freshly. Detailed guidelines for collection of clean catch midstream urine are mentioned in appendix VI.

4.3 SPECIMEN EVALUATION

Before proceeding with any testing, 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 labelled 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.

4.4 MACROSCOPIC EXAMINATION

It included the observation of the color and transparency of the collected urine sample.

4.5 MICROSCOPIC EXAMINATION

Ten 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 included the detection of WBC (pus cells) and RBC. Number of WBC and RBC will be estimated as number per high power field (HPF) i.e. 40X objective of microscope.

4.6 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 is accepted) and known volume(0.001ml) of mixed uncentrifuged urine to inoculate on the surface of 5% Blood Agar and MacConkey Agar .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.

4.7 EXAMINATION OF CULTURE PLATE

The culture plates were observed after 24 hours of incubation at 37ºC. The bacterial isolates in the plates was identified incase of significant growth (10^5 CFU/ml). The plates were reincubated for additional 24 hours if the growth was not observed or if bacterial growth was unidentifiable after 24 hours of incubation or if only tiny colonies

were observed. After incubation, the approximate numbers of colonies were counted on each plate and organisms per ml were estimated in accordance to the volume of urine inoculated previously. For example, 100 colonies on inoculating 0.001ml of urine would correspond to 10^5 CFU/ml. The plates were discarded if growth was not observed after 48 hours incubation and reported as sterile. Mixed growth of contaminating organism was neglected. Blood agar was observed for haemolysis and MacConkey agar for lactose fermentation and lactose non-fermentation.

The agar plates producing homogenous colony morphology were subjected for identification.

4.8 IDENTIFICATION OF ISOLATES

Identification of significant isolates was done by using microbiological techniques as described in the Bergy's manual which involves morphological appearance of the colonies, staining reactions, biochemical properties and serotyping if required in specific cases (Forbes *et al*, 2002; Cheesbrough, 1984; Collee *et al*, 1996).

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 MacConkey agar and for gram-positive the same from blood agar 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 on 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, Oxidation Fermentation test, Triple Sugar Iron (TSI) test, Motility test and Gas production tests.

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

Purity plate

Purity plate culture of each biochemical test was performed to observe whether the tests were preceded in an aseptic condition or not. The 4 hours incubated broth culture prepared for biochemical test was inoculated on one half of the nutrient agar plate just before preceding the biochemical test. The other half of the same nutrient agar plate was inoculated immediately after completing the biochemical test .The plate was incubated at 37℃ for overnight. The pure growth of same organism in both the pre and post-inoculated portion of the plate is the indication of maintenance of aseptic condition throughout the experiment.

4.9 ANTIBIOTIC SUSCEPTIBILITY TEST

The antimicrobial susceptibility testing of the isolates towards the various antimicrobial disks was done by modified Kirby- Bauer discs diffusion technique as recommended by (NCCLS, 1999) using Mueller Hinton agar. The inoculum was prepared by suspending

colonies from freshly grown on agar plate (non selective medium) in sterile physiological saline and compared the turbidity of the bacterial suspension with that of the Mc Farland standard. The turbidity was adjusted as necessary by dilution or addition of more colonial growth. Antibiotic susceptibility test was performed as follows:

- Pure colonies of organisms was transferred to 5 ml Nutrient broth and incubated at 37º C for 4 hours. The prepared inoculum was compared with Mc Farland tube number 0.5.
- A sterile cotton- wool swab was dipped into the suspension and the excess liquid was removed by turning the swab against the side of the tube. The inoculum was spread evenly over the entire surface of the Mueller Hinton Agar plate by swabbing in three directions.
- The plate was allowed to dry before applying discs and it should be applied to the surface of the agar within 15 min of inoculation.
- The paper discs of selected antibiotics were gently pressed into the organism carpeted MHA plate at a distance of 15mm away from the edge and 24 mm apart of each other.
- Plates were inverted and incubated at 37ºC for 24 hours under aerobic conditions within 15 min of disc application.
- The diameter of zone of inhibition was measured and compared with standardized zone interpretative chart provided by the company. The diameters of zones of inhibition were measured to the nearest mm with a ruler, calipers or an automated zone reader.

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.

4.10 QUALITY CONTROL

To obtain reliable microbiological result it is necessary to maintain quality control. During this study, quality control was applied in various areas.

- During sample collection, aseptic technique was followed for collecting midstream urine in sterile bottles in order to avoid contamination.
- During sample processing, all the tests were carried out appropriately in aseptic conditions.
- While using readymade dehydrated media, the manufacturer's instructions for preparation, sterilization and storage were followed to prevent the alteration of the nutritional, selective, inhibitory and biochemical properties of media.
- The performances of newly prepared media were tested using control species of bacteria (i.e. known organisms giving positive and negative reactions).
- For stains and reagents, whenever new batches of them were prepared or a control smear was stained to ensure correct staining reaction.
- Control strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) were used for the standardization of the Kirby- Bauer test and also for correct interpretation of zone of diameter.

4.11 DATA ANALYSIS

Chi- square test was used to determine significant association of bacteriuria and gender, bacteriuria and hospitalization of patients and pyuria versus bacteriuria in various groups of patients. Test of present work are shown in appendix XI. Similarly calculation of sensitivity, specificity, positive predictive and negative predictive and efficiency of pus cell and erythrocytes count were done and are shown in appendix X.

CHAPTER-V

5. RESULTS

5.1 INDOOR AND OUTDOOR DISTRIBUTION OF PATIENTS

Out of total 185 patients, 130 cases (70.27%) were from outdoor patients and 55 (29.73%) were from indoor patients. Out of 108 total female patients, the distribution between outdoor and indoor patients was 79 (73.14%) and 29 (26.85%) respectively. Similarly out of 77 male patients, 26(33.77%) were from indoor patients and 51(62.23%) were from outdoor patients. In comparison to males higher numbers of urine samples were collected from females which account for 108(58.37%). The results are tadulated in table 4.

Table 4: Indoor and outdoor distribution of patients visiting TUTH

Gender	Indoor patients	Outdoor patients	Total Number	%
Male	26	51	77	41.62
Female	29	79	108	58.37
Total	55	130	185	
%	29.73	70.27	100	100

5.2 AGE AND GENDER WISE DISTRIBUTION OF PATIENTS VISITING TUTH

In this study, the age of the patients ranged from 2 years to 90 years. The highest number of patients 66 (35.67%) belonged to the age group 20-30 followed by 31(16.75%) belonged to 30-40, 21 (11.35%) belonged to 40-50, 19 (10.27%) belonged to 10-20 age group. Larger number of female patients 45 (41.67%) were found in the age group 20-30 followed by 17 (15.74%) in age group 30-40.Similarly higher number of male patients 21 (27.27%) were found in age group 20-30 followed by 14 (18.18%) in age group 30-40. The results are shown in table 5.

Table 5: Age and gender wise distribution of patients

Age group	Inpatients				Out Patients				Total
	Male		Female		Male		Female		
	No.	%	No.	%	No	%	No	%	
0-10	0	0.00	0	0.00	2	1.08	2	1.08	4
10-20	3	1.62	4	2.16	4	2.16	8	4.32	19
20-30	7	3.78	8	4.32	14	7.56	37	20.00	66
30-40	2	1.08	6	3.24	12	6.48	11	5.95	31
40-50	3	1.62	5	2.70	3	1.62	10	5.40	21
50-60	4	2.16	2	1.08	4	2.16	6	3.24	16
60-70	3	1.62	3	1.62	7	3.78	4	2.16	17
70-80	4	2.16	1	0.54	3	1.62	0	0.00	8
80-90	0.0	0.00	0	0.00	2	1.08	1	0.54	3
Total	26	14.05	29	15.67	51	27.56	79	42.70	185

5.3 PATTERN OF CULTURE RESULTS

Out of total 185 Midstream urine samples (MSU), 41 (22.16%) samples showed significant bacteriuria (i.e. 10^5CFU/ml), 135 (72.97%) were sterile and 9 (4.86%) samples showed mixed growth.

Table 6: Pattern of culture results

Growth	No of samples	Percentage of samples
Significant growth	41	22.16
No growth	135	72.97
Mixed growth	9	4.86

5.4 GROWTH PATTERN IN INDOOR AND OUTDOOR PATIENTS

Out of 130 total samples from outdoor patients, 25 (19.23%) showed significant growth and out of 55 samples from indoor patients 16 (29.09%) showed significant growth. The results are shown in table 7.

Table 7: Growth pattern in indoor and outdoor patients

Source	Total no. of patient	Significant growth	%
Outpatients	130	25	19.23
Inpatients	55	16	29.09
Total	185	41	

5.5 GROWTH PATTERN IN MALE AND FEMALE PATIENTS

Of the total 77 samples from male patients, 19 (24.67%) samples were found to show significant growth. Similarly 22 (20.37%) samples from female patients showed significant growth .The results are shown in table 8.

Table 8: Growth pattern in male and female patients

Gender	Total no of patient	Significant growth	%
Male	77	19	24.67
Female	108	22	20.37
Total	185	41	22.16

5.6 GROWTH PATTERN IN VARIOUS AGE GROUPS

Among the 41 significant growth samples highest number of samples was obtained from age group 30-40 (26.82%), followed by age group 20-30 (24.39%). However, in female patients, higher status of significant growth was found in the age group 20-30 (19.51%), followed by 30-40 (17.07%). The least number of samples with significant growth was obtained from age group 80-90 years (2.44%). The results are shown in table 9.

Table 9: Growth pattern in various age groups

Age group	Male		Female		Total	
	No	%	No	%	No	%
10-20	0	0.00	2	4.88	2	4.88
20-30	2	4.88	8	19.51	10	24.39
30-40	4	9.75	7	17.07	11	26.82
40-50	2	4.88	1	2.44	3	7.32
50-60	4	9.75	0	0.00	4	9.75
60-70	3	7.32	3	7.32	6	14.63
70-80	3	7.32	1	2.44	4	9.75
80-90	1	2.44	0	0.00	1	2.44
Total	19	46.34	22	53.66	41	100.00

5.7 PATTERN OF BACTERIAL ISOLATES CAUSING UTI

Out of 41 isolates isolated from MSU samples in this study, 8 different species were isolated which are tabulated in table 10. Among the isolates *E. coli* (65.85%) was found to be most predominant organism followed by *Klebsiella pneumoniae* (9.75%) and *Staphylococcus aureus* (7.32%).

Table 10: Pattern of bacterial isolates causing UTI

S.N	Organisms isolated	Number of isolates	Total Percentage (N= 41)
	Gram negative bacteria		
1	*Escherichia coli*	27	65.85
2	*Enterobacter* spp	1	2.44
3	*Klebsiella pneumoniae*	4	9.75
4	*Klebsiella oxytoca*	1	2.44
5	*Pseudomonas aeruginosa*	2	4.88
6	*Proteus vulgaris*	1	2.44
	Gram positive bacteria		
1	*Staphylococcus aureus*	3	7.32
2	*Streptococcus faecalis*	2	4.88
Total		41	100

5.8 BACTERIAL GROWTH PATTERNS IN MALE AND FEMALE PATIENTS

Out of 41 growth positive patients, 22 (53.66%) female and 19 (46.34%) male patients were found to be infected by different types of bacteria. Among the different species of bacteria isolated, *E. coli* was the most frequent isolate in female 16 (59.26%) than in male 11 (40.74%). The results are shown in table 11.

Table 11: Bacterial growth pattern in male and female patients

S.N	Organism isolated	Male		Female		Total
		No	%	No	%	
	Gram negative bacteria					
1	*E. coli*	11	40.74	16	59.26	27
2	*Enterobacter spp*	1	100.00	0	0.00	1
3	*K. pneumoniae*	3	75.00	1	25.00	4
4	*K. oxytoca*	0	0.00	1	100.00	1
5	*P. aeruginosa*	0	0.00	2	100.00	2
6	*P. vulgaris*	1	100.00	0	0.00	1
	Gram positive bacteria					
1	*S. aureus*	2	66.67	1	33.33	3
2	*S. faecalis*	1	50.00	1	50.00	2
	Total	19	53.66	22	46.34	41

5.9 PYURIA VERSUS BACTERIURIA

Out of total 185 centrifuged urine samples, pus cells were absent in 46 (24.86%) samples and all of these showed negative growth. Similarly out of 82 samples with 0-5 pus cells/HPF, 10 samples showed significant growth and among 16 samples with 5-10 pus cell/HPF, 6 samples showed significant growth. On the other hand four samples with 20-25 pus cells /HPF, only 1 sample showed significant growth. In the same way, out of 21 samples with plenty of pus cells/ HPF, 17 samples showed significant growth. The results are tabulated in table 12.

Table 12: Pyuria versus bacteriuria

Pus cells /HPF	No of samples	% (N= 185)	Significant growth	
			No	%
0	46	24.86	0	0.00
0-5	82	44.32	10	12.19
5-10	16	8.65	6	37.50
10-15	9	4.86	4	44.45
15-20	7	3.78	2	28.57
20-25	4	2.16	2	50.00
Plenty	21	11.35	17	80.95
Total	185	100.00	41	

5.10 PYURIA VERSUS BACTERIURIA IN MALE AND FEMALE PATIENTS

Table 13 describes the gender wise distribution of pyuria versus bacteriuria. As number of pus cells increase per HPF the chance of getting culture positive results are also high. This pattern is equally likely to occur in both male and female as seen by percentage increase in positive growth results with increasing numbers of pus cells seen per HPF in centrifuged urine samples.

Table 13: Presence of pus cells (5per HPF) versus significant growth

Pyuria	No. of samples		Total
	Significant growth	Non significant growth	
Significant	31	26	57
Insignificant	10	118	128
Total	41	144	185

Table 13 describes the pattern of pyuria (5 pus cells per HPF) of urine sediment against the number of samples showing significant bacteriuria.

Table 14: Pyuria versus bacteriuria in male and female Patients

Pus cells /HPF	No of sample	Male		Female	
		Culture negative (%)	Culture positive (%)	Culture negative (%)	Culture positive (%)
0	T= 46, M= 20 F =26	20 (100.0)	0 (0.00)	26 (100.00)	0 (0.00)
0-5	T= 82, M = 29 F =53	25 (86.20)	4 (13.89)	47 (88.68)	6 (11.32)
5-10	T =16, M=8 F=8	6 (75.00)	2 (25.00)	4 (50.00)	4 (50.00)
10-15	T=9, M=7 F=2	4 (57.14)	3 (42.85)	1 (50.00)	1 (50.00)
15-20	T=7,M=3 F=4	2 (66.66)	1 (33.33)	3 (75.00)	1 (25.00)
20-25	T=4, M=2 F=2	1 (50.00)	1 (50.00)	1 (75.00)	1 (25.00)
Plenty	T=21, M=8 F=13	0 (0.00)	8 (100.00)	4 (30.77)	9 (69.23)
Total	T=185, M=77 F=108	58 (75.3)	19 (24.6)	86 (79.6)	22 (20.3)

Key: T= Total, M= Male, F= Female

5.11 HAEMATURIA VERSUS BACTERIURIA

Out of total 185 centrifuged urine samples, 139 samples were not found to contain any RBC per HPF. Among the samples in which RBC were nil, 30 sample showed significant growth. On the other hand 24 samples out of 185 showed RBC <3 per HPF out of which 5 samples showed significant growth .Out of 16 samples with 3 pus cell, 3 samples showed significant growth. In the same way 6 samples out of 185 were found to contain plenty RBC per HPF, of which 3 samples showed significant growth.

Table 15: Haematuria versus bacteriuria

RBC / HPF	No of samples	% (N= 185)	Significant growth	
			No	%
0	139	75.13	30	21.58
<3	24	12.97	5	20.84
3	16	8.65	3	18.75
Plenty	6	3.24	3	50
Total	185	100	41	

Table 16: Presence of erythrocytes (3perHPF) versus significant growth.

