CHAPTER-I

1. INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism due to insulin deficiency and /or insulin resistance, evolving from interaction of variety of genetic and environmental factors. Insulin is necessary for the mobilization of glucose from the blood to the inside of the cells. So its deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body's system. The characteristic feature of diabetes is hyperglycemia. Therefore diabetes represents a heterogeneous group of disorders that have a common feature of hyperglycemia (Murray *et al,* 2000).

There is growing evidence to suggest that diabetes mellitus (DM) is heterogeneous in etiology, clinical presentation and susceptibility to complication and response to treatment. The spectrum is so wide that diabetes is presently regarded as a syndrome rather than disease entity. DM is the most frequent endocrine disease whose prevalence and incidence varies widely among study population (Reitsma *et al,* 1991).

In general, the prevalence of diabetes is 2.0 to 4.0% in western countries (Reitsma *et al,* 1991). Recently compiled data show that approximately 150 million people have DM worldwide and the number may double by the year 2025 (WHO, 2002).

It has been noted that diabetic patients can have severe medical complications. These often appear to be more insidious and may occur with a greater intensity and severity than their peers without DM. The complications of diabetes are retinopathy, neuropathy, nephropathy, cardiovascular complications and stroke. The patients with DM have increased risk of infection due to their weakened immune system (Hoepelman, 1994).

Urinary tract infections (UTI) are very often encountered in patients with diabetes mellitus. They may present themselves as asymptomatic bacteriuria, but may also lead to

more serious infection. Women with diabetes are at a particularly increased risk of urinary tract infection (Geerlings *et al,* 2000).

Various studies demonstrate greater susceptibility of diabetic than nondiabetic patients to UTI. Suggested mechanisms are decreased antimicrobial activity due to sweet urine, defects in neutrophil function and increase adherence to uroepithelial cells. When diabetic patients get infected, they are more severe as they are compromised host (Reiber *et al,* 1998).

Primarily, bacteria that colonize in the bowel and are capable of proliferating in urine cause UTI. Urinary tract infection is defined as condition of multiplication of the organisms in the urinary tract and the presence of more than 10^5 organisms /ml of mid stream urine. Bacteriuria may indicate infection of urinary tract, contamination by exogenous bacteria or the removal of normal urethral flora with first flow of urine. Discovering over 10^5 bacteria / ml of voided urine indicates significant bacteriuria, a presumably pathological condition (Forbes *et al,* 2002).

Gram negative bacilli and Enterococci are the primary enteric microorganisms capable of proliferating in the human urine. *Escherichia coli* is the most common cause of UTI. Among the microorganisms causing UTI, *E. coli* is responsible for 74.6%, *Proteus species* is responsible for 8.0%, *Klebsiella species* is responsible for 2.0%, *Pseudomonas species* is responsible for 2.0% and other organisms are responsible for 13.3% of the total cases of UTI (Herm *et al,* 2003).

Classically, the severity of complications applies also to wound healing in diabetic patients. It is likely that many of the serious problems associated with cause and resolution of wounds in the diabetic patient are due to the persistent elevated blood glucose and its effects. It appears equally likely that control of glucose over the years will reduce the incidence of these wound complications and the wound healing deficits in diabetes mellitus (Reiber *et al,* 1998).

Hyperglycemia causes defective phagocytosis and migration of cells important in the inflammatory response. Fibroblasts may become dysfunctional when glucose is unavailable for aerobic metabolism and lead to an impairment of collagen deposition and endothelial proliferation. Vasodilatation may not occur in the diabetic patient as a response to injury and as part of the repair process as in others (Stadelmann *et al,* 1998).

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 the six commonly 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,* 2001).

In context to our country, most of the people are not financially sound to have a routine check-up of their health status and even those who are financially capable do not care to do so. People generally seek for medical services only when the symptoms of the disease begin to become evident. In such cases, asymptomatic diseases in diabetic patients are simply ignored and in many cases, this negligence ultimately leads to serious complications. In our country, this is commonly true with different types of infections because most of the people do not have access to proper hygiene and good sanitation practices. UTI in diabetic patients also poses a threat to the Nepalese community.

In this context, the present study was carried out for patients attending the outpatient department with diabetes. Clinical laboratory records of cases of diabetes were studied for the spectrum of bacterial isolates causing UTI and their antibiotic susceptibility results were analyzed for recommending suitable therapy and to investigate the association between diabetes and UTI. The next goal of this study was to define the current status of multi-drug resistant strains among UTI isolates.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To determine the incidence of urinary tract infection in diabetic patients visiting “OM” Hospital and Research Center.

2.2 SPECIFIC OBJECTIVES

- To estimate fasting and post prandial blood sugar in patients suffering form diabetes.
- To detect the urinary sugar and protein in diabetic patients.
- To detect the pyuria in diabetic patients.
- To isolate and identify the bacteria associated with the urinary tract infection.
- To analyze the antibiotic susceptibility pattern of the isolated organisms.
- To interpret the MDR strains of the urinary isolates.
- To compare the results with nondiabetic control patients.

CHAPTER-III

3. LITERATURE REVIEW

3.1 DIABETES

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism due to insulin deficiency and /or insulin resistance, evolving from interaction of variety of genetic and environmental factors. Insulin is necessary for the mobilization of glucose from the blood to the inside of the cells. So its deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body's system (Murray *et al,* 2000).

Diabetes mellitus is a group of metabolic disorders with one common manifestation: hyperglycemia. Chronic hyperglycemia causes damage to the eyes, kidneys, nerves, heart and blood vessels. Diabetes mellitus is due to insufficient action of insulin, owing either to its absence or to resistance to its action. The cardinal manifestation of diabetes mellitus is hyperglycemia which results from (i) decreased entry of glucose into cells. (ii) decreased utilization of glucose by various tissues. (iii) Increased production of glucose by liver (Murray *et al,* 2000).