Haematuria	No of samples		Total
	Significant growth	Non significant growth	
Significant	6	16	22
Insignificant	35	128	163
Total	41	144	185

Table 16 describes the pattern of presence of 3erythrocytes per HPF of urine sediment against the number of samples showing significant bacteriuria.

5.12 ANTIBIOTIC SUSCEPTIBILITY PROFILE OF BACTERIAL ISOLATES

5.12.1 Gram-negative bacteria

The most effective antibiotic among the test against gram-negative bacteria (excluding *P. aeruginosa, K. oxytoca* and *K. pneumoniae*) was found to be Nitrofurantoin(58.33%) followed by Ceftriazone(50%). Ampicillin(82.75%)was found to be least effective drugs among the tested antibiotics for gram-negative bacteria.

Table 17: Antibiotic susceptibility profile of gram-negative bacteria

S.N	Antibiotics Used	Number	Susceptibility Pattern					
			Resistant		Intermediate		Sensitive	
			No	%	No	%	No.	%
1	Ampicillin	29	24	82.75	0	0.00	5	17.24
2	Ciprofloxacin	36	21	58.33	0	0.00	15	41.67
3	Cephalexin	36	20	55.55	4	11.11	12	33.34
4	Norfloxacin	36	21	58.33	0	0.00	15	41.67
5	Nitrofurantoin	36	13	36.11	2	5.55	21	58.34
6	Cotrimoxazole	34	17	50.00	3	8.82	14	41.17
7	Ceftriazone	36	18	50.00	0	0.00	18	50.00
8	Amikacin	7	1	14.28	0	0.00	6	85.71
9	Cefotaxime	2	0	0.00	0	0.00	2	100.00

5.12.2 Gram-positive bacteria

The most effective antibiotics against gram-positive bacteria were found to be Novobiocin (100%) followed by Erythromycin (Sensitivity 60%).

Table 18: Antibiotic susceptibility profile of gram-positive bacteria

S.N	Antibiotics used	Number	Susceptibility pattern					
			Resistant		Intermediate		Sensitive	
			No	%	No	%	No	%
1	Ampicillin	5	3	60	0	0	2	40
2	Ciprofloxacin	5	3	60	0	0	2	40
3	Cephalexin	5	3	60	0	0	2	40
4	Erythromycin	5	2	40	0	0	3	60
5	Cloxacillin	5	3	60	0	0	2	40
6	Cotrimoxazole	5	3	60	0	0	2	40
7	Novobiocin	5	0	0	0	0	5	100

5.13 ANTIBIOTIC SUSCEPTIBILITY PROFILE OF DIFFERENT GROUP OF BACTERIA

5.13.1 Antibiotic susceptibility profile of *Escherichia coli*

Incase of *E. coli*, sensitivity pattern to different drugs was as follows: Nitrofurantoin (66.67%) followed by Ceftriazone (48.14%) and Cotrimoxazole (40.74%). Sensitivity towards Ciprofloxacin, Cephalexin and Norfloxacin was found to be moderate. Similarly Ampicillin was found to be least sensitive (18.52%). The results are shown in Table 19.

Table 19: Antibiotic susceptibility profile of *E. coli*

Organism isolated	No.	Antibiotics Used	Antibiotic Susceptibility Profile					
			Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%
E. coli	27	Ampicillin	22	81.48	0	0.00	5	18.52
		Ciprofloxacin	18	66.66	0	0.00	9	33.34
		Cephalexin	14	51.85	3	11.12	10	37.03
		Norfloxacin	17	62.96	0	0.00	10	37.03
		Nitrofurantoin	7	25.92	2	7.40	18	66.67
		Cotrimoxazole	13	48.14	3	11.12	11	40.74
		Ceftriazone	14	51.85	0	0.00	13	48.14

5.13.2 Antibiotic susceptibility profile of *Klebsiella spp*

K. pneumoniae was found to be highly sensitive towards Amikacin, Ceftriazone and lower sensitivite towards Cephalexin and Cotrimoxazole. Sensitivity towards Ciprofloxacin, Nitrofurantoin and Norfloxacin was found to be 50%. Similarly on the other hand, *K. oxytoca* was found to be 100% sensitive towards Amikacin, Ciprofloxacin, Cephalexin, Norfloxacin and Ceftriazone and resistant to Nitrofurantoin and Cotrimoxazole.

Table 20: Antibiotic Susceptibility Profile of *Klebsiella spp*

Organism isolated	No	Antibiotics Used	Susceptibility Profile					
			Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%
K. pneumoniae	4	Amikacin	1	25	0	0	3	75
		Ciprofloxacin	2	50	0	0	2	50
		Cephalexin	2	50	1	25	1	25
		Norfloxacin	2	50	0	0	2	50
		Nitrofurantoin	2	50	0	0	2	50
		Cotrimoxazole	3	75	0	0	1	25
		Ceftriazone	1	25	0	0	3	75
K. oxytoca	1	Amikacin	0	0	0	0	1	100
		Ciprofloxacin	0	0	0	0	1	100
		Cephalexin	0	0	0	0	1	100
		Norfloxacin	0	0	0	0	1	100
		Nitrofurantoin	1	100	0	0	0	0
		Cotrimoxazole	1	100	0	0	0	0
		Ceftriazone	0	0	0	0	1	100

5.13.3 Antibiotic susceptibility profile of gram-negative bacteria other than *E. coli* and *Klebseilla spp*

P. aeruginosa was showing good susceptibility to Amikacin and Cefotaxime while Cephalexin showed least sensitivity towards *P. aeruginosa.*

Similarly *P. vulgaris* was found to be 100% sensitive towards Ciprofloxacin, Cotrimoxazole and Ceftriazone and resistant to Ampicillin, Cephalexin, Norfloxacin, and Nitrofurantoin. *Enterobacter spp* was found to be 100 % sensitive to Ciprofloxacin, Norfloxacin, Cotrimoxazole and resistant to Ampicillin, Cephalexin, Nitrofurantoin and Ceftriazone.

Table 21: Antibiotic susceptibility profile of gram-negative bacteria other than *E coli* and *Klebseilla spp.*

Organism isolated	No	Antibiotics Used	Susceptibility pattern					
			Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%
P. aeruginosa	2	Amikacin	0	0	0	0	2	100
		Ciprofloxacin	1	50	0	0	1	50
		Cephalexin	2	100	0	0	0	0
		Norfloxacin	1	50	0	0	1	50
		Nitrofurantoin	1	50	0	0	1	50
		Cefotaxime	0	0	0	0	2	100
		Ceftriazone	1	50	0	0	1	50
P. vulgaris	1	Ampicillin	1	100	0	0	0	0
		Ciprofloxacin	0	0	0	0	1	100
		Cephalexin	1	100	0	0	0	0
		Norfloxacin	1	100	0	0	0	0
		Nitrofurantoin	1	100	0	0	0	0
		Cotrimoxazole	0	0	0	0	1	100
		Ceftriazone	1	100	0	0	0	0
Enterobacter species	1	Ampicillin	1	100	0	0	0	100
		Ciprofloxacin	0	0	0	0	1	100
		Cephalexin	1	100	0	0	0	0
		Norfloxacin	0	0	0	0	1	100
		Nitrofurantoin	1	100	0	0	0	0
		Cotrimoxazole	0	0	0	0	1	100
		Ceftriazone	1	100	0	0	0	0

5.13.4 Antibiotic susceptibility profile of other gram-positive bacteria

Ampicillin, Ciprofloxacin, Erythromycin and Cotrimoxazole were found to be 66.66% sensitive towards *S. aureus* and Novobiocin was found to be 100 % sensitive. Similarly Erythromycin and Cloxacillin was found to be 50% sensitive and 100% sensitive towards Novobiocin.

Table 22: Antibiotic susceptibility profile of other gram-positive bacteria

Organism isolated	No	Antibiotics used	Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%
S. aureus	1	Ampicillin	1	33.33	0	0	2	66.67
		Ciprofloxacin	1	33.33	0	0	2	66.67
		Cephalexin	2	66.66	0	0	1	33.34
		Erythromycin	1	33.33	0	0	2	66.67
		Cloxacillin	2	66.66	0	0	1	33.34
		Cotrimoxazole	1	33.33	0	0	2	66.67
		Novobiocin	0	0	0	0	3	100
S. faecalis	2	Ampicillin	2	100	0	0	0	0
		Ciprofloxacin	2	100	0	0	0	0
		Cephalexin	2	100	0	0	0	0
		Erythromycin	1	50	0	0	1	50
		Cloxacillin	1	50	0	0	1	50
		Cotrimoxazole	2	100	0	0	0	0
		Novobiocin	0	0	0	0	2	100

5.14 RESISTANCE PATTERN OF ISOLATES AGAINST COMMONLY USED ANTIBIOTICS

Most of the pathogens isolated were found to be resistant to >3 drugs[(23/ 41 positive isolates) 56.09%].It was found that 9.75% were found to be sensitive to all antibiotics used in the study and14.63% isolates were resistant to 1 drug, 12.19% isolates were resistant to 2 drugs and 7.31% isolates were resistant to 3 drugs. The results are shown in Table 23.

Table 23: Resistance pattern of bacterial isolates against commonly used antibiotics

Organism	Total	Resistant to									
		0 drug		1 drug		2 drug		3drug		>3drugs	
		N0	%	No	%	No	%	No	%	No	%
E.coli	27	3	11.12	3	11.12	2	7.40	2	7.40	17	62.9

Enterobacter spp	1	0	0	0	0	0	0	1	100	0	0
K. pneumoniae	4	0	0	2	50	0	0	0	0	2	50
K. oxytoca	1	0	0	1	100	0	0	0	0	0	0
P. aeruginosa	2	0	0	0	0	2	100	0	0	0	0
P. vulgaris	1	0	0	0	0	0	0	0	0	1	100
S. aureus	3	1	33.33	0	0	1	33.33	0	0	1	33.3
S. faecalis	2	0	0	0	0	0	0	0	0	2	100
Total	41	4	9.75	6	14.63	5	12.19	3	7.31	23	56.0

5.15 MULTIPLE DRUG RESISTANT PATTERNS OF BACTERIAL ISOLATES

Total multiple drug resistant (MDR) cases were 56.09 %(23 / 41). Among the total 27 samples of *E. coli* isolates 17 (62.96%) were found to be MDR. The results are tabulated in Table 24.

Table 24: Distribution MDR among the bacterial isolates in UTI

S.N	Organism	Total cases	Total MDR	
			No	%
1	*E. coli*	27	17	62.96
2	*Enterobacter spp*	1	0	0
3	*K. pneumoniae*	4	2	50
4	*K. oxytoca*	1	0	0
5	*P. aeruginosa*	2	0	0
6	*P. vulgaris*	1	1	100
7	*S. aureus*	3	1	33.33
8	*S. faecalis*	2	2	100
Total		41	23	56.09

CHAPTER-VI

6. DISCUSSION AND CONCLUSIONS

6.1 DISCUSSION

Nepal is one of the developing countries of south East Asia having comparatively very poor health status than many other developed countries of the world. Some of the main reasons for possessing low health status among Nepalese population are illiteracy, lack of hygienic and sanitary knowledge, malnutrition, economic status and lack of proper techniques in using medical procedures. Due to such problems, people are usually victimized by many infectious diseases which can be prevented with prior knowledge.

Urinary tract infections are a serious health problem affecting millions of people each year.UTI is among the most common bacterial infections that lead patients to seek medical care. It has been estimated more than 6 million out patients visit and 300,000 hospital stays every year are due to UTI (Palac, 1986; Stamm, 1992; Wong, 1983). UTIs are common type of bacterial infection accounting for reasonably high health care expenditures in people of all ages with more than 35 million medically treated infections each year (Mindbranch Inc, 2004). Young women, the elderly and those undergoing genitourinary instrumentation or catheterization are also at risk (Kunin *et al*, 1993). Urinary tract infections account for about 8.3 million doctor visits each year (CDC, 2004). There are an estimated 150 million urinary tract infections per annum worldwide (Stamm and Norrby, 2001). According to annual report published by Department of Health Services, Kathmandu (1996/1997) the morbidity of UTI is 0.42% of the total population and urine sample appears as the second commonest sample (98%) to be submitted to the laboratory after blood (48%) for examination. The geographical distribution of UTI amongst the Nepalese population is 0.57% in the mountains, 0.45 % is estimated to be in planes (DoHS, 2000).

The present study was conducted to address the isolation of etiological agent and to determine the antibiotic susceptibility of urinary pathogens. The study was conducted by

collecting midstream urine samples from patients of 2 to 90 years attending at TUTH during June 2006 to August 2006.

Out of total patients, 130 cases (70.27%) were from out patients department and 55(29.73%) were from patients admitted to the Hospital. Similarly 108 (58.37%) of the patients were female and 77(41.62%) were male. The age of the patient visiting hospital ranged from 2 to 90 years.

According to table 5, the highest number of patients 66(35.67%) belonged to the age group 20-30 years. Similarly the higher number of female patients 45 (41.67%) were found in the age group 20-30 years whereas higher number of male patients 21(27.27%) were also found in the same age group 20-30.

Out of 185 midstream urine samples (MSU), 41(22.16%) samples showed the growth of significant bacteriuria (table no. 6). Such a low rate of growth positivity for UTI was also observed by Dhakal, 1999; Gautam *et al,* 1998; Karki *et al*, 2004; Levitt, 1993; Manandhar, 1996 and Obi *et al*, 1996. 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 media we used.

This finding was also lower than those reported by other investigators in the valley. Study carried out in a tertiary medical center, the Tribhuvan University Teaching Hospital located in northern part Kathmandu valley have shown bacterial growth positivity in 44.5% and 42.8% of outpatients and inpatients respectively (Tuladhar *et al*, 1989) . But the growth positive rate has been found to be reduced (37.3%) (Rai *et al,* 2001) at the same hospital. The low growth positive rate observed in present study could be due to relatively small sample size and difference in the study population.

However very low growth positivity (4.6%) has also been reported from elsewhere (Talukder, 1987).

In this study, higher percentage of growth positive culture was obtained from Inpatients (hospitalized) rather than from the outpatients (patients from the community). The study results correlates with the research findings of Shrestha, 2004 and Tuladhar *et al*, 1990. The statistical analysis failed to show the significant association of positive growth between outpatients and inpatients ($P>0.05$).

Out of total growth positive 22.16% samples, higher numbers of growth positive samples were obtained from male patients (24.67%) as compared to female patients (20.37%). The results were expressed in table 8.Association of presence of significant growth between male and female patients was found statistically insignificant ($P>0.05$). In general or in most of the literatures, we found the significant bacteriuria is more common in female as compared to male. However scientists are not sure why women have more UTI than men. The reasons may be the women's urethra much shorter and closer to the anus than in males. This may allow bacteria quick access to the bladder.

But in present study, it was found the high prevalence of significant bacteriuria in male as compared to female. This may be due to inclusion of higher no of male patients suffering from renal stones and kidney infection in our study. This fact is supported by the study done by Pendse and Singh, 1996 who demonstrated UTI infection as the principle risk factor for urolithiasis and vice versa.

Result of this study also supports the findings of various others studies that document the higher prevalence of UTI in males than in females due to prostatic hypertrophy: Dhakal, 1999; Kosakai *et al,* 1990; Leigh, 1990 and Nordenstan *et al,* 1986. Present study contradicts the statement of Leigh, 1990 that the relative infrequency of urinary tract infection in men may be attributable to the length of the male urethra and the bactericidal activity of the prostatic fluid.

Table no.9 shows the significant growth pattern in various age groups. According to which 26.82% of the infected patients belong to the age group 30-40 years (total growth positive samples). The high incidence (19.51%) of the infected females were found in the age group 20-30 years (8 out of total positive), this finding may be correlated well with

sexual activity and pregnancy. Asymptomatic bacteriuria is common during pregnancy due to physiological changes like hormone level changes etc. Its average prevalence is 6% during pregnancy. It is important risk factors for acute pyelonephritis, hypertension, preaclampsia, foetal wastage, low birth weight and prematurity as said by Abdul Razak 1992. In males, asymptomatic bacteriuria is almost always secondary to chronic prostatitis or urinary tract obstruction. A number of studies suggest that intercourse is important in the pathogenesis of bacteriuria in susceptible women. So bacteriuria is high in sexually active ages. Recent sexual intercourse and spermicide use increase the risk of cystitis several fold. History of UTI is also an important risk factor (Hooton *et al,* 1996).

The frequency of bacteriuria greatly varies between sexes and age and is influenced by development of pathological conditions that favor UTI (Kunin, 1987).

In this study, there is constant rise of cases with age in both the sexes' upto 60 years. This finding is in correlation with the result of Fowler,1990 ; Kosakasi *et al*, 1990 and Manandhar , 1996 who has stated that the prevalence of infection in females correlates directly with age increasing from about 1%-6% between puberty and 60 years. The result was also found to in correlation with Leigh's study (1990) in which he noted that the increased prevalence of bacteriuria in elderly age in both males and females. In our study prevalence of bacteriuria was 14.63% in age group 60-70 years. According to Kosakai *et al* 1990, the bacteriuria in male is quite common in latter stage of life. This is supported by our finding in which 7.32% of bacteriuria was found in age 70-80 years which is comparatively low 2.44% in female. The finding of Nordenstam *et al*, 1986 suggest that 15% of men of 70 years old have histories of UTI.

Elderly males are predisposed to UTI due to the benign prostatic hypertrophy, decreased amount of prostatic secretion and Tamm-Horsfall protein. Similarly elderly females are prone to UTI due to poor perineal hygiene and low estrogen level that favor colonization of distal urethra and vulva with pathogenic organisms. Presence of uterine prolapse with cystocoele, if any, results into residual urine in the bladder and contributes to infection (Sweny *et al,* 1989).

Out of total 41 bacterial isolates 87.80% were gram-negative bacilli and only 12.20% were gram-positive cocci. This study is similar to study done by Okada *et al*, 1994 in Japan, 70.2% isolates were gram-negative bacilli and 29.8% were gram-positive bacteria. The higher incidence of UTI by gram-negative bacteria is also accounted in the study done by Dhakal, 1999; Manandhar, 1996; Shrestha, 2004 and Tuladhar *et al*, 1987.

From this study and the study done by other researchers, we can conclude that high incidence of UTI is caused by gram-negative bacteria in comparision to gram-positive bacteria. This statement is further supported by Kosakai *et al*, 1990 in Japan and Sandhu *et al,* 1992 in India.

Pattern of bacteriuria isolated during the study are tabulated in the table7. Out of total 41 bacterial isolates, 8 different species were isolated from 185 Midstream urine samples. The bacteria isolated were *E. coli, Enterobacter spp K. pneumoniae, K. oxytoca, P. aeruginosa, P. vulgaris, S. aureus and S. faecalis.* Leigh (1990) had stated that infecting organisms are most commonly derived from the patient's own flora and in women they are usually present on the perineal skin before infection occurs. This statement is more or less related to our results because some of the isolates belong to normal faecal flora i.e. *E. coli, Enterobacter spp* and *S. faecalis*. This result also supports the report of Manges *et al,* 2001.

According to published literatures, commonest invading agent for UTI is *E. coli* which is present in about 80-90% of cases. *E. coli* are present on between 80 and 90% of UTI (Delzell & Lefevre, 2000) and up to 95% of acute pyelonephritis other isolated gram-negative rods are *Proteus mirabilis, K. pneumoniae.*

In present study also *E. coli* was isolated as the most predominant species and accounted for 65.85% of the total bacterial isolates (27 / 41 isolates). Higher prevalence of *E. coli* in this study also resemble to the study done by Chhetri *et al*, 2001; Dhakal , 1999 ; Jha and Yadav, 1992; Manandhar, 1996; Sharma , 1983 and Tuladhar *et al* ,1989 in Nepal. The result is also in harmony with the study done at international context: Achari, 1989(India); Baron and Finegold, 1990; Corrado *et al*, 1990(USA); Fowler and Mariano,

1990; Glikberg and Brawer-Ostrovsky, 1993(Israel); Hendry *et al*, 1975(Australia); Kosakai, 1990(Japan); Leigh, 1990; Nahar *et al*, 1989(Bangladesh); Sandhu *et al,* 1992(India) and Steenberg *et al*, 1969.

But in few study done in India, *Pseudomonas aeruginosa* was found as the predominant organism followed by *E. coli* and *Klebsiella spp* (Bais *et al*, 1980).

E. coli can bind to the glycocongugate receptor (Gal 1| 4 Gal) of the uroepithelial cells of human urinary tract (Block *et al,* 1990) so it can initiate infection itself. *E. coli* is isolated in 90% 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, 08,079) 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.

One study suggests that even when infected cells lining the bladder die and slough off, carrying the *E. coli* bacteria with them, some bacteria can invade into deeper tissue in the bladder where they survive to reinfect the patient later on.

E. coli infection is high in female as compared to male. In present study also *E. coli* infection was found to be 59.26% in female whereas in male 40.74 %(out of total *E. coli* isolates). Similar type of result was found by Dhakal, 1999; Gautam *et al,* 1998; Kosakai *et al*, 1990; Vorland *et al*, 1985.

Studies by Svanborg Eden and Devian have shown stronger binding of *E. coli* to the genitourinary tract epithelial cells of infection prone Women that o the cells of non infected control subject. Bacteriuria in ambulatory adult women is caused primarily by gram- negative bacilli derived from the faecal flora (Fowler and Mariano, 1990). This statement resembles with present work as well as the finding of above workers.

Klebsiella spp were isolated as second commonest pathogen in frequency to cause UTI. *Klebsiella spp* (*K. pneumoniae* and *K. oxytoca*) accounted for 12.19 %(5 / 41 isolates). Our finding is in harmony with the study done by Astal and Sharif*,* 2002; Das *et al*, 2006;Gautam *et al*,1998; Ghimire *et al,*1995; Kumari *et al,*2005; Manandhar *et al*, 1995; Sharma, 1983 and Tuncer *et al* , 1988.

In present study, the incidence of *K. pneumoniae* was found to be 9.75% and that of *K. oxytoca* was 2.44%, which follows the statement of Fowler and Mariano (1990)-*"K pneumoniae* is the primary pathogen in the genus although *K. oxytoca* may also cause bacteriuria." *K. oxytoca* was isolated only from female patients whereas 75% of *K .pneumoniae* was isolated from male patients. In present study, *Klebsiella spp* was found slightly greater in male as compared to female, the organism may be favored by the alkaline pH conditions in the urogenital tract of male especially in benign prostatic hyperplasia. But this finding is not found elsewhere in literatures collected.

Only one episodes of *Enterobacter spp* was isolated during the study period and belonged to the male patient.

Pseudomonas aeruginosa accounted for 4.48% of total bacterial isolates. *P. aeruginosa* plays an important role in the bladder carcinogenesis (Kaji, 1994) and is considered as primary pathogen in compromised hosts(Dolan *et al,* 1989 ; Fowler and Mariano 1990), Hospitalized patients (Lohr *et al,* 1989) and in complicated urinary tract infections Kosakai *et al,* 1990; Miyata *et al,* 1985;Ogata *et al,* 1989) of gram-negative bacilli glucose non-fermenter gram-negative bacilli including *P. aeruginosa* are somewhat increasing by *Serratia* has drastically decreased. Many of these organisms are part of the patient's endogenous bowel flora but they can also be acquired by cross-contamination from other patients or hospital personnel or by exposure to contaminated solutions or non- sterile equipments (Mc Leod, 1958 and Selden *et al*, 1971).

Proteus vulgaris accounted for 2.44% of total bacterial isolates. *Proteus spp* produce urease resulting in rapid hydrolysis of urea with liberation of ammonia. Thus in UTI with *Proteus spp*, the urine becomes alkaline promoting stone formation and making

acidification virtually impossible. The rapid motility of *Proteus spp* may contribute to its invasion of the urinary tract (Jawetz *et al*, 2004).Formation of infectious urinary calculi is the most common complication accompanying UTI by the member of the genus *Proteus* supported by earlier studies (Li *et al,* 2002 and Torzewska *et al,* 2003) *Proteus vulgaris* was only isolated from male patient 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).

Among gram-positive bacteria, *Staphylococcus aureus* was the most common gram-positive cocci prevalent in both sexes. In this study, the incidence was found to be 7.32% of total bacterial isolates. Isolation of *S. aureus* from the urine should around suspicion of bacteremic infection of the Kidney acquired by haematogenous spread so a pure culture of *S. aureus* is considered to be significant regardless of the number of CFU (Baron and Finegold, 1990).

Streptococcus faecalis accounted for 4.88% of total bacterial isolates. Urinary tract infections due to *Streptococcus faecalis* are usually associated with the use of instruments or catheterization (Leigh, 1990). But Streptococci are seldom primary pathogens of the urinary tract. Enterococci are present in normal faeces and are commonly found on the perineum, they grow at low temperature and so are often found as contaminants in urine.

Streptococcus faecalis is a normal commensal of the vagina and intestinal tract. Kosakai *et al*, 1990 reported that *Streptococcuus faecalis* was more frequent in males and complicated urinary tract infections (10-12%) (Leigh, 1990).

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, red cells. Finding of equal to or more than five leucocytes (WBC) per HPF is of great importance for urinary tract infection diagnosis 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 leucocyturia>10 WBC/ HPF in urine sediments predicts a

positive urine culture and hence indicates urinary tract infection. But other many workers (Abyad, 1991; Block, 1990; Chakraborty, 1995; Ziloski and Smucker, 1989 and Wargotz *et al*, 1987) concluded that pyuria is significant if more than 5 leucocytes are seen per HPF.

In this study, positive predictive value of WBC count of 5per HPF for growth positive culture was found out to be 54.38% and sensitivity accounting for 75.61% .Pus cells was nil in 24.86% of sample of which significant growth was not found in any sample. Similarly 82 (44.32%) of sample showing 0-5 pus cells/ HPF showed 12.19% significant growth. Plenty of pus cells observed in 11.35% of samples showed 80.95% of significant growth (17 / 21 plenty pus cells/ HPF).

Table 14 describes the gender wise distribution of pyuria versus bacteriuria. In general as the number of pus cells increase per HPF, the chance of getting culture positive results are also high. This pattern was also found in our study and was equally likely to occur in both male and female. Male patients with plenty of pus cells/ HPF showed 100% culture positive whereas 70% of culture positive was observed in female patients. But the association of pyuria between culture positive male and female was found statistically insignificant ($P>0.05$).

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. Gram- positive bacteria such as *S. epidermidis* and *S. faecalis* produced minimal white cell response even in high colony counts but gram-negative and fungal organisms elicited significant pyuria (Anderson and Hsieh- Ma, 1983).

On the other hand PPV and sensitivity of microscopy of RBC i.e. haematuria (3 erythrocytes per HPF) in centrifuged urine sample was calculated to be 27.27% and

14.63% respectively. This implies that finding of WBC in urine samples is more significant than findings of RBC for the prediction of positive urine culture.

According to table 15, samples with plenty of RBC/ HPF showed 50 % significant growth (3 out of 6 plenty RBC/ HPF) .Schumann and Schweitzer (1991) suggested that the observation of 0 to 2 RBC per HPF on microscopic examination of the sediment is normal both in males and females. But Steward *et al,* 1995 and Wargotz *et al*, 1987 put forward for consideration that reported they are abnormal if the red blood cell count was greater or equal to 3 per high power field. 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.

Nepal is a developing country. Most of the people are illiterate and do not know antibiotics and its mechanism against bacteria and resistant developing mechanisms. So they are not aware of effect of irrational use of drugs, wrong dose and dose taken insufficient length of time. Drugs resistance microorganisms are increasingly important public health concern. Therefore selection of drugs for treatment and susceptibility testing is most important.

In present study, the purpose of antibiotic susceptibility testing for UTI is to know the susceptibility pattern of bacterial isolates causing UTI.

In this study, Nitrofurantion was found to be the most effective antibiotics against gram-negative bacteria (excluding *P. aeruginosa, K. oxytoca and K. pneumoniae*). Sensitivity of Nitrofurantoin against gram-negative bacteria was found to be 58.34% which is similar to the study done by Arosio *et al*, 1978; Gautam *et al,* 1998; Jha and Yadav, 1992; Karki *et al,* 2004 and Levitt ,1993 reported that majority of their isolates were sensitive to Nitrofurantoin.

Nitrofurantoin should be considered as drug of choice for acute, uncomplicated UTI particularly in view that it continues to show such low *in vitro* resistance (Obi *et al,* 1996 and Spencer *et al,* 1994). Though Nitrofurantoin seems to be the most effective

antibiotics against gram-negative bacteria, it is not generally prescribed in UTI caused by *Proteus spp* as this urease producing organisms render urine alkaline and thus decreasing the potency of drug *in vivo* (Collee *et al*, 1996).

On the other hand, the other penicillin Ampicillin used in routine test was found to be least effective drugs against gram negative bacteria (82.75% resistant). Resistance to penicillins may be determined by the organisms due to the production of penicillin destroying enzymes (- lactamase). Similarly 81.48% of *E. coli* was 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 was found by Sharma, 1983, who also showed Ampicillin resistance was present in more than 93% cases with *E. coli.*

Ceftriazone sensitivity towards *Klebsiella spp* was found to be high (*K. oxytoca* 100%, *K. pneumoniae* 75%) and 100% *Enterobacter spp* was found to be resistant to Ceftriazone. This finding is in accordance with statement of Benner *et al,* 1965 that *Klebsiella spp* are at one time usually sensitive to Cephalosporins resistant.

Transferable enzymatic resistance to third generation Cephalosporins during a nosocomial outbreak of infection with multi resistant strains has been documented by Brun Buission *et al,* 1987.

The other Cephalosporin Cephalexin used in routine antibiogram during the study has only 33.34% sensitivity which does not support the findings of the similar studies (Pandet *et al*, 2002 and Shrestha, 2004).

Ceftriazone was found as second effective drug against the gram- negative bacteria and sensitivity was found to be 50%.

According to table 17, 41.67% of gram-negative bacteria were sensitive to Ciprofloxacin and Norfloxacin. Similarly Resistivity of Ciprofloxacin and Norfloxacin against gram-negative bacteria was found to be 58.33%. In contrast to the result, Norfloxacin has been recommended as highly effective antimicrobial (Chattopadhyay and Mandal, 1993; Esko

and Renkonen 1985). Resistance of *E. coli* to quinolones has remained rare until recently and until their use increased (Oteo *et al*, 2001) .The intimate mechanisms of *E. coli* antibiotic resistance have been studied and explained in numerous publications (Henquell *et al*, 1995; Huovinen, 1997; Lehn *et al,* 1996; Piddock, 1999).

Similarly resistivity of Ciprofloxacin and Norfloxacin against *P. aeruginosa* was found to be 50%. Repeated use of quinolone therapy for complicated UTI, particularly *P. aeruginosa* infection results in emergence of quinolone- resistant organisms (Ena *et al,* 1995). This may particularly be a problem in the institutional setting where the prevalence of quinolone resistance may be high due to intense antimicrobial use (Flournoy, 1994). Emergence of resistance of *P. aeruginosa* to a quinolone while on therapy has been repeatedly reported with rates up to 18% observed (Boerema and van Saene, 1986).