The plasma glucose level rarely exceeds 120 mg /dL in normal humans but much higher levels are routinely found in patients with deficient insulin action (generally >80 mg/dL in humans), with the maximum level of renal tubular reabsorption of glucose is exceeded and sugar is excreted in the urine (Murray *et al,* 2000).

According to the 1998 WHO criteria, diabetes mellitus was defined as fasting glucose concentration of at least 6.1 mmol/ L (110 mg/dL) or a two-hour post prandial glucose (2 hr PPG) concentration of at least 10.0 mmol/L (180 mg/dL) or the use of glucose-lowering medication (Nicolle, 2000).

3.2 CLASSIFICATION OF DIABETES

In June 1997, an international expert committee released a report with new recommendations for the classifications and diagnosis of diabetes mellitus (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). These new recommendations were the result of more than two years of collaboration among experts from the American Diabetes Association (ADA) and the World Health Organization (WHO). The use of classification systems and standardized diagnostic criteria facilitates a common language among patients, physicians, other health care professionals and scientists.

The new classification system identifies four types of diabetes mellitus: type I, type II, other specific types and gestational diabetes. Each of the types of diabetes mellitus identified extends across a clinical continuum of hyperglycemia and insulin requirements.

3.2.1 Type I diabetes mellitus

Type I diabetes mellitus (formerly called Type 1, Insulin dependent diabetes mellitus or juvenile diabetes) is characterized by beta cell destruction caused by and autoimmune process, usually leading to absolute insulin deficiency (National Diabetes Data Group 1995; Report of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). This type of diabetes is reported in about 10.0 % total people suffering form diabetes (life time risk of 0.5%) This is manifested by elevated glucose level (Murray *et al,* 2000). The onset is usually acute, developing over a period of a few days to weeks. Over 95.0 % of persons with type I diabetes mellitus develop the disease before the age of 25, with an equal incidence in both sexes.

3.2.2 Type II diabetes mellitus

Type II diabetes mellitus (formerly called Type 2, Non Insulin dependent diabetes mellitus or adult onset diabetes) is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the β cell. Type II diabetes was defined as the

combination of resistance to insulin action and an inadequate compensatory insulin secretory response (National Diabetes Data Group 1995; Report of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). About 90.0% of persons (life time risk is 5.0-7.0 %) with diabetes mellitus have this type of DM and is highly associated with a family history of diabetes, older age, obesity and lack of exercise. These patients have elevated plasma insulin level but have down regulated insulin receptor (Murray *et al*, 2000). It is more common in women with a history of gestational diabetes and in Negroid races. Insulin resistance and hyperinsulinemia eventually lead to impaired glucose tolerance. Defective β cell become exhausted, further fuelling the cycle of glucose intolerance and hyperglycemia. The etiology of type II diabetes mellitus is multifactorial and probably genetically based, but also has strong behavioral components.

3.2.3 Other specific types

Types of diabetes mellitus of various known etiologies are grouped together to form the classification called “other specific types” (formerly called MODY or maturity- onset diabetes in youth). This type of diabetes is reported in less than 5% people suffering form diabetes. This group includes persons with genetic defects of β cell function or with defects of insulin action, persons with diseases of the exocrine pancreas, such as pancreatitis or cystic fibrosis, persons with dysfunction associated with other endocrinopathies (e.g. acromegaly) and persons with pancreatic dysfunction caused by drugs, chemicals or infections (National Diabetes Data group 1995; Report of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997).

3.2.4 Gestational diabetes

Gestational diabetes mellitus is an operational classification (rather than a pathophysiologic condition) identifying women who develop diabetes mellitus during gestation. (National Diabetes Data Group, 1995) Women with diabetes mellitus before pregnancy are said to have pregestational diabetes and are not included in this group. Women who develop type I diabetes mellitus during pregnancy and women with undiagnosed asymptomatic type II diabetes mellitus that is discovered during pregnancy

are classified with gestational diabetes mellitus. However, most women classified with gestational diabetes mellitus have normal glucose homeostasis during the first half of the pregnancy and develop a relative insulin deficiency during the last half of the pregnancy, leading to hyperglycemia. The hyperglycemia resolves in most women after delivery but place them at increased risk of developing type II DM later in life.

3.3 DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS

The oral glucose tolerance test previously recommended by the National Diabetes Data Group has been replaced with the recommendation that the diagnosis of diabetes mellitus be based on two fasting plasma glucose levels of 126 mg/dL (7.0 mmol / L) or higher. Other options for diagnosis include two two- hour post prandial plasma glucose (2 hr PPG) readings of 200 mg/dL (11.1 mmol /L) or higher after a glucose load of 75 g (the criterion recommended by WHO) or two casual glucose readings of 200 mg/dL (11.1 mmol/L) or higher. Fasting plasma glucose was selected as the primary diagnostic test because it predicts adverse outcomes (e.g. retinopathy) as well as the2 hr PPG test but is much more reproducible than the oral glucose tolerance test or the 2 hr PPG test and easier to perform in a clinical setting.

According to the 1998 WHO criteria, diabetes mellitus was defined as fasting glucose concentration of at least 6.1 mmol/L (110 mg/dL) or a two-hour post prandial glucose concentration of at least 10.0 mmol/L (180 mg/dL) or the use of glucose-lowering medication (Nicolle , 2000).

Blood glucose levels above the normal level but below the criterion established for diabetes mellitus indicate impaired glucose homeostasis. Persons with fasting plasma glucose levels ranging from 110 to 126 mg/dL (6.1 to 7.0 mmol/L) are said to have impaired fasting glucose, while those with 2 hr PPG level between 140 mg/dL (7.7 mmol/L) and 200 mg/dL (11.1 mmol/L) are said to have impaired glucose tolerance. Both impaired fasting glucose and impaired glucose tolerance are associated with an

increased risk of developing type II diabetes mellitus. Lifestyle changes, such as weight loss and exercise, are warranted in these patients (Mayfield, 1998).