Among gram-positive bacteria, the most effective drug was found out to be Novobiocin (100%) followed by Erythromycin (Sensitivity 60%). *Staphylococcus aureus* and *Streptococcus faecalis* was found to be 100% sensitive towards Novobiocin while *Streptococcus faecalis* was found only 50% sensitive towards Erythromycin and Cloxacillin.

Antimicrobial resistance is a global problem. It is now generally accepted as major public health issue and has significant implication in health and patient care. Resistance to antimicrobial drugs is causing increasing morbidity and mortality from infectious diseases. The problem of the drug resistance to antimicrobial drug is more troublesome to developing countries.

The World Health Organization (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.

MDR isolates were defined as those isolates resistant to three or more group of antimicrobial agents used in the study (Rijal *et al*, 2004). According to table 22, 56.09% bacteria were found out to be Multidrug resistant (23 out of 41). Multidrug resistance

(MDR) in this study is higher than other studies that have same MDR criterion (Fluit *et al*, 2000; Sahm *et al,* 2001).Similar lower prevalence of MDR , 13.92 % was found in the study done by Oteo *et al*, 2001 when MDR criterion was resistance to 3 or more antibiotics. The higher finding of MDR strains in this study may be due to higher prevalence of positive growth seen among the inpatients compared to outpatients because the hospitalized group of patients has higher chances of getting exposure to resistant strains.

In the study done by Tuladhar *et al,* 2003 in TUTH hospital, MDR bacterial strain were detected in 35.21% cases in which the most predominant was *E. coli* (22.17%) followed by *Klebsiella spp* 28 (6.08%) and *Staphylococcus aureus* (2.17%). But in this study, MDR were detected in 100% in *Proteus vulgaris* (1 out of 1) and *Streptococcus faecalis* (2 out of 2). Similarly MDR in *E. coli* were found to be 62.96% (17 out of 27).

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, 1991).

Resistant strains are now reported against all available classes of antibiotics (Kunin, 1993 and Levy, 1991). The morbidity and mortality rates because of MDR strains among the very young, elderly population and among immuno-compromised patients are very high (Kunin, 1983). The recent increase of MDR strains in hospital has started to pose great difficult in selecting antimicrobial agents for the management of the infection they caused and obviously the cost in the management of infection caused by MDR strains will be definitely high because of need of acquiring new drug which is of course will be high in cost as well as the cost of prolong staying in the hospital. Some factors responsible for the emergence of resistant strains in hospital include the indiscriminate use of antibiotics the prolonged hospitalization, the increase in uses of insertion devices etc. Moreover results obtained from these surveillance systems must be used to implement preventive programmes and policy decisions to prevent the emergence and spread of antimicrobial resistance.

6.2 CONCLUSIONS

The study revealed that there is no significant difference of positive growth among male and female patients and outpatient and inpatients ($P > 0.05$). On the statistical analysis of parameter like pyuria was also failed to show significant association between culture positive male and female patients.

Gram negative bacteria were the major cause of urinary tract infection. *E. coli* was found the most predominant multi- drug resistant isolate than others.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

A prospective study was conducted among patients suspected of UTI attending outpatients department and hospitalized patients of Tribhuvan University Teaching Hospital, Kathmandu Nepal. The study period was three months from June 2006 to August 2006. The purpose of study was to isolate different bacteria causing UTI, to correlate bacteriuria with pyuria, to determine antibiotic susceptibility profiles of isolated organisms and to analyze the MDR strains. One hundred and eighty five midstream urine samples collected were investigated by conventional semi-quantitative culture technique, microscopy and antibiotic susceptibility tests.

1. Out of 185 midstream urine samples, 41 (22.16%) were growth positive with significant number of bacteria, 135 samples (72.97%) were sterile and rest 9 samples (4.86%) showed mixed growth.
2. Out of total 41 bacteria, 8 different species were isolated from growth positive urine samples. *E. coli* 27(65.85%) was found the most predominant organism followed by *Klebsiella spp* [*K. pneumoniae* (9.75%), *K. oxytoca* (2.44%)], *S. aureus* (7.32%), *P. aeruginosa* (4.88%), *S. faecalis* (5.26%), *P. vulgaris* (2.44%) and *Enterobacter spp* (2.44%).
3. The infection rate was found higher in males (24.67%) than in females (20.37%). Association of significant bacteriuria and gender of patients was found to be statistically insignificant ($P > 0.05$).
4. The incidence of bacteriuria was found higher in inpatients (29.09%) than in outpatients (19.23%). But statistically it was found that there was no significant association of significant bacteriuria and hospitalization of patients.
5. Higher prevalence of significant growth was obtained from the age group 30-40 years (26.83%i.e 11/ 41).

6. Greater prevalence of bacteriuria in males was found in age groups 30-40 years (9.75%) and 50-60 years (9.75%).
7. Greater than or equals to 5 pus cells per HPF in centrifuged urine was obtained in 57 samples, out of which 31 (54.38%) samples were culture positive with significant number of bacteria. Positive predictive value (PPV) of presence of 5 pus cells per HPF for growth positive culture was found out to be 54.38%.
8. Greater than or equals to3 RBC per HPF was found in 22 samples out of which 6 (27.27%) samples were culture positive with significant number of bacteria. Positive predictive value of 3 RBC per HPF for growth positive culture was found out to be 27.27%.
9. The most effective antibiotic to overall gram-negative bacteria (excluding *P. aeruginosa, K. oxytoca and K. pneumoniae*) was found out to be Nitrofurantoin (58.34%) followed by Ceftriazone (50%). Amikacin was also effective as Nitrofurantoin against *P. aeruginosa, K. oxytoca* and *K. pneumoniae* (sensitivity 85.71%). The least effective drugs was found out to be Ampicillin (Resistant rate 82.75%).
10. The most effective antibiotic to overall gram-positive bacteria was found out to be Novobiocin (Sensitive rate 100%) and Erythromycin (60%).
11. Out of total 41 bacterial isolates, 9.75% bacterial isolates were found out to sensitive to all antibiotics, 14.63% was found to be resistant to 1 drug, 12.19% was resistant to 2 drugs, 7.31% was resistant to 3 drugs and 56.09% was found to be resistant to more than 3 drugs.
12. MDR was observed in 56.09% (23/41) bacterial isolates of which the most predominant was *E. coli* 62.96 %(17/27) and *K. pneumoniae* (50%) (2/4).

7.2 RECOMMENDATIONS

1. This study was confined on TUTH, Kathmandu and our case patients probably represent the most visible part of the population so it does not necessarily reveal the total picture of the whole country therefore systematic prospective

surveillance of this type of study should be carried out throughout the year covering wide geographical region in order to obtain information regarding seasonable, geographical and ethnic variation of pathogens and their antibiotic susceptibility profile.

2. The study should be continued and MDR strains should be detection for ESBL producing strains and further studied up to genetic level to acquire their detailed genetic makeup and to characterize the mechanism of drug resistance.

3. There are some limitations in this study. The organisms are tested against very few panels of antibiotics of first line. Hence it is recommended that the study should be carried on using wide range of antibiotics including the second line drugs.

4. Microscopic examination of urine prior to culture is useful for correlating pyuria and bacteriuria. Thus it should be done routinely.

REFERENCES

Abayad AR (1991) Screening for asymptomatic bacteria in pregnancy: urinanalysis vs urine culture. J Pharm Pract 33:471-474

Achari V (1989) Study of UTI: Changing pattern in bacteriology. Ind Med J 83: 12-14

Agrawal M, Wilkinson R and Goldstein R (1989) Molecular epidemiology of adhesion and hemolysin virulence factors among uropathogenic *Escherichia coli.* Infect Immun 57:303-313

Anderson R and Hsieh-Ma ST (1983) Association of bacteriuria and pyuria during intermittent catheterization after spinal cord injury. J Urol 130:299-301

Arosio E, Spiazzi G and Boninsegna F (1978) Frequency and antibiotic sensitivity of gram-negative bacteria in urinary tract infection in relation to predisposing factors. Ann Sclavo 20: 162-170

Asscher AW and Sussman M (1966) Urinary tract infection. Lancet 2:1037

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

Bais CS, Bais B and Joshi KR (1980) *In vitro* sensitivity of bacteria isolated from suspected urinary tract infections. Rasjasthan Med J 19:13-18

Baron EJ and Fingold SM. (1990) Balley and Scott's Diagnostic Microbiology.8th ed. St. Louis Missouri Baltimore: CV Mosby- Year Book Inc

Basic Laboratory Procedures in Clinical Bacteriology (2004) WHO, ISBN 924154545

Bauer AW, Kirby WMM, Sherris JC and Truck M (1966) Antibiotic susceptibility testing by a standardized single disc method. Am J Pathology 45:493-496

Block B (1990) Urinary Tract Infection. Am Fam Physician 33:172-185

Boerema JBJ and van Saene HKF (1986) Norfloxacin treatment of complicated urinary tract infection. Scand J Infect. Dis 48: 20-26

Brun-Buisson C, Legrand P, Philippon A, Montravers F, Ansquer F and Duval J (1987) Transferable enzymatic resistance to third- generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. Lancet 6:302-306

Caprioli A, Fallbo V, Ruggeri FM, Baldassarri L, Bisicchia R, Ippolito G, Romoli and Donelli G (!987) Cytotoxic necrotizing factor production by hemolytic strains of *Escherichia coli* causing extraintestinal infections. J Clin Microbiology 25:146-149

Car J and Sheikh A (2003) Recurrent urinary tract infection in women. BMJ 327:1204

Centers for Disease Control and Prevention. Preventing emerging infectious diseases.Online.2004(Cited20June2006):http://www.cdc.gov/ncidod/emerplan/plan98

Chakraborty P (1995) A text book of Microbiology, 1st ed. New Central Book agency Ltd, Calcatta

Chattopadhyay AK and Mandal AK (1993) Microbes associated with urinary tract infection in Burdwan. West Bengal. Ind J Phisiology and Allied Science 47:67-72

Cheesbrough M (1984) Medical Laboratory Practice for tropical countries Part-II Microbiology 1st ESBL ed. Cambrige University press

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

Chhetri PK, Rai SK, Pathak UN, Thapa JB, Devkota KC, Shrestha BO and Shrestha RR (2001) Retrospective study on urinary tract infection at Nepal Medical College Teaching Hospital, Kathmandu. Nep Med J 3:83-85

Collee JG, Frasier AG, Marmion BP and Simmons A (1996) Mackie and McCartney Practical Medical Microbiology, 14th ed. Churchill Livingstone New York

Corrado ML, Roylance PJ, Struble WE and Lancaster MS (1990) Review of efficacy of oral norfloxacin in pyelonephritis and nosocomial urinary tract infection. Eur Urol 17:24-29

Curhan GC, Willett WC, Rimm EB, Speizer FE and Stamfer MJ (1998) Body size and risk of kidney stones. J Am Soc Nephrol 9: 1645-52

Das RN, Chandrashekhar TS, Joshi HS, Gurung M, Shrestha N and Shivananda PG(2006) Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal. Singapore Med J 47:281-5

Delzell JE and Lefevre ML (2000) Urinary tract infection during the pregnancy. Am fam physician 61:713-721

Department of Health Services (2059/60) Annual Report. HMG Ministry of Health, DOHS

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

Dhungel R (2001) Prevalence of common bacterial pathogens in different clinical samples submitted at TUTH and their sensitivity test profile. A dissertation submitted to Central Department of Microbiology. T.U. Kathmandu

Dolan JG, Bordly DR and Polito R (1989) Initial Management of serious urinary tract infection: Epidemiologic guidelines. J Gen Intern Med 4:190-194

Donnenberg MS and Welch RA (1996) Virulence determinants of uropathogenic *Escherichia coli* p. 135-174. In: Mobley LT and Warren JW (ed) Urinary tract infection: molecular pathogenesis and clinical management. American Society for Microbiology, Washington, DC

Eisinger RP, Asghar F, Kosala C and Weinstein MP (1997) Does pyuria indicate infection in asymptomatic dialsis patients? Ckubucal Nephrology 47:50-51

Ena J, Amador C, Martinez C and Oritz de la (1995) Risk factors for acquisition of urinary tract infections caused by ciprofloxacin resistant *Escherichia coli* J Urol 153:117-120

Esko E and Renkonen OV (1985) Comparative *in vitro* activity of Norfloxacin and 7 other antimicrobial agents against clinical isolates from urinary tract infection. Scand J Infect Dis 17:95-98

Fair WR and Wehner N (1971) Antimicrobial action of spermine: Effect on urinary tract pathogens.J Applied microbiology 21:6

Fair WR, Couch J and Wehner N (1976) Prostatic antibacterial factor: Identify and Significance. Urol 7:169

Flournoy DJ (1994) Antimicrobial susceptibilities from nursing home residents in Oklahoma .Gerontology 40: 53-56

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

Fowler JE and Mariano M (1983) Bacterial infection and male infertility: Absence of immunoglobulin A with specificity for common *Escherichia coli* O-serotypes in seminal fluid of infertile man. J Urol 130:171

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

Freedman LR, Phair JP and Seki M (1965) The epidemiology of urinary tract infections in Hiroshima. Yale J Biol Med 37:262

Fridlender B, Chejanovsky N and BeckerY (1978) Selective inhibition of herpes simplex virus type 1 DNA polymerase by zinc ions. Virology 84:551

Froom P, Gross M and Froom J (1986) The effect of age on prevalence of asymptomatic microscopic haematuria. Am J Clin Pathol : 656-657

Gallacher DJA, Montgomerie JZ and North JDK (1965) Urinary tract infection .Brit Med J 1:62

Gautam P (1998) Prevalence of urinary tract infection in children .A Dessertation submitted to the Central Department of Microbiology. T.U. Kathmandu

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

Gip L and Molin L (1970) Study on the inhibitory activity of human prostatic fluid on *Candida albicans*. Mykosen 13:61

Gleckman R and Esposito A (1978) Sterile pyuria in the elderly. Am Fam Physician 19: 109-111

Glikberg F and Brawer-Ostrovsky Y (1993) Urinary tract infection in the community. Harefuah 125:16-1863

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

Greenwood D, Slack RBC and Peutherer JF (2001). Medical Microbiology. 23rd edn, McGraw Hill Publishers

Hamilton-Miller JMT (1994) The urethral syndrome and its management. J Antimicrob Chemother 33*:* 63

Hendry PIA, Gibson BS and Nicholson S (1975) Antibiotic sensitivity of bacteria from urine. Pathology 7:293-297

Henquell C, Chanal C, Sirot D, Labia R and Sirot J (1995) Molecular characterization of none different types of mutants among 107 inhibitor-resistant TEM- lactamase from clinical isolates of *Echerichia coli*. Antimicrob Agents Chemother 39:427-430

Hooton TM and Stamm WE (1991) Mnagement of acute uncomplicated UTI in adults. Med Clin North Amr 75:339-57

Hooton TM, Scholes D and Hughes JP *(*1996) A prospective study of risk factors for symptomatic urinary tract infection in young women. New Eng J Med 335: 468-74

Hugo WB and Russel AD (1981) Pharmaceutical microbiology, 2nd edn. Black well scientific publication

Hugo WB and Russel AD (1983) Pharmaceutical microbiology. 5th edn. Black well scientific publication

Huovinen P (1997) Increases in rates of resistance to trimethoprim. Clin Infect Dis 24:63-66

Jawetz E, Melnick JL and Adelberg EA (2004). Medical Microbiology, 23rd edn McGraw Hill companies.Inc

Jha VC and Yadav JN (1992) Bacterial species isolated from urine of UTI suspected patients and their sensitivity to commonly available antibiotics. J Nep Med Assoc 30:222-225

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

Kaji SI (1994) Experimental study on the role of bacteria from the urinary tract infection on the bladder carcinogenesis. Fukoka Acta Medica 85: 294-308

Karki A, Tiwari BR and Pradhan SB (2004) Study of Bacteria isolated from Urinary tract infection their sensitivity Pattern. J Nep Med Assoc 43:200-203

Kass EH and Miall WE (1978) Infections of the urinary tract. In: Kass EH, Brumfitt W Uiversity of Chicago press, Chicago

Kaye DB (1968) Urinary tract infection in the elderly .J Clin Invest 47: 2374

Kosakai N, Kumamoto Y and Hirose T (1990) Comparative studies on activities of antimicrobial agents against causative organisms isolated from urinary tract infection: Background of patients. Jpn J Antibiotics 43:454-967

Krieger JN and Rein MF (1982) Canine prostatic secretions kill *Trichomonas vaginalis.* Infect Immun 37:77

Kumari N, Ghimire G, Magar JK, Mohapatra TM and Rai A(2005) Antibiogram of isolates from UTI cases in eastern part of Nepal. Nep Med coll J 7:116-8

Kunin CM (1983) Antibiotic resistance-A World Health problem we can not ignore. Ann Int Med 99:859-60

Kunin CM (1993) Resistance to antimicrobial drugs- A world wide calamilty. Ann Int Med J 115:1050-5

Kunin CM (1997) Detection, Prevention and Management of Urinary Tract Infection.4th ed. Philadelphia: Lea and Febiger

Kunin CM, White LV and Hua TH (1993) A reassessment of the importance of low-count bacteriuria in young women with acute urinary symptoms. Am Int Med J 119: 454

Lehn N, Stower-Hoffmann J, Kott T, Strassner C, Wagner H, Kronke M and W. Schneider-Brachert (1996) Characterizationn of clinical isolates of *Escherichia coli* showing high levels of fluoroquinolone resistance. J Clin Microbiology 34:597-602

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

Leigh DA (1993) Prostatitis:An increasing problem for diagnosis and management. J Antimicrob Chemother 32:1-9

Lenette EH, Balows A, Hauser WJ and Shadomy J (1985) Manual of clinical microbiology 4th ed. American society of microbiology

Levitt PN (1993) Analysis of pathogens isolated from urinary tract infection in Barbados. West Ind Med J 42:72-76

Levy SB (1991) Antibiotic availability and use: Consequences to men and his environment. J Clin Epidemial 44:635-875

Li X, Zhao H, Lockatell CV, Drachenberg CB, Johnson DE and Mobley HL (2002) Visualization of Proteus mirabilis within the matrix of urease-induced bladder stones during experimental urinary tract infection. Infect Immun 70:389-94

Lohr JA, Donowitz LG and Sadler JE (1989) Hospital acquired urinary tract infection. Pediatrics 83:193-199

Lundrigan MD and Webb RM (1992) Prevalence of omp T among *Escherichia coli* isolates of human origin. FEMS Microbiol Lett 97: 51-56

Madigan MT, Martinko JM and Parker J (2003) Biology of Microorganism. 10th ed. Pearson Education International, Inc

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

Manges AR, Johnson JR , Foxman B, O'Bryan TT , Fullerton KE and Riley LW (2001) Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. New Eng J Med 345:1007-1013

Manual of basic techniques for a health Laboratory (2005) WHO, ISBN 9241545305

Mardh PA, Coleen S and Sylwan J (1980) Inhibitory effect on the formation of chlamydial inclusions in McCoy cells by seminal fluid and some of its components. Invest Urol 17: 510

Matthew A, Mulvey D, Schilling J and Scott J (2001) Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. Infect Immun 69: 4572–4579

McCabe WR and Jackson GG (1962) UTI. Archives of Internal medicine. 110:847

McLeod J W (1958) The hospital urine bottle and bed pan as reservoirs of infection by *Pseudomonas*. Lancet 1:394-395

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

Mindbranch Inc (2004) Stakeholder Opinions: Urinary Tract Infections -Ciprofloxacin Leads the Way. Data monitor, USA

Miyata K, Furukawa M and Kishi M (1985) Statistical studies on bacteria isolated from urinary tract infection in recent five years (1970-983). Nishinihon J Urol 47: 431-442

Nahar N, Huq F and Anwar KS (1989) Prevalence of bacteriuria in adult Bangladesh Females. Bangladesh J Microbiol 6(2):37-42

National Committee for Clinical Laboratory Standards (1999) Performance standards for antimicrobial susceptibility testing, 9th informational supplement, 18, number Wayne, Pa: National Committee for Clinical Laboratory Standards

Nickel JC (1996) Prostatitis in antibiotic therapy in urology. (ed) pp.57-69 Lippincott-Raven, Philadelphia

Nordenstam GR, Brandberg CA, Oden AS, Svanborg Eden CM and Svanborg A (1986) Bacteriuria and mortality in an elderly population. New Eng J Med 314:152-1156

Norrby SR (1990) Antibiotics for treatment of urinary tract infection. Workshop on treatment of urinary tract infection

O' Grady F and Cattell WR (1996) Urnary tract infection. Brit J Urol 38:156

O' Sullivan DJ and Fitzgerald MG (1961) Urinary tract infection. Brit Med J 1: 786

Obi CL, Tarupiwa A and Simango C (1996) Scope of urinary pathogens isolated in the public Health Bacteriology Laboratory. Harare: Antibiotic susceptibility patterns of isolates and incidence of haemolytic bacteria. Central African J Med 42: 244-249

Oelschlaeger TA, Dobrindt U and Hacker J (2002) Virulence factors of uropathogens. Curr Opin Urol 12:33-8

Ogata M, Yoshida H and Shigeta S (1989) Comparative studies on activities of antimicrobial agents against causative organisms isolated from urinary tract infections: Background of patients. Jpn J Antbiot 42: 2175-2188

Okada K, Usui Y and Wantanabe R (1994) Statistic studies on bacteria isolated from urinary tract infections (Report 6: Isolation rate and drug sensitivity from 1988 through 1989). Acta Urol Japonica 40:175-185

Oteo J, Campos J, Baquero F and Spanish members of the European Antimicrobial Resistance Surveillance System (EARSS) (2001) *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance system(2001) J Antimicrobial Chemotherapy

Palac DM (1986) Urinary tract infection in women. A physician's perspective pp17-25

Pandey R (2002) A Hospital based study of Urinary tract infection among women visiting antenatal clinic of Tribhuvan University teaching Hospital. A dissertation submitted to Central Department of Microbiology.T.U. Kathmandu

Pelczar MJ, Chan ECS and Krieg NR (1993) Microbiology. 5th edition Tata Mc Graw-Hill Publisher

Pendse AK and Singh PP (1986) The etiology of urolithiasis in Udaipur (western part of India). Urol res 14:59-6

Pfau A and Sacks TG (1970) Urology International. J Urol 25:326

Piddock LJ (1999) Mechanisms of fluoroquinolone resistance:an update 1994-1998 Drugs.58:11-18

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

Rahaman T , Haq F, Begum J and Khan IH (1990)Urinary tract infection in diabetic and non-diabetic patients :A comparative bacteriological study. Bangladesh Renal J 9:8-12

Rai CK, Pokhrel BM and Sharma AP (2001) A prospective study on antibiotic sensitivity profile of the organisms associated with clinical infections among the patients attending TU Teaching Hospital. J Nep Assoc Med Lab 3:13-6

Reynolds EA, Hoberman A and Wald S (1996) Is urine culture necessary to rule out urinary tract infection in young febrile children? Pediatric Infect Dis J 15: 304-309

Rijal B P, Tuladhar N R and Pokhrel B M (2004) The Prevalence of -lactamase production in hospital isolates and their antimicrobial resistance patterns . J Ins Med 26: 27-28

Roberts AP and Beard RW (1965) Urinary tract infection. Lancet 1:1133

Roberts JA (1996) Tropism in bacterial infections: Urinary tract infection. J Urol 156: 1552-1559

Robins DG, Rogers KB, White RH and Osman MS(1975) Urine microscopy as an aid to detection of bacteriuria. Lancet 1: 476- 478

Rubin RH, Shapiro ED, Andrriole VT, Davis RJ and Stamm WE (1992) Evaluation of new anti-infective drugs for the treatment of urinary tract infection. Clin Infect Dis 15:216-227

Sahm DF, Marsilio MK and Piazza G (1998) Antimicrobial resistance in key bloodstream bacterial isolates: electronic surveillance with the surveillance Dornbusch K, King A, Legakis N, Incidence of antibiotic resistance in blood and urine isolates from hospitalized patients. Report from a European collaborative study group on antibiotic resistance (ESGAR). Scand J Infect Dis 30:281-288

Sahm DF, Thornsberry C, Mayfield DC, Jones ME and Karlowsky J (2001) Multidrug resistant urinary tract isolates of *Escherichia coli*: Prevalence and patient demographics in the United States in 2000. J Antimicrobial agents Chemotherapy 45:1

Saini S, Kaur H, Sabharwal U and Malik AK (1983) Coagulase negative *Staphylococci* in the urinary tract. Ind J Med Res 78: 26-28

Sandhu KS, Arora AK and Dwivedi PN (1992) Recent trends in the sensitivity pattern of the organisms isolated from urine of human origin in Punjab. Ind Med J 16:57

Schaeffer AJ and Chmiel J (1983) Urethral meatal colonization in the pathogenesis of catheter associated bacteriuria. J Urol 130:1096-1099

Selden R, Lee S and Wang WLL (1971) Nosocomial *Klebsiella* infections: intestinal clonization as a reservoir. Ann Intern Med 74:657-664

Sharma J (2004) Prospective study on microbiology of lower respiratory tract infection and antibiotic sensitivity profile with special interest in multidrug resistant and extended spectrum of -lactamase strain. A dissertation submitted to Central Department of Microbiology.T.U. Kathmandu

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

Shrestha P (2004) A prospective study of urinary tract infection in patients attending Kathmandu Model Hospital. A Dissertation submitted to Central Department of Microbiology. T.U. Kathmandu

Shumann GB and Schweitzer SC (1991) Examination of urine In: Henry JB (ed) Clinical diagnosis and management by laboratory method. 18th edition. Hacourt Brace Jovanovich, Inc. USA: W.B. Saunders Company 387-444

Stamey TA (1975) Editorial: A clinical classification of urinary tract infections based upon origin. South Med J 68: 934

Stamey TA (1980) Pathogenesis and treatment of Urinary tract infections. William and Wilkins, Baltimore

Stamey TA (1981) Urinary Tract infections. Williams and Wilkins

Stamey TA, Goven DE and Palmer JM (1965) The localization and treatment of urinary tract infections: The role of bacterial urine levels as opposed to serum level. Medicine 44:1

Stamey TA, Timothy M and Millar M (1971) Recrrent urinary infections in adult women. California Med 115:1

Stamm WE (1992) Criteria for the diagnosis of urinary tract infection and for the assessment of therapeutic effectiveness infection. 20-151

Stamm WE and Norrby SR (2001) Urinary tract infections: disease panorama and challenges infectious disease. 183: 1- 4

Stamm WE, Counts GW, Running KR, Fihn S, Turck M and Holmes KK (1982) Diagnosis of coliform infection in acutely dysuric women. New Eng J Med 307:463-468

Steenberg J,Bartels ED and Bay-Nielsen H (1969) Epidemiology of urinary tract diseases in general practice. Brit Med J 4:390

Stephens FA and Lengaham D (1962) Urinary tract infection. J Urol 187:669

Steward DK, Wood GL and Cohen RL (1985) Failure of the urinanalysis and quantitative urine culture in diagnosing symptomatic urinary tract infection in patients with long term urinary catheters. Am J Ifect Contr 13: 154-160

Sweny P, Farrington K and Moorhead JF (1989) The kidney and its disorder, Blackwell scientific publication: 621-37

Talukder MAS (1987) Sensitivity pattern of positive urine culture in a Teaching Hospital. Bangladesh Renal J 6:49-52

Torzewska A, Staczek P and Rozalski A (2003) Crystallization of urine mineral components may depend on the chemical nature of Proteus endotoxin polysaccharides. J Med Microbiol 52:471-7

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

Tuladhar NR, Nakanishi M, Shrestha HG, Duffy J, Tomiyoshi Y, Pandey G and Sharma SP (1990) Nosocomial infections in surgical patients at Tribhuvan University Teaching Hospital. J Nep Med Assoc. 28: 152-156

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

Tuncer I, Sengil AZ and Findik D (1988) Microorganisms and their antibiotic resistance that were isolated from the urine of urinary infection suspected patients. Mikrobiyol Bul2:296-230

Urinary tract infection (2006) Urology channel online. Last update June 2006 Cited (August 2nd, 2006): http://.urologychannel.com.htm

Valenti WM and Reese RE (1993) Genitourinary tract infections in a practical approach to infectious diseases. Reese RE, Douglas RG (eds). Little, Brown and company, Boston, Toronto.491-499

Vivaldi E, Munoz J and Vosti KL (1965) Intestinal Receptors for Microbial Attachment, In: Kass EH(ed) Progress in pyelonephritis, Davis, Philadelphia: 103

Vorland LH, Carlson K and Aalen O (1985) An epidemiological survey of urinary tract infection among out patients in Northern Norway. Scand J Infect Dis 17:277-283

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

Warner L and Kim YS (1989) Intestinal receptors for microbial attachment. In: Farthing MJG

Yagisawa T, Hayashi T and Yoshida A (1999) Metabolic characteristics of the elderly with recurrent calcium oxalate stones. Brit J Urol Int 83:934-4

Ziloski MW and Smucker DR (1989) Urinary tract infection in elderly. Am Fam Physician 39: 125-134

APPENDIX-I

QUESTIONNAIRE

CLINICAL AND MICROBIOLOGICAL PROFILE OF PATIENT

Clinical Profile:

Name of Patient: …………

Sex:……..	Lab No:…….
Age: ….	Patient : OPD/ INDOOR
Address:…..	Ward :……….
Date:…….	Bed No:……

Clinical History:

Patient on Antibiotics: a. yes b. No

If yes, Antibiotic taken: a. Ciprofloxacin   b.Ofloxacin   c.Norfloxacin

d. Nalidixic Acid   e. Nitrofurantoin   f.Others:

Duration of Antibiotics taken:………..

Others (If any)   :………..

Microbiological Profile :

DAY 1

Time of sample Collection:……….

Specimen :

Method of Sample Collection:………..

Macroscopic Observation:

Colour: ……….   Transparency:…………

Others:………..

Direct Microscopic Observation:

Wet mount preparation of centrifuged urine

Observation	Number per HPF	Comments
Pus Cells		
RBC		
Epithelial Cells		
Others		

Culture of specimen on: a. MacConkey / Blood Agar

Incubation a. Aerobic b.Anaerobic c. Microaerophillic

Incubation Temperature and period:………….

DAY2: Reading of culture plates

Colony Characteristics on MacConkey Agar/Blood Agar

Media used	shape	size	colour	texture	Hydrolysis on BA	Lactose fermentation	growth
MacConkey							
Blood Agar							
Others							

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: …….. Others:…………..

Serotyping: If Required

Organism Identified as: …………………………

Antibiotic sensitivity test (Kirby- Bauer Method)

Media Used	Test Organism	Antibiotics Used	Reference diameter of zone of inhibition ATCC strain(control	ZOI of test organism	Interpretation

			organism)		
		Ciprofloxacin			
		Norfloxacin			
		Nitrofurantoin			
		Ampicillin			
		Cotrimoxazole			
		Cephalexin			
		Cloxacillin			

Comments on Drug Resistance Pattern: MDR/ non MDR

Resistant to …………….. number of antibiotics.