3.4 SYMPTOMS OF DIABETES

The symptoms of the type I diabetes are classic symptoms like polyuria, polydipsia and weight loss. The symptoms may be less marked in type II diabetes. In this form, it can happen that no early symptoms appear and the disease is diagnosed several years after its onset, when complications are already present (Murray *et al*, 2000).

The urine volume is increased owing to osmotic diuresis and coincident obligatory water loss (polyuria) and this in turn leads to dehydration (hyperosmolarity), increased thirst and excessive drinking (polydipsia), glycosuria causes a substantial loss of calories. This loss when coupled with the loss of muscle and adipose tissue result in severe weight loss in spite of increased appetite (polyphagia) and normal or increased caloric intake (Murray *et al*, 2000).

3.5 INSULIN IN RELATION TO DIABETES

Langerhans identified the islets in 1860s; Von Mering and Minksowski were the first to demonstrate that pancreatectomy leads to symptoms of acute diabetes in 1889. The link between the islets and diabetes was suggested by de Mayer who coined the epochal name insulin in 1909 and by Sharpey and Schaffer in 1917.

It was Banting and Best who proved that diabetes was the result of deficiency of insulin and islets, the source of this hormone.

Insulin was the first protein proved to have hormone action, the first protein crystallized (Abel, 1926), the first protein sequenced (Sanger *et al*, 1955), the first protein synthesized by chemical technique (Du *et al*, 1964), the first protein synthesized as a large precursor molecule (Steiner *et al*, 1967) and the first protein prepared for commercial use by recombinant DNA technology . Yalow and Berson, by 1961 develop

the method of Radio immuno assay of insulin in biological fluids and paved the way for better understanding of pathophysiology of diabetes and number of related condition (Murray *et al*, 2000).

3.5.1 Insulin synthesis, secretion and its regulation

Insulin is a polypeptide consisting of two chains A and B, linked by two interchain disulfide bridges that connect A7 and B7 and A20 to B19. A third intrachain disulfide bridge connects residues 6 and 11 of the A chain. The A and B chains have 21 and 30 aminoacid respectively, having molecular mass 5.734 KDa (Murray *et al*, 2000).

Insulin is synthesized and secreted by β cells of islets of langerhans. Adult human pancreas contain about 200,000 to 18,00,000 islets. Insulin is synthesized as a preprohormone with molecular weight 11,500 KDa. The hydrophobic 23 amino acid, pre or leader sequence directs the molecules into the cisternae of the endoplasmic reticulum and is removed resulting preinsulin having molecular weight 9000 KDa. Its length varies from 78 to 86 aminoacids. The synthesis of proinsulin occurs in ribosomes and the enzymatic removal of the leader peptide sequence, disulfide bond formation and folding occur in the cisternae of endoplasmic reticulum. The proinsulin molecule is transported to the golgi apparatus wherein proteolysis results into equimolar amount of insulin and C peptide and packaging into secretory membrane bound granules begin. Granules continue to mature as they traverse the cytoplasm toward the plasma membrane. Proinsulin and insulin both combine with zinc to form hexamers but since about 95.0% of the proinsulin is converted to insulin it is the crystals of the latter that confer morphological distinctness to the granules. Upon appropriate stimulation the granules into β cells utilize energy derived from glucose metabolism and the tubules propel the hormone to the surface of the cell where the sacs fuse with the plasma membrane and discharge their content into extracellular fluid by exocytosis (Murray *et al*, 2000).

Around 200 units of insulin are usually stored in healthy pancreas. Insulin requirement of healthy adult has been estimated to around 40-60 units per day. Glucose is a prime

regulator of insulin secretion and insulin is the prime modulator of blood glucose. Physiologically minute fluctuation of blood glucose affects insulin release from β cells. It is hypothesized that glucose acts in the β cells both by activating glucoreceptors situated on the surface and stimulating intracellular mechanism after entry into the cell. Prompt and immediate response depends on release of second signal (activated adenylcyclase and cAMP) from the site of the receptors (Murray *et al*, 2000).

The glucose stimulated insulin release is biphasic in nature. Within half to one minute of rise in concentration of glucose in extracellular fluid, the first or quick phase of insulin release is obtained, the peak of which lasts for only a few minute. The second phase of insulin secretion starts about 15 minutes later and continues for more than an hour. The first phase is solely contributed from the stored insulin, inside the β cell. The second phase comprises mostly of newly synthesized insulin. In some diabetics the quick phase of insulin secretion is usually abolished and onset of second phase is delayed but may be more or less pronounced (Murray *et al*, 2000).

3.5.2 Physiological action of insulin

Insulin is a powerful anabolic hormone. It conserves glucose as glycogen, amino acids as protein and increases deposition of neutral fat. Further it promotes preferential oxidation of glucose to provide energy and spares fat and protein breakdown.

Primarily insulin facilitates transfer of glucose across the cell membrane in tissues other than liver, brain, peripheral nerves, blood cells and tubules and medulla of the kidney. In addition, insulin stimulates the intracellular enzyme glucokinase and accelerates phosphorylation of glucose and enhances glucose oxidation by activating some intracellular steps as yet undefined. It promotes glycogen formation and its deposition in liver and muscles by activating glycogen synthetase and prevents glycogenolysis by inhibiting phosphorylase (Burtis and Ashwood, 1998).

Insulin is a lipogenic hormone. It enhances synthesis of free fatty acids from glucose both in adipose tissue and liver and inhibits lipolysis. The above actions lead to lowering of blood sugar and decrease in serum levels of free fatty acid. Insulin stimulates transfer of amino acids at cell membrane level and acting on ribosomes, it increases protein synthesis. It counteracts the effect of catabolic hormones and inhibits gluconeogenesis (Burtis and Ashwood, 1998).