Performed by ……………….. Checked by………………

APPENDIX –II

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

A. EQUIPMENTS:

1. Hot air oven: Sakura (Japan)
2. Incubator: Sanyo (Japan)
3. Autoclave: Sakura (Japan)
4. Refrigerator: Toshiba (Japan)

5.	Microscope:	Olympus (Japan)
6.	Centrifuge:	Hitachi (Japan)
7.	Weighing Machine:	Chyo MP 300(Japan)
8.	Water Distillation Plant:	Yamato (Japan)
9.	Laminar Flow:	Dalton (USA)
10.	Water bath:	NSW (India)

B. 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:

1. Ampicillin (10mcg)
2. Cefotaxime (30mcg)
3. Cephalexin (30mcg)
4. Ciprofloxacin (5mcg)
5. Cloxacillin (1mcg)
6. Cotrimoxazole (1.25/23.75mcg)
7. Nitrofurantoin (300mcg)
8. Norfloxacin (10mcg)
9. Novobiocin (30mcg)
10. Ceftriazone(30mcg)
11. Erythromycin(15mcg)
12. Amikacin(30mcg)

APPENDIX-III

I. 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, Hampsire, 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 Digest	2.5
Yeast extract	5.0
Sodium Chloride	5.0
Agar	12.0

Final pH (at 25^0C) 7.3±0.2

Preparation: 40 grams of the blood agar base medium was suspended in 1000 ml distilled water ,dissolved by boiling and sterilized by autoclaving at 121^0C (15lbs pressure) for 15 minutes. After cooling to 40-50^0C, 5-7% 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

Preparation: 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.

3. **Mueller Hinton Agar (Oxoid, England)**

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

Preparation: 38 grams of the medium was suspended in 1000 ml distilled water and the medium was warmed to dissolve completely. Then the medium was sterilized by autoclaving at 121℃ (151lbs pressure) for 15 minutes.

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

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

5. Nutrient Broth (Hi- Media)

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

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

II. COMPOSITION AND PREPARATION OF DIFFERENT BIOCHEMICAL TESTS MEDIA

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

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

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

2. Hugh and Leifson's Medium (Hi-media laboratories)

Ingredients	gm/liter
Tryptone	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Bromothymol Blue	0.08

Agar	2.0
Final pH (at 25^0C)	6.8±0.2

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

3. Sulphide Indole Motility (SIM) medium (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

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

4. 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

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

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

Ingredients	gm/litre
Lablemco 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	0.024
Agar	12.0
Final pH (at 25^0C)	7.4±0.2

Preparation: 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

6. Christensen Urea Agar

Ingredients	**gm/litre**
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Dipotassium Phosphate	1.2
Mono-potassium Phosphate	0.8

Phenol Red	0.012
Agar	15.0
Final pH (at 25^0C)	7.4±0.2

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

III. 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

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

(b) Lugol's Iodine

Potassium Iodide	20.0 g
Iodine	10.0 g
Distilled Water	1000 ml

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

(c) Acetone-Alcohol Decoloriser

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

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

(d) Safranin (Counter Stain)

Safranin	10.0 g
Distilled Water	1000 ml

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

2. Normal saline

Sodium Chloride	0.85 g
Distilled Water	100 ml

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

Biochemical Tests Reagents

a. For Catalase test

Catalase Reagent (3% H_2O_2)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

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

b. For Oxidase Test

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

Tetramethyl *p*-phenylene

diamine dihydrochloride (TPD)	1 gm
Distilled Water	100 ml

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

c. For Indole Test

Kovac's Indole Reagent

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

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

d. For Methyl Red Test

Methyl Red Solution

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

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

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

Solution A

-Naphthol	5.0 g
Ethyl alcohol (absolute)	100 ml

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

Solution B

Potassium hydroxide	40.0 g
Distilled Water	1000 ml

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

4. **McFarland nephlpmeter tube (No. 0.5)**

0.5 ml of 0.048 M $Bacl_2$ (1.17% w/v $BaCl_2H_2$ O) was added to 99.5ml of 0.18 M H_2 SO4 (1% w/v) with constant stirring. The McFarland standard was thoroughly mixed to ensure that it is evenly suspended. Using matched cuvettes with a 1cm 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 10-30 seconds.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface.
6. The slide was rinsed with tap water, shaking off excess.
7. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer. Thicker smear requires more aggressive decolorizing.

8. The slide was flooded with counter-stain (safranine) for 30 seconds and washed off with tap water.
9. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 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 produce 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.*

Reduced flavoprotein reacts directly with gaseous oxygen by way of electron reduction to form hydrogen peroxide, which is an oxidative end product of the aerobic breakdown of sugars.

$FPH_2 + O_2$ | $FP + H_2O_2$

Reduced Flavoprotein   Oxidized Flavoprotein

Hydrogen peroxide thus formed is toxic to bacteria, resulting in their death.

Catalase enzyme breaks down hydrogen peroxide into water and oxygen

$2H_2O_2 \rightarrow 2H_2O + O_2$

catalase

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.This oxidase reaction is due to the presence of a cytochrome oxidase system which activates the oxidation of reduced cytochrome by molecular oxygen, which in turn acts as an electron acceptor in the terminal stage of the electron transfer system.

Organisms capable of growing in the presence of oxygen i.e, aerobic or facultative anaerobic organisms are mostly oxidase positive and at the same time they produce the enzyme catalase. Obligate anaerobic organisms lack oxidase activity since they are unable to live in the presence of atmospheric oxygen and do not possess a cytochrome oxidase system.

The test is used for screening species of *Nesseria, Alcaligens, 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 (Wattman'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. Oxidation-Fermentation test

This test is done to determine the oxidative or fermentative metabolism of carbohydrate resulting in production of various organic acids as end product. Some bacteria are capable of metabolizing carbohydrates (as exhibited by acid production) only under aerobic conditions, while others produce acid both aerobically and anaerobically. Most medical bacteria are facultative anaerobes.

Procedure: The test organism was stabbed into the bottom of two sets of tubes with Hugh and Leifson's media, bromothymol blue being the pH indicator. The inoculated medium in one of the tubes was covered with a 10 mm deep layer of sterile paraffin oil. The tubes were then incubated at 37°C for 24 hours. After incubation the tubes were examined for carbohydrate utilization as shown by yellow color of media that denotes the acid production (indicator in media is phenol red).

Fermentative organism utilizes the carbohydrate in both the open and sealed tubes as shown by a change in color of the medium from green to yellow. Oxidative organisms, however, are able to use the carbohydrate only in the open tube.

D. 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 (Para dimethyl aminobenaldehyde in avid ethanol) 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.

E. 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. The methyl red test uses a pH indicator in the form of methyl red, to determine the hydrogen ion concentration (pH) arising out of fermentation of glucose by an organism. The hydrogen ion concentration depends on gas ratio (CO_2 and H_2), which in turn is an index to the different pathways of glucose metabolism exhibited by various organism. The different

fermentation patterns are due to variations in enzymes concerned with pyruvic metabolism present in the organism. Methyl red positive organisms produce stable acids: lactic, succinic, acetic and formic acid, maintaining a high concentration of hydrogen ions until a sudden concentration is reached.

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.

F. 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 enterobacterial group is usually either methyl red positive and Vogues- 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.

G. 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. Normally citrate metabolism involves condensation of acetyl group with coenzyme A and oxaloacetate to enter the Kreb's cycle. In bacteria the cleavage of citrate involves an enzyme system without the intervention of the coenzyme A; this enzyme is called citratase or citrate desmolase. The product obtained from citrate metabolism depends upon pH of the medium.

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.

H. Motility test

This test is done to determine if an organism was motile or non-motile. Bacteria are motile by means of 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 24 hours. Motile organisms migrate from the stab line and diffuse into the medium causing turbidity. Whereas non-motile bacteria show the growth along the stab line, and the surrounding media remains colorless and clear.

I. 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, NoH2S 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.

J. 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.

K. 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.

Two types of coagulase are produced by most of the *Staphylococcus aureus*

a. Free coagulase, which converts fibrinogens to fibrin by activating a coagulase reacting factor present in plasma. It can be detected by the appearance of fibrin clot in the tube coagulase test.

b. Bound coagulase, also known as clumping factor, converts fibrinogen directly to fibrin without requiring a coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide coagulase test.

a. 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.

b. 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 in 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 MIDSTREAM URINE

It cannot be overemphasized that considerable importance is attached to the method of collection of urine specimens, transport to the laboratory and the initial efforts by the laboratory to screen and culture the urine. It is the responsibility of laboratory to provide patient with sterile, wide mouthed, glass or plastic jars, beakers or suitable receptacles. They should have tight- fitting lids or be covered with papers or foils prior to sterilization by dry heat or autoclaving.

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

Midstream urine (MSU) for microbiological examination is as follows:

WOMEN

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 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 thoroughly with soap and water and dry them with a clean towel.
2. Pull back the foreskin (if not circumcised) and wash the glans thoroughly using sterile cotton gauze pads and warm soapy water.
3. Rinse the glans thoroughly with warm water and dry with a sterile gauze pad.
4. Pull back the foreskin and pass a small amount of urine. Still holding back the foreskin, the patient should pass most of the remaining urine into a sterile container. This is a midstream urine specimen.
5. 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

MORPHOLOGY AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM URINE SAMPLE

Bacteria	Morphological Characteristics	Cultural Characteristics
Escherichia coli	Gram negative rod of 1-3µm×0.4-0.7µm size, aerobic and anaerobic, nonsporing, motile, noncapsulated	On BA: Large 1-4 mm in diameter, grayish white, moist, smooth, convex, and opaque. The colonies may appear mucoid and some strains are haemolytic. On MA: Bright pink colonies due to lactose fermentation ,smooth, glossy and translucent.
Klebsiella spp	Gram negative, short and thick rod of 1-2µm ×0.8µm size, nonsporing, nonmotile and capsulated.	Large dome shaped moist and usually viscid or mucoid colonies when cultured on BA and MA. Most *Klebsiella species* are lactose fermenting.
Pseudomonas aeruginosa	Gram negative slender rod of 1.5-3 µm × 0.5µm size, nonsporing, motile with a single polar flagellum, most strains produce slime, strict aerobe.	Six different colonial types of *Pseudomonas aeruginosa* are encountered (Philips, 1969). Type 1 is the most common: colonies are large, low convex, rough in appearance and often oval, sometimes surrounded by a thin serrated skirt of growth. Type 2 colonies are small, domed and smooth and are described as coliform like. Colony type 3 and 4 are small and appear rough and rugose respectively. The mucoid alginate – producing type 5 colony result in merging colonial growth after overnight incubation, the drawf colony type 6 is the smallest colony form. It has characteristic sweet musty odour and the distinctive blue-green appearance due to fluorescein and pyocyanin pigment On BA: Large, flat colonies showing haemodigestion. On MA: Pale, nonlactose fermenting, colourless translucent colonies.
Proteus species	Gram negative rod of 1-3 µm×0.4-0.6µm size, noncapsulated.	On BA: when cultured aerobically, most strains are swarming type and have a characteristic fishy odour. On MA: *Proteus species* produces individual nonlactose

	nonsporing motile rods.	fermenting colonies after overnight incubation at 35ºC to 37ºC. Swarming is prevented on MA because this media contains bile salts.
Enterobacter species	Gram –ve rod, none sporing, noncapsulated.	About 2 to 3 mm in diameter, moist, yellowish coloured, LF, motile organism.
Enterococcus faecalis	Gram positive, spherical cocci occurring in pairs or short chains. They are non capsulated and the majority are nonmotile.	Enterococci are aerobic organism capable of growing over a wide temperature range, 10-45ºC On BA: Enterococci are mainly non haemolytic but some strains show *alpha* or *beta*- haemolysis. On MA: *Enterococcus faecalis* ferments lactose, producing small dark red magneta colonies. *Enterococcus species* are also able to grow in the presence of 6.5% Nacl and 40% bile. When grown in media containing aesculin, Enterococci hydrolyze the aesculin producing black colonies.
Staphylococcus aureus	Gram positive, spherical cocci, 0.8-1 µm in diameter, nonsporing, 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 consistence and opaque and pigments appearance. 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.

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,fermentation of lactose; inos, 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- -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 Agent	ol	ontent	ant (mm or less)	ediate (mm)	ve more)
Amikacin		μg	14	15-16	7
mpicillin esting gram-negative enteric organisms esting Staphylococci esting *Streptococcus* other than *S. pneumoniae*		μg	13 28 18	14-16 - 19-25	7 9 6
Cefotaxime		μg	14	15-22	3
Ceftriaxone		μg	13	14-20	1
Cephalexin		μg	14	15-17	8
Cotrimoxazole		.75μg	10	11-15	6
Ciprofloxacin		μg	15	16-20	1
Cloxacillin		μg	12	12-13	4
Erythromycin esting Staphylococci esting Streptococci		μg	3 15		3 1
Norfloxacin		μg	12	13-16	7
trofurantoin		μg	14	15-16	7
Novobiocin		μg	17	18-21	2
ancomycin esting Enterococci esting other gram positive organisms			14 -		-

(Source: Product Information Guide, Hi-Media Laboratories Pvt. Limited, Bombay, India and Cheesbrough, 2000)

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)
Pus cell count	31	26	10	118
Erythrocytes count	6	16	35	128

Calculation of sensitivity

Sensitivity can be calculated as:

Sensitivity = a/ (a+c) ×100%

Sensitivity of Pus cell count = 31/(31+10)×100%=75. 61%

Sensitivity of Erythorcyte count = 6/ (6+35) ×100%=14.63%

Calculation of Specificity

Specificity can be calculated as

Specificity=d/ (b+d) ×100%

Specificity of Pus cell count=118/ (26+118) ×100%=81.95%

Specificity of Erythrocyte count=128/ (16+128) ×100=88.89%

Calculation of Predictive value Positive (PVP)

PVP can be calculated as

PVP=a/ (a+b) ×100%

PVP of Pus cell count =31/ (31+26) ×100%=54.38%

PVP of Erythrocyte count=6/ (6+16) ×100%=27.27%

Calculation of Predictive Value Negative (PVN)

PVN can be calculated as

PVN=d/(c+d) ×100%

PVN of Pus cell count =118/ (10+118) ×100=92.19%

PVN of Erythrocyte count=128/ (35+128) ×100=78.52%

Calculation of Efficiency

Efficiency can be calculated as

Efficiency= a+d/ (a+b+c+d) ×100%

Efficiency of Pus cell count=31+118/ (31+26+10+118) ×100%=80.54%

Efficiency of Erythrocyte count=6+128/ (6+16+35+128) ×100%=72.43%

APPENDIX-XI

DATA ANALYSIS (CHI-SQUARE TEST)

1. Association of presence of significant bacteriuria in males and females

	Presence of bacteriuria	Absence of bacteriuria	Total
Male Patients	19	58	77
Female Patients	22	86	108
Total	41	144	185

Test statistic is ^{2}

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

H_1: There is significant association of presence of significant bacteriuria in male and female patients.

From ^{2}= (0-E) ^{2}/E we find ^{2}=0.47

Thus ^{2} cal (0.47) < ^{2} tab at =0.05 and d.f = 1 i.e.3.841

Hence, Ho is accepted i.e. there is no significant association of presence of significant bacteriuria in male and female patients.

2.Association of presence of significant bacteriuria in indoor and out-patients

	Presence of bacteriuria	Absence of bacteriuria	Total
Admitted Patients	16	39	55
Outdoor Patients	25	105	130
Total	41	144	185

Test statistic is 2

Ho: There is no significant association of presence of significant bacteriuria between indoor and outdoor patients.

H1: There is significant association of presence of significant bacteriuria between admitted and outdoor patients.