3.5.3 Mechanism of insulin action

The mechanism, by which insulin activates cell membrane and subcelllular structure, is not yet fully understood. Apparently its major action is on cell membrane itself. Recent report suggests internalization of insulin in hepatocyte but for its action it may have to enter into the cell. Plenty of information is by now available regarding presence of insulin receptors in the surface of cells viz, hepatocyte, adipocyte, myocyte and monocyte. Molecules of insulin are bound at the receptor sites and facilitate transfer of metabolites through cell membrane and also influences intracellular mechanism by generating signals. Through this it influences the metabolism of cells, which do not require the hormone for the purpose of membranes transfer (Burtis and Ashwood, 1998).

Insulin has been found to decrease intracellular availability of cAMP, possibly by activating phosphodiesterase. This leads to decrease of phosphokinase inside the target cell. Thus it counterchecks the action of most of the anti-insulin hormones (Burtis and Ashwood, 1998).

3.5.4 Metabolic effects of insulin deficiency

Insulin deficiency may be relative or absolute. Normal serum insulin level in a non obese fasting person varies from 5-15 micro units/ml and it rises by 2-5 folds at peak hours after carbohydrate and protein meals (Burtis and Ashwood, 1998).

In some forms of diabetes there is an absolute decrease in the amount of available insulin but in others there may be physiological evidence of insulin lack even when the plasma levels are fairly high (Burtis and Ashwood, 1998).

In case of mild insulin deficiency state, fasting blood sugar level is usually normal. But hyperglycemia occurs after loads of carbohydrate. Normally after glucose loads, 70% is trapped in liver. This occurs due to prompt release of insulin, which rapidly reaches liver through portal vein. Failure of this mechanism in early diabetes leads to hyperglycemia because of entry of larger amount of glucose into general circulation.

Moderate insulin deficiency results in fasting hyperglycemia. Blood sugar remains high for a period of 4-5 hours after a meal. It continues to remain high postprandially due to over production of glucose by gluconeogenesis and glycogenolysis. Moreover, there is diminished peripheral utilization of glucose (Burtis and Ashwood, 1998).

In severe diabetic state, gross failure of insulin action produces hyperglycemia. Due to lack of utilization of glucose, free fatty acid and amino acid derived from adipose tissue and muscles are utilized to provide alternative fuels. In a situation of gross insulin lack, the production of free fatty acids exceeds than can be utilized by peripheral tissues. (Murray *et al*, 2000).

3.6 COMPLICATIONS ASSOCIATED WITH DIABETES

3.6.1 Long term complications

Diabetic retinopathy, heart disease, diabetic neuropathy, diabetic foot diseases and renal complications are considered to be long term complications.

3.6.2 Short term complications

Short term complication includes glycosuria, meaning sugar in urine. Glucose is present in glomerular filtrate but is reabsorbed by the kidney's proximal tubule. If the blood glucose level exceeds the capacity of the tubule to reabsorb all the glucose present in the glomerular filtrate, the renal threshold is reached and glucose spills into the urine. The

renal threshold for glucose is approximately 160 to 190 mg/dL of blood. Occasionally glycosuria may be the normal finding, such as after eating a heavy meal or during times of emotional stress. Some individuals have a benign condition in which they have a lower than usual renal threshold for glucose, but have normal blood glucose levels. But the most common reason for glycosuria is diabetes mellitus.

3.6.3 Infectious complications

It is strongly believed that diabetic patients are susceptible to infection (Tattersall and Gate, 1975). Several aspects of immunity are altered in patients with diabetes. Ploymorphonuclear leucocytes function is depressed, particularly when acidosis is present. Leucocytes adherence, chemotaxis and phagocytosis may be affected (Delamaire *et al*, 1997; Gallacher *et al*, 1995; Valerius *et al*, 1982).

3.6.4 Diabetes and infection

Patients with diabetes mellitus are more predisposed to infections (Murphy *et al*, 1981; Wheat, 1980). This predisposition is due to a combination of angiopathy, neuropathy, and hyperglycemia (Murphy *et al*, 1981). Impaired host defense mechanisms such as impaired wound healing, impaired granulocyte function, decreased cellular immunity, impaired complement function and decreased lymphokine response may be influenced by glycemic control. Most physicians believe that diabetic individuals are not only predisposed to infections but that infection also complicates the control of the diabetes (Leibovici *et al*, 1996; McMahon and Bistrian, 1995; Murphy *et al*, 1981).

3.7 COMMON INFECTIONS

- **Foot infections**

Diabetic patients are particularly susceptible to foot infections. These infections are the leading cause of limb loss in the United States and are responsible for the majority of hospitalizations of diabetic patients (Gibbons and Habershaw, 1995).

- **Skin infections**

Diabetic patients appear to have skin infections more often than their non- diabetic counterparts. Poor host defense mechanism and a general environment conducive to

bacterial growth combine to make the diabetic patient more susceptible to skin infections due to bacteria and fungi.

- **Upper extremity soft tissue infections**

The incidence of hand and upper extremity infections is higher among diabetic patient (Archibald *et al*, 1997; Gill *et al*, 1998; Gonzalez *et al*, 1999; Pinzur *et al*, 1997). Golden *et al* (1999) believes that hyperglycemia may be an independent predictor of postoperative infectious complications in diabetic patients and suggest a target glucose concentration of less than 200 mg/dL to reduce the risk of postoperative infection.

- **Respiratory infections**

Diabetic patients may be more predisposed to lower respiratory tract infections (Koziel and Koziel, 1995).

- **Helicobacter infection**

Gentile *et al* (1998) reported a higher frequency of *Helicobacter pylori* infection among dyspeptic diabetic patients than among nondiabetic patients.

- **Urinary tract infection (UTI)**

Diabetes leads to decreased resistance to infection and urinary tract infections are common. In addition, complications from UTI are more common. Diabetes predisposes the patient to renal abscesses and UTI may increase this risk. Infections increase the risk of nephropathy, which may place the patient at greater risk for developing kidney failure (Carton *et al*, 1992; MacFarlane *et al*, 1986).

Most infections in diabetic patients are located in the urinary tract (Carton *et al*, 1992; MacFarlane *et al*, 1986). An autopsy study in 1940 showed that 18% of patients with diabetes mellitus had a serious infection of the urinary tract (Baldwin and Root, 1940). Symptomatic UTIs are also more common in diabetic women compared with non diabetic women (Patterson and Andriole, 1997)

Urinary tract infections are very often encountered in patients with diabetes mellitus. They may present themselves as asymptomatic bacteriuria, but may also lead to more serious infection (Hoepelman, 1994). Anatomic and functional abnormalities of the

urinary tract are also associated with diabetes (Stapleton, 2002).Women with diabetes are at a particularly increased risk of urinary tract infection (Geerlings *et al,* 2000).

The occurrence of bacteria i.e. the single species of bacteria within the urinary tract is the common denominator of this disorder. Confirmation of UTI is based on finding appreciable number of pathogenic bacteria in the bladder urine (White *et al,* 1977).

3.8 URINARY TRACT INFECTION

The presence of bacteria in urine is called bacteriuria (Cheesbrough, 2000).

Infection of urinary tract is defined as bacteriuria, the multiplication of the organisms in urinary tract and the presence of more than a hundred thousand organisms per ml in the midstream sample of urine (Chakraborty, 2001).

Urinary tract infection simply means the presence of bacteria undergoing multiplication in urine within urinary drainage system (Leigh, 1990).

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

The term urinary tract infection (UTI) refers to the invasion of the urinary tract by a non-resident infectious organism. UTI encompasses a wide variety of clinical entities whose common denominator is microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus. Infection of the prostate and epididymis is also included in the definition (Pokhrel, 2004).

Confirmation of UTI based on finding appreciable numbers of pathogenic bacteria in the bladder urine (White *et al,* 1977)

In order to confirm UTI with reasonable confidence, the criteria of clinical features,

bacteriuria and pyuria must be met. Significant bacteriuria is defined as the presence of bacteria in the urine. Organisms are actually multiplying in the urine and present in a count, which is excessively high or unexplainable by urethral contamination (Pokhrel, 2004).

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

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

Low count significant bacteriuria subject to the following conditions

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

UTI is among the most common reasons patients seek medical care. It is estimated that approximately 10.0% of humans will have UTI at sometime during their lives. The incidence ratio of UTIs in middle-aged women to men is 30:1; however, during later decades of life, the ratio of infection in women to men with bacteriuria progressively decreases (Boscia and Kaye, 1987)

The urethra has resident microflora that colonize its epithelium in the distal portion. The resident microflora of urethra includes coagulase negative staphylococci (excluding *S. saprophyticus*), viridans and non-hemolytic streptococci, diptheroids, anaerobic cocci and anaerobic Gram negative bacilli. Potential pathogens, including Gram negative aerobic bacilli primarily *Enterobacteriaceae* and occasional yeasts, are also present as

transient flora, which contaminate urine in passage, so the voided urine may contain small number of bacteria in absence of urinary tract infection (Brooks *et al,* 2004).

Diabetes leads to decreased resistance to infection and urinary tract infections are common. In addition, complications from UTI are more common. Diabetes predisposes the patient to renal abscesses and UTI may increase this risk. Most infections in diabetic patients are located in the urinary tract (Carton *et al,* 1992; MacFarlane *et al,* 1986). Patients with diabetes mellitus have 10 folds increased risk of UTI (Goswami *et al,* 2001). An autopsy study in 1940 showed that 18.0% of the patients with diabetes mellitus had a serious infection of the urinary tract (Baldwin and Root, 1940). Symptomatic UTIs are also more common in diabetic women compared with nondiabetic women (Patterson and Andriole, 1997).

Diabetes leads to several abnormalities of the host defense system and higher glucose concentration in urine may serve as a culture medium for pathogenic microorganisms. The risk of developing infection in diabetic patients is higher (Carton *et al,* 1992; Pozzilli and Lesli, 1994) and urinary tract is the most common site for infection (MacFarlane, 1986; Wheat, 1980). Serious complications of urinary infection, such as emphysematous cystitis, pyelonephritis, renal or perinephric abscess, bacteremia and renal papillary necrosis occur more commonly in diabetic patients (Nicolle, 1980). Many Urinary Tract Infections (UTIs) are asymptomatic and whether the symptomatic UTIs are preceded by asymptomatic bacteriuria (ASB) is not known (Vejlsgaard, 1966). Development of ASB in diabetic woman is much more common than in nondiabetic woman (Wheat, 1980). Various risk factors for ASB in women with diabetes have been suggested including sexual intercourse, age, and duration of metabolic control and complications of diabetes (Osterby, 1964; Zhanel *et al,* 1995). In the study on ABS and diabetes done by Geerlings *et al* (2000) the prevalence of ASB was 29.0% in type II diabetes. Risk factors for ASB in type II diabetic women included age, macroalbuminuria, low BMI and UTI during the previous year.

UTI are very often encountered in patients with diabetes mellitus, they may present themselves as asymptomatic bacteriuria, but may also lead to more serious infections. In

most cases the kidney is involved, although signs and symptoms or renal infection may not be present (Hoepelman, 1994).

Patients with diabetes often have increased complications of UTI, including rare complications such as emphysematous cystitis and pyelonephritis, fungal infections and increased severity and unusual manifestations (e.g. gram negative pathogens other than *E. coli*). Anatomical and functional abnormalities of the urinary tract are also associated with diabetes (Stapleton, 2002).

3.9 MICROBIOLOGY OF UTI IN DIABETIC PATIENTS

The bacteria causing UTIs in diabetic patients are the same as in complicated UTIs in non diabetic patients. Bacteria and yeast are the major pathogens. Enteric bacteria are common pathogens especially *E. coli* and *Klebsiella* spp. (MacFarlane *et al*, 1986). *P. aeruginosa* is found in patients with previous experience of antimicrobial therapy. *Streptococcus agalactiae* may be found in diabetic patients with poor glycemic control. Though *Staphylococcus saprophyticus* is the second most common organism grown in Western nondiabetic subjects with UTI, it is rare in India (Hoepelman, 1994). Among diabetic individuals with UTI, *Proteus* spp. and *S. aureus* account for the remaining infections (Svanborg and Godaly, 1997).