From $^2 = \frac{(O-E)^2}{E}$ we find $^2 = 2.175$

Thus $^2_{cal}$ (2.175) < $^2_{tab}$ at = 0.05 and d.f =1 i.e 3.841

Hence, Ho is accepted i.e. there is no significant association of significant bacteriuria between indoor and outdoor patients

3. Association of significant and non significant pyuria in culture positive samples among male and female patients.

	Significant pyuria	Non significant pyuria	Total
Male patients	15	4	19
Female patients	16	6	22
Total	31	10	41

Test statistic is 2

Ho: There is no significant association of significant and non significant pyuria in culture positive samples between male and female patients.

H1: There is significant association of significant and non significant pyuria in culture samples between male and female patients.

From $^2 = \frac{(O-E)^2}{E}$ we find $^2 = 1.613$

Thus $^{2}_{cal}$ (1.613) < $^{2}_{tab}$ at = 0.05 and d.f =1 i.e 3.841

Hence, Ho is accepted i.e. there is no significant association of significant pyuria in culture positive male and female patients.

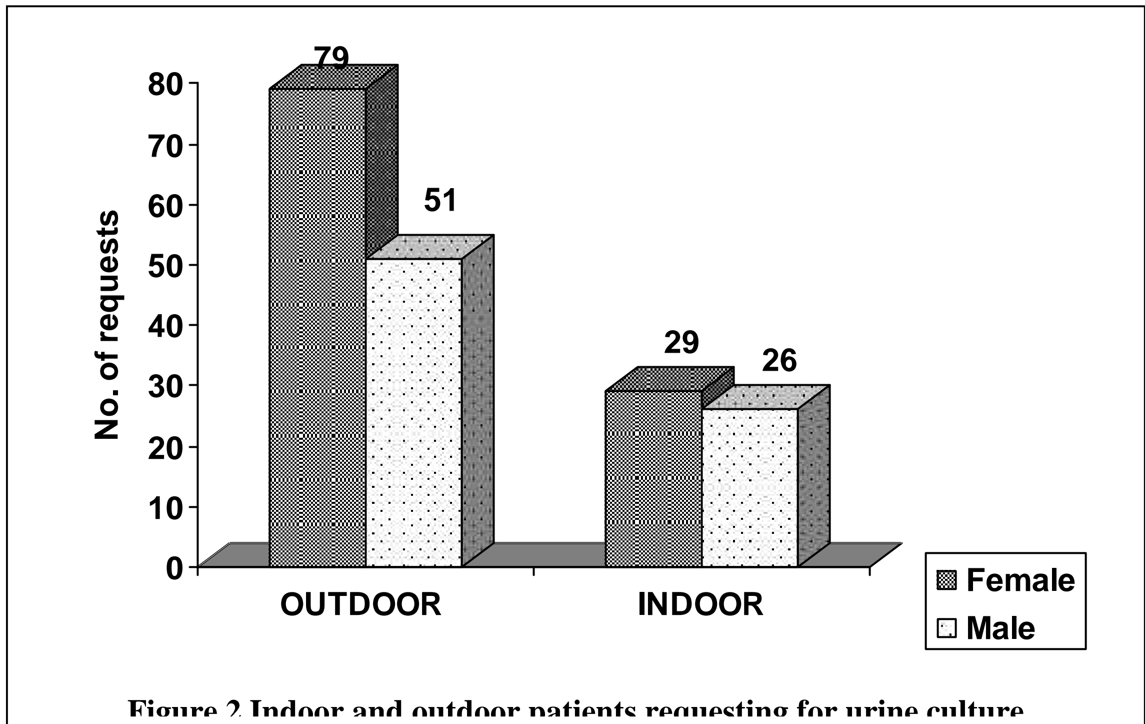


Figure 2 Indoor and outdoor patients requesting for urine culture

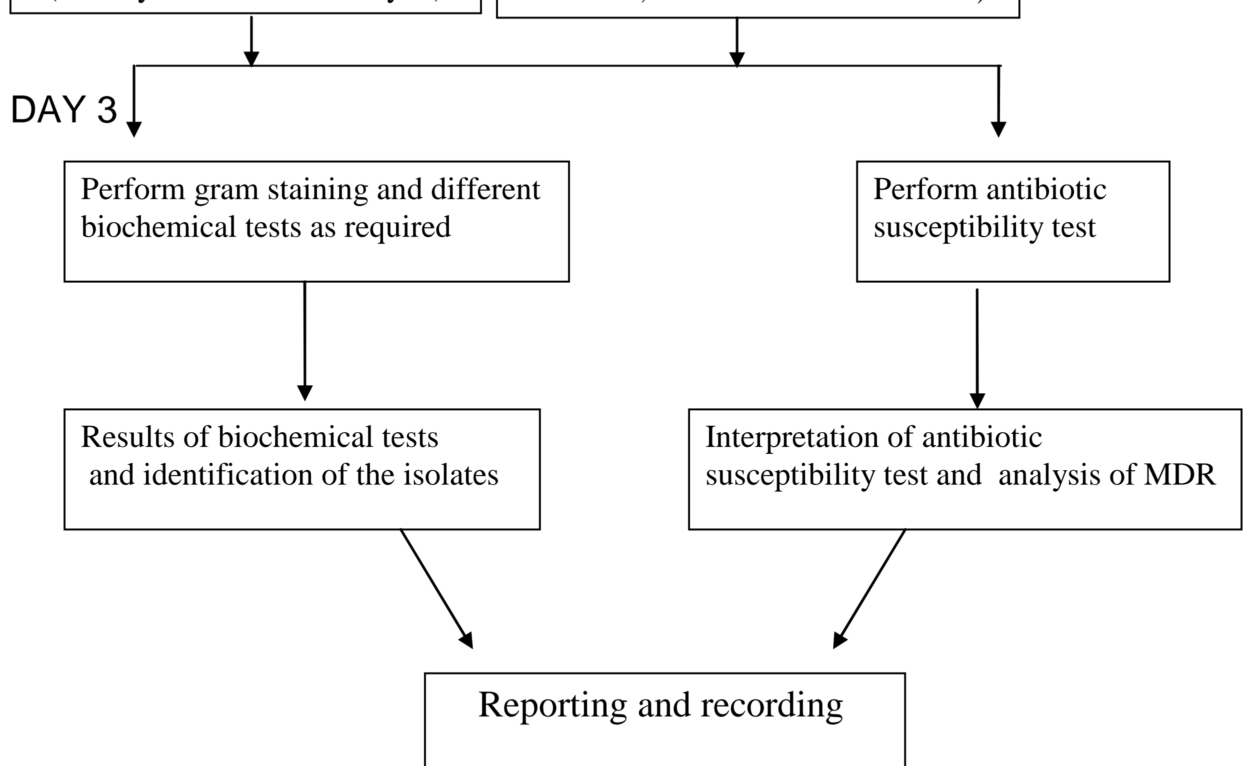


Figure1 Flow Diagram Showing Processing of Urine Sample
(Source: Cheesbrough, 2000)

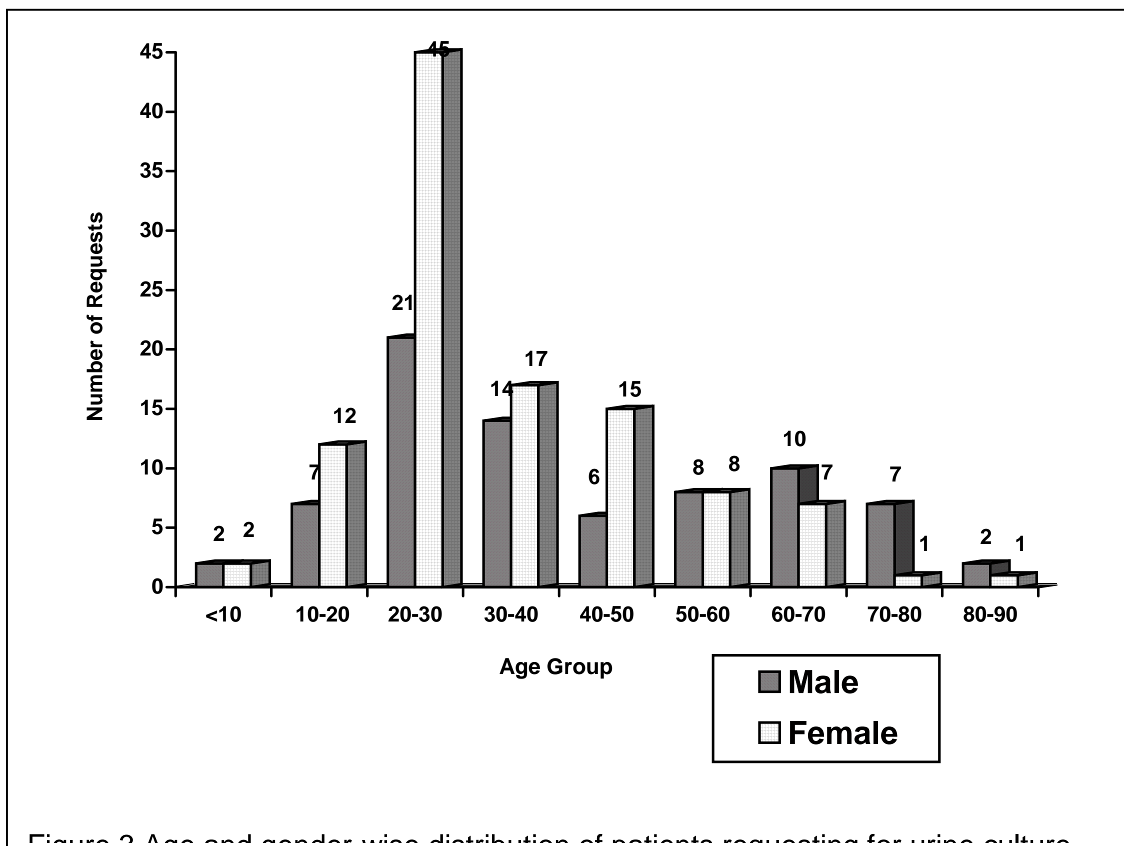


Figure 2 Age and gender wise distribution of patients requesting for urine culture