3.9.1 Etiological agents of urinary tract infection

Bacteria of only a limited number of species are able to initiate infection in the urinary tract. The causative agents are listed below:

Gram negative *E. coli, P.vulgaris, Proteus mirabilis, Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp, *Serratia* spp., *M. morganii and P. aeruginosa*

Gram positive *S. aureus, S. saprophyticus,* Group B streptococci and *E. faecalis*

Other pathogens *Chlamydia (Chlamydia trachomatis), Mycoplasma (Ureaplasma urealyticum), Candida* spp. *and Mycobacterium tuberculosis.*

(Source: Cheesbrough, 2000; Fowler, 1990)

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

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

In a study on bacteriology of urinary calculi in relation to UTI, out of 52 patients, 37.0% patients had calculi associated UTI with *E. coli* and *P. mirabilis* being the most common causative microorganisms (Nass *et al*, 2001).

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

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

Gram positive pathogens such as *E. faecalis, S. saprophyticus* and group B streptococci can also infect the urinary tract. Urinary tract infections due to *E. faecalis* are usually associated with the use of instruments or catheterization. Novobiocin resistant *S. saprophyticus* is a true primary pathogen of the urinary tract, which is responsible for 20.0% of urethritis and cystitis in sexually active but otherwise healthy young women.

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

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

3.9.2 Site of infection

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

3.9.3 Routes of infection

Infectious microorganisms can invade and spread within the urinary tract by following routes:

- **Ascending Route**

The ascending route of infection is the most important means by which bacteria from the fecal flora spread to the perineum before ascending into the bladder and subsequently urinary tract becomes infected (Stamey, 1981). The distal urethra and the vaginal introitus are normally colonized by lactobacilli, streptococci, diptheroids and staphylococci.

- **Haematogenous Route**

Haematogenous infection is much less common, but urinary infection secondary to bacteraemia may occur. 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* spp., *Listeria* spp. (Leigh, 1990). The kidney may be the site of staphylococcal abscess in patients with bacteraemia or endocarditis due to *S. aureus* (Smellie *et al,* 1975).

- **Lymphatic Route**

Direct extension of bacteria from the adjacent organ via lymphatics may occur in unusual circumstances such as a severe bowel infection or retroperitoneal abscesses. There is little evidence that lymphatic routes play a significant role in the vast majority of UTI (Schaeffer, 1998).

3.9.4 Factors predisposing to infection

There is no doubt that certain anatomic abnormalities, systemic diseases, and manipulative procedures carry an increased risk of UTI. A common abnormality is obstruction of urine flow. Some of the most common factors associated with an increase on UTI are as follows:

- Anatomical abnormalities of the urethral orifice, changes in bladder-neck function and weakness of the pelvic floor and uterine prolapse during old age increase the risk of UTI. Obstructions to the flow of urine by anatomical or pathological abnormalities are major predisposing factors at any age.

- Pregnancy appears to be associated with an increased incidence of bacteriuria. About 2.0% to 11.0% of pregnant women have asymptomatic bacteriuria and, of those, 13.0% to 27.0% will develop a kidney infection late in their term. It is possible that increased incidence is related in part to the urethral dilatation that occurs during pregnancy.

- Neurologic dysfunction predisposes to UTI because of the inability to initiate or control bladder emptying. This inability is most frequently seen in patients with spinal cord transection, spinal cord tumors, spina bifida, and diabetic neuropathy.

- Catheterization or the instrumentation of the bladder is the most common factor predisposing to the development of UTI in the hospital. Over 20.0% of hospital-acquired infections are of urinary tract and about 75.0% of these follow the use of instruments in

the urinary tract (Leigh, 1986). Indwelling bladder catheters are often used in the treatment of neurological dysfunction contribute to the increased incidence of UTI (Yasumasu *et al,* 1984).

Diabetes mellitus has a number of long term effects on the genitourinary system. These effects predispose to bacterial UTIs in the patient with diabetes mellitus. Bacteriuria is more common in diabetic women than in non diabetic women because of a combination of host and local risk factors. Upper tract infection complications are also more common in this group. Diabetic patients are at higher risk for intrarenal abscess, with a spectrum of disease ranging from acute focal bacterial pyelonephritis to renal urine contains sufficient amount which help maximum growth for bacteria (Foreland *et al,* 1977). Predisposing factors are longer duration of diabetes, macroalbuminuria, UTI in previous year, lower BMI and peripheral neuropathy and risk factors are the presence of ASB for women with type II diabetes and sexual intercourse (Geerlings *et al,* 2000).

3.9.5 Categorization of urinary tract infection

A recent categorization of UTI is most helpful clinically because it divides patient into groups based on clinical factors and their impact in morbidity and treatment.

Urinary tract infection in children and infants

Urinary tract infection are the most common bacterial childhood infection and are responsible for about 5.0% of febrile bacterial illness in children under 2 years of age (Hoberman, 1993). It can be potentially fatal in neonatal period because of associated septicemia and meningitis. At the beginning of the 20^{th} century, a mortality rate of up to 20.0% was reported among infants and neonates hospitalized for acute pyelonephritis (Gupta, 2002). In later childhood, UTI are responsible for less severe illness but in rare situation progress to chronic pyelonephritis, hypertension and end stage renal disease.

Urinary tract infection in adults

Urinary tract infection in adults include the following conditions

Acute uncomplicated cystitis in young women

Acute uncomplicated cystitis is a common cause of morbidity in women those most at risk are sexually active young women. It is estimated that between 20.0% and 50.0%

suffer from UTI at sometime (Smith and Easmon, 1990).Recurrent cystitis occurs in more than three episodes per year. About 20.0% of young women with an episode of cystitis have recurrent infection (Stamm, 1993). Occasionally, such recurrence are due to persistent focus of infection, but well over 90.0% of recurrence in young women are episodes of exogenous re-infection (Stamm and Hooton, 1993). About 95.0% of all recurrent infections in female are re-infection of urinary tract (Schaeffer, 1998).

Uncomplicated pyelonephritis

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

Complicated urinary tract infection

This is the situation where bacteria leaving residual inflammatory changes have repeatedly invaded urinary tract. Complicated UTI occurs in a patient who have functionally, metabolically or anatomically abnormal urinary tract or those caused by pathogens that are resistant to antibiotics (Schaffer, 1998). A broad range of bacteria can cause complicated infections and many are resistant to multiple antimicrobial agents (Stamm, 1998).

Asymptomatic bacteriuria

It is defined as the presence of more than 10^5 cfu/ml (i.e. significant numbers of bacteria) of voided urine in patients with no symptoms of urinary tract infection. Many urinary tract infections are asymptomatic but may lead to serious infections and it is not known whether symptomatic UTIs are preceded by asymptomatic bacteriuria (Hansen, 1964; Vejlsgaard, 1966). Severe complications of these UTIs and renal involvement, even without the presence of symptoms, are frequently seen in this patient group (Forland *et al,* 1977; Forland and Thomas, 1985; Ooi, 1974).

Symptomatic urinary tract infection

Symptomatic UTI involving the lower urinary tract is frequently termed acute cystitis and is characterized by urgency, frequency, suprapubic pressure and dysuria, and absence of systemic symptoms such as fever (Leigh, 1990). Symptomatic UTI of upper tract is

termed as acute pyelonephritis, which is a serious and common UTI, with about 250,000 acute cases occurring each year (Lyannae *et al,* 1994).

Catheter-associated urinary tract infection

Catheter-associated UTIs account for 40.0% of nosocomial infection and are the most common source of Gram negative bacterimia in hospitalized patients (Warren, 1997).

Complications of urinary tract infection in diabetes

Complications from UTI frequently seen in diabetes include acute lobar nephronia, intrarenal abscess, perinephric abscess, emphysematous cystitis, emphysematous pyelonephritis, papillary necrosis and metastatic infections.

3.9.6 Pathogenesis

Most UTIs result from the ascending route, only a minority occurs after bacteraemia. The first step in the pathogenesis is the colonization of the periurethral tissue with uropathogens. Secondly, these uropathogens may gain access to the urethra. A symptomatic or asymptomatic infection of the bladder may result. A few organisms may finally ascend the ureters to the kidneys. These entire steps take place depends on the inoculum size, the virulence properties of the invading microorganism and the defense mechanisms of the host. Micturition is the most important defense mechanism against UTIs and therefore obstruction, stasis and reflux of the urinary tract predispose to infection (Sobel and Kaye, 1984 and 1985).

Uropathogenic *E. coli* causes 90.0% of the urinary tract infections in anatomically normal, unobstructed urinary tracts. The bacteria colonize from feces or perineal region and ascend the urinary tract to the bladder. The adhesin that has been most closely associated with uropathogenic *E. coli* is the P fimbriae. The fimbriae bind not only to red cells but to a specific galactose disaccharide that is found on the surfaces uroepithelial cells in approximately 99.0% of the population.

E. coli with P fimbriae also possess the gene for Type 1 fimbriae, and there is evidence that P fimbriae are derived from Type 1 fimbriae by insertion of a new fimbrial tip protein to replace the mannose-binding domain of Type 1 fimbriae. Another factor

thought to be involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement dependent bactericidal effect of serum.

Uropathogenic strains of *E. coli* usually produce siderophores that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce hemolysins, which are cytotoxic due to formation of transmembranous pores in host cells

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

Once introduced into the urinary tract, *Proteus* strains appear to be uniquely suited to cause significant disease in the urinary tract. These strains are able to facilitate their adherence to the mucosa of the kidneys. Also, *Proteus* spp. is able to hydrolyze urea via urease production, which results in an increase in urine pH that is directly toxic to kidney cells and also stimulates the formation of kidney stones. Similar findings have been made with *Klebsiella* spp. and *S. saprophyticus* also adheres better to uroepthelial cells than do *S. aureus* and *S. epidermidis.*

Motility may be important for organisms to ascend to the upper urinary tract against the flow of urine and cause pyelonephritis (Baron and Finegold, 1990).

Urinary tract infections are very often encountered in patients with diabetes mellitus. The suggested mechanisms of an increased susceptibility to UTI are

- Decreased antibacterial activity due to 'sweet urine'
- Defects in neutrophil function
- Increased adherence to uroepithelial cells.

UTI in diabetics should be treated as complicated UTI with agents reaching high tissue levels for 10-14 days (Hoepelman, 1994). Poor control of diabetes, impaired renal function secondary to glomerulosclerosis and non-specific or specific immunity have not been shown to be substantial contributors to the increased occurrence of morbitiy of UTI

among patients with diabetes. Hyperglycemia by itself does not predictably increase bacterial rates of multiplication, (Geerlings *et al,* 1999) although neutrophil function is impaired in the presence of higher urinary or tissue glucose concentration. This in itself has not been shown to be a major determinant of either the incidence of bacteriuria or its subsequent complications (Balasoiu *et al,* 1997; Delamaire *et al,* 1997). Micturition abnormalities secondary to diabetic neuropathy occurs in 10% to 40% of patients with longstanding diabetes and increased residual urine. This presumably accounts for some of the increased morbidity as well as most of the increased susceptibility to infection (Sawers *et al,* 1986).

Tamm–Horsfall protein is an important defense as it traps type I fimbriated *E. coli* in uromucoid present on epithelial cells and prevents adherence and cell entry (Leeker *et al,* 1997). This protein is markedly reduced in some patients with diabetes (Torffvit and Agardh, 1993).