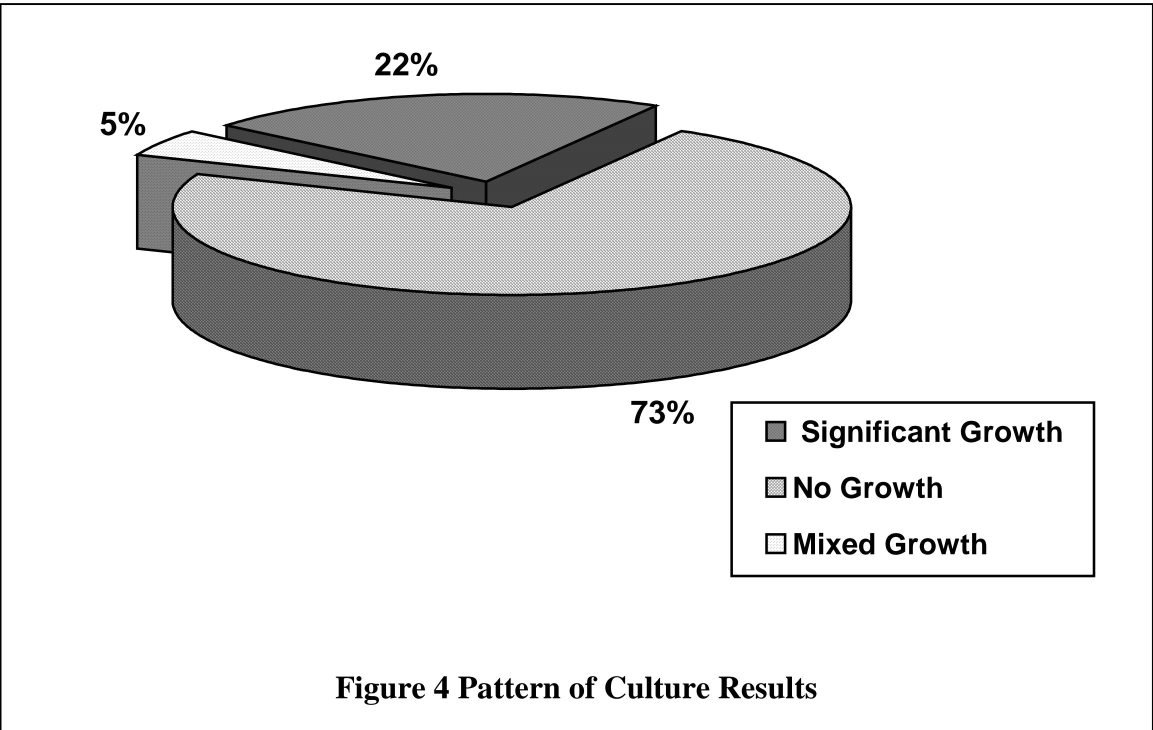


Figure 4 Pattern of Culture Results

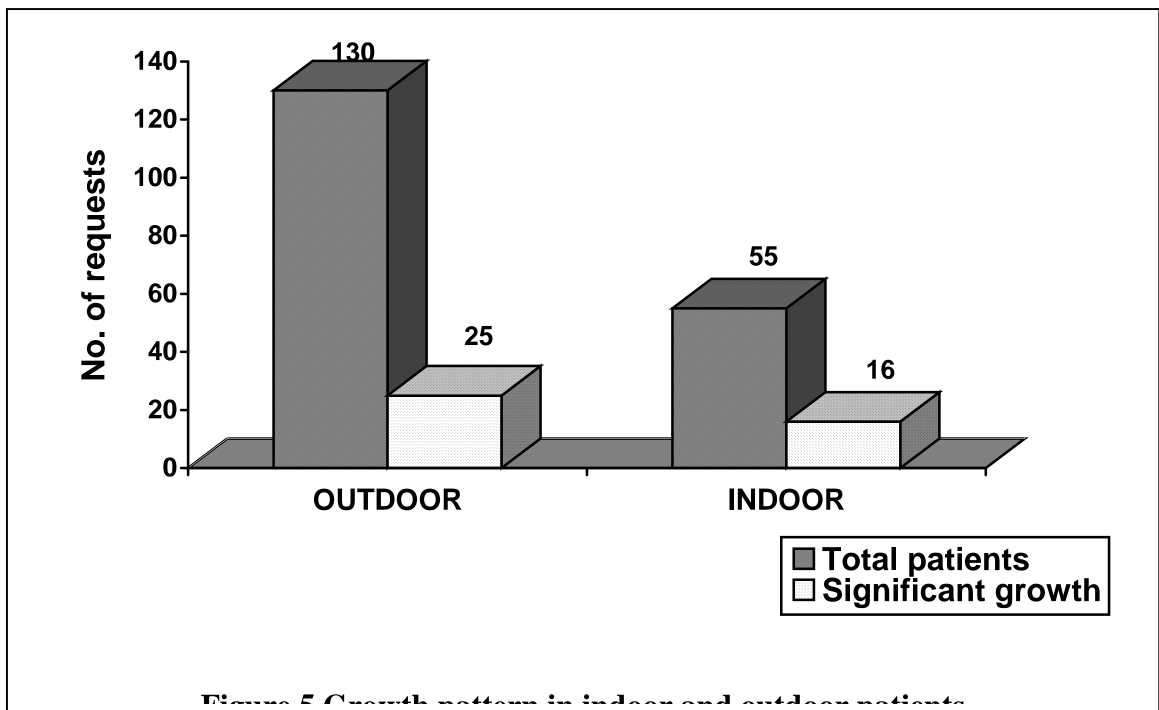


Figure 5 Growth pattern in indoor and outdoor patients

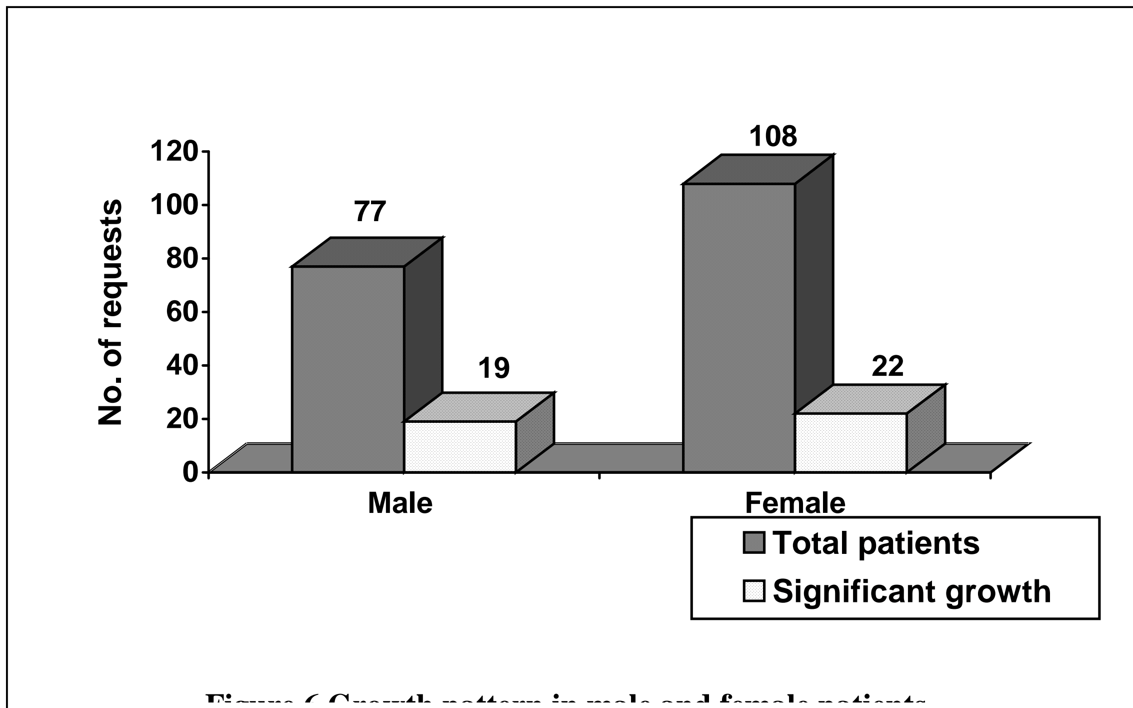
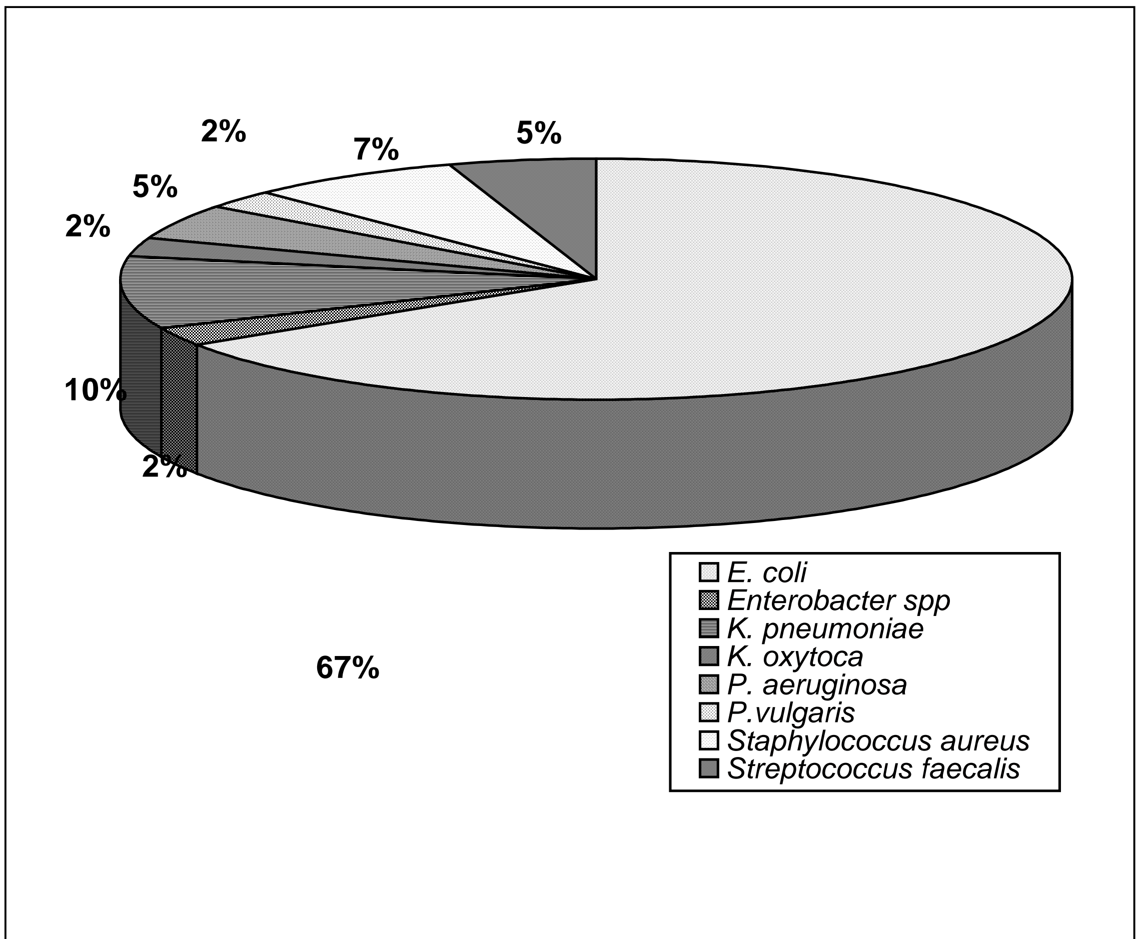
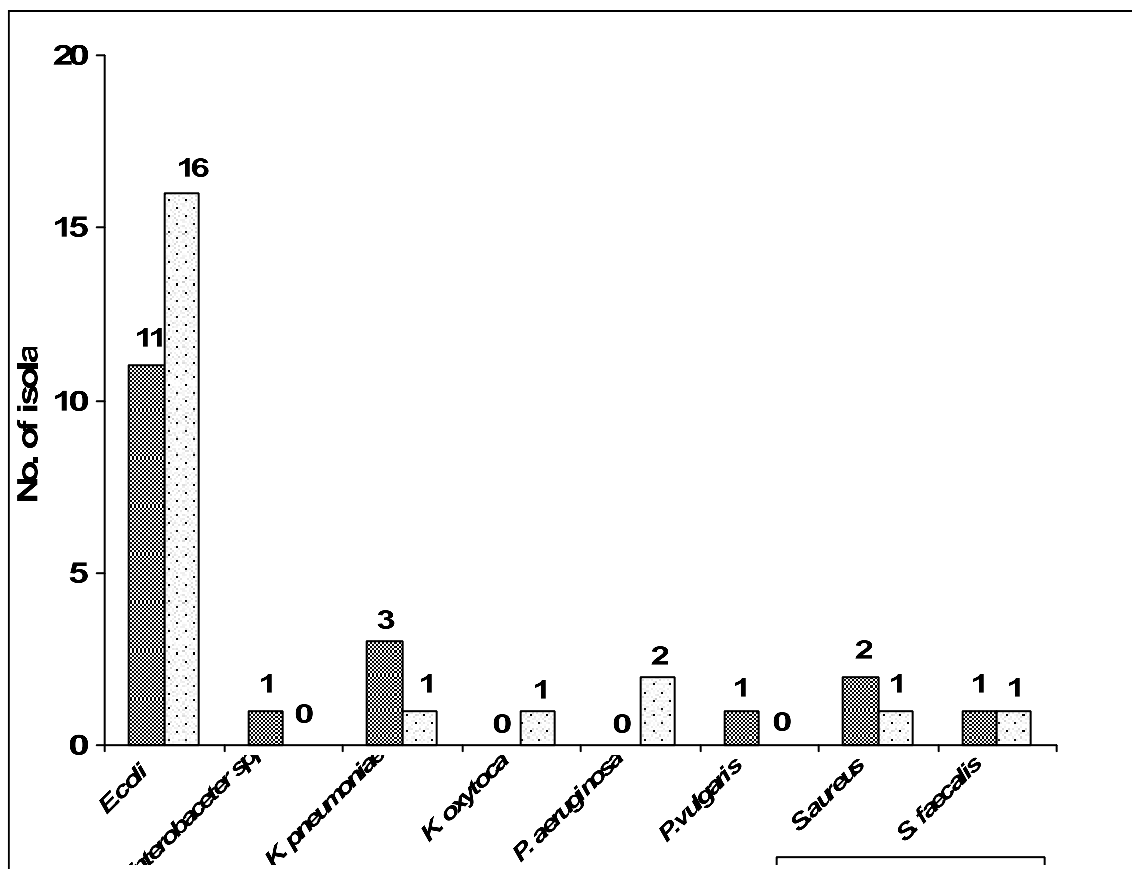


Figure 6 Growth pattern in male and female patients


2%
7%
5%
5%
2%
10%
2%
67%
E. coli
Enterobacter spp
K. pneumoniae
K. oxytoca
P. aeruginosa
P.vulgaris
Staphylococcus aureus
Streptococcus faecalis



No. of isola
20
15
10
5
0
11
16
1
0
3
1
0
1
0
2
1
0
2
1
1
1
E.coli
nterobacter sp
K. pneumoniae
K. oxytoca
P. aeruginosa
P.vulgaris
S.aureus
S. faecalis

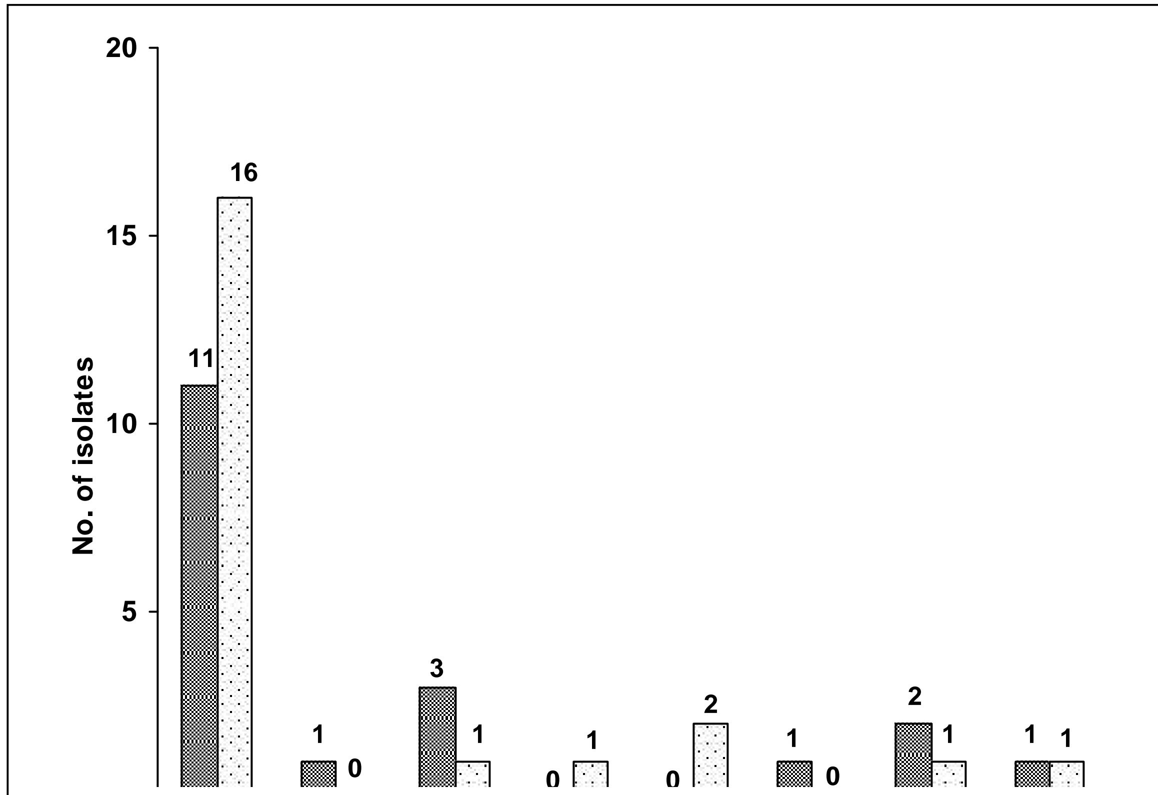

20
15
10
5
No. of isolates
16
11
1
0
3
1
0
1
0
2
1
0
2
1
1
1

Figure 9 Pyuria versus bacteriuria

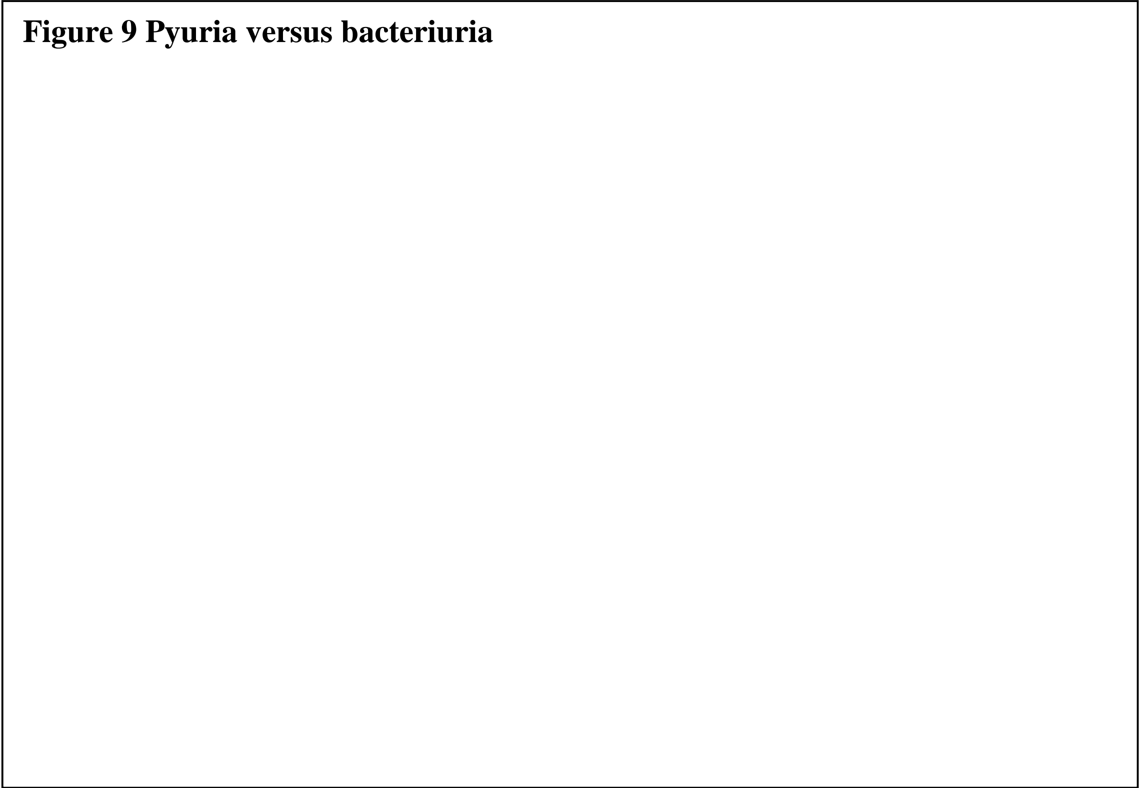


Figure 10: Antibiotic susceptibility pattern of gram-negative bacteria

Figure 11: Antibiotic susceptibility pattern of gram- positive bacteria