Bacterial virulence factors have been investigated in patients with bacteremia and strains causing bacteremia have more virulence markers than fecal *E. coli* strains from healthy controls (Johnson 1998).

No study has, however, compared bacteremic strains in patients with and without diabetes, and there is no evidence yet to prove that *E. coli* strains are indeed different between these populations.It is also possible that in the presence of diabetes, there is impaired transport of metabolic end products perhaps due to impaired tissue perfusion (Huang and Tseng, 2000; Yang and Shen, 1990).

Intra-renal abscesses or renal carbuncles are most commonly due to *S. aureus* with 30–50.0% of patients having concomitant diabetes.

3.9.7 Host defense mechanism

The presence of bacteria in the urinary tract does not necessarily result in infection. The size of the inoculum, virulence of the organism, and defense mechanisms inherent in the urinary tract will determine whether infection is established.

Urine itself is inhibitory to some of the urethral flora such as anaerobes. In addition, a low pH, high or low osmolality, high urea concentration, or high organic acid content of urine may inhibit even those organisms that can grow in urine.

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

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

Prostatic antibacterial factor (PAF) appears to be the most significant antimicrobial constituent of prostatic fluid (Fair *et al,* 1976). This also inhibits the growth of viruses (Fridlender *et al,* 1978), yeast (Gip and Molin, 1970), trichomonads (Krieger and Rein, 1982) and *C. trachomatis* (Mardh *et al,* 1980). Prostatic fluid is also rich in spermine, which has some activity against gram positive bacteria (Fair and Wehner, 1971). A remarkable local immune response of the prostate to bacteriuria and bacterial prostatitis has been demonstrated (Fowler and Marioano, 1982 and 1984; Shortliffe *et al,* 1981).

3.9.8 Establishment and multiplication of bacteria in urine

The bladder and urinary tract are normally sterile. The urethra however may contain a few commensals and also the perineum, which can contaminate urine when it is collected (Cheesbrough, 2000). Although urethra has a resident bacterial flora, these organisms do not commonly cause bladder infection in normal person (Leigh, 1990).

There is a dynamic culture system in the urinary tract in which bacteria undergo multiplication while urine is continuously being added by glomerular filtration and lost by micturition.

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

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

The ability of the urine to support bacterial growth is related to urinary pH, osmolality, and chemical constituents such as glucose, amino acids and organic acids. Optimal bacterial growth occurs within a pH range of 6.0-7.0. A lowered osmolality of the urine encourages bacteriuria (Kaye, 1980).

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

3.9.9 Manifestations of urinary tract infection

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

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

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

3.10 DIAGNOSIS OF URINARY TRACT INFECTION

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

3.10.1 Methods of specimen collection

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

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

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

) Clean-catch, midstream urine (CC-MSU)

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

) Straight catheterized urine specimen

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

) Suprapubic bladder aspiration

Urine is withdrawn directly into a syringe through a percutaneously inserted needle, thereby ensuring a contamination-free specimen. The bladder must be full before

performing the procedure. If good aseptic techniques are used, this procedure can be performed with little risk in premature infants, infants, small children, and pregnant women and other adults with full bladders.

- **Indwelling catheter**

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

3.10.2 Screening procedures

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

3.10.3 Macroscopic examination of urine

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

3.10.4 Microscopic examination of urine

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

Erythrocytes

Erythrocytes are found in small numbers in normal urine. In normal male and female, occasional red cells (0-2/hpf or 3-12/µL) may be seen on microscopic examination of the sediment. Under high power, unstained RBC appears as pale discs, usually 7 µm in diameter. They may become created in hypertonic urine and appear as small, rough cells with crinkly edges. Increased number of erythrocytes in the urine may be present in renal diseases like glomerulonephritis, extra renal disease like acute appendicitis, urinary

schistosomiasis, leptospirosis, infective endocarditis, malignancy of urinary tract and hemorrhagic conditions. The finding of RBC count greater than 3/hpf is considered as abnormal (Froom *et al,* 1986; Steward *et al,* 1985; Wargotz *et al,* 1987). The study done by Froom *et al* (1987) concluded that the examination of the urine sediment by hpf method is not sufficiently sensitive to be used as a screening test for the detection of UTI in asymptomatic subjects. Microscopic haematuria may be present in 40.0% to 60.0% of patients with UTI (Faro and Fenner, 1998).

Leucocytes

These are round 10 to 15 µm in diameter cells that contain granules (Cheesbrough, 2000). Normal urine contains 2-3 pus cells/hpf. Pyuria is usually regarded as significant when moderate or many pus cells are present i.e. > 10WBC/ml (Cheesbrough, 2000).

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

Pyuria is significant if more than or equal to 5 white blood cells or pus cells are seen per high power field in the sediment (Abyad, 1991; Block, 1990; Chakraborty, 2001; Merila *et al,* 1987; Steward *et al,* 1985; Wargotz *et al,* 1987). Three or more fresh leucocytes per hpf suggest infection and are rarely found in normal non-bacteriuric patients (Stamm *et al,* 1981). Pyuria with sterile routine culture may be found with renal tuberculosis, gonococcal urethritis, *C. trachomatis* infection and leptospirosis or when a patient with urinary infection has been treated with antimicrobials (Cheesbrough, 1984).

Epithelial Cells

It is normal to find few epithelial cells in urine. These cells are cuboidal in shape having small nuclei and granular cytoplasm (Fowler, 1990). When seen in large number, however, they usually indicate inflammation of the urinary tract or vaginal contamination of the specimen (Cheesbrough, 2000). Normally few cells (3-5/hpf) from genitourinary tract can be found in urine due to sloughing off of old cells (Godkar, 2001). Increased

number of tubular epithelial cells suggests tubular damage. It can occur in pyelonephritis, acute tubular necrosis, salicylate intoxication and kidney transplants rejection (Godkar, 2001). Wargotz *et al* (1987) reported that greater than or equal to five squamous epithelial cells per high power field is considered as abnormal.

3.10.5 Chemical examination of urine

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

3.10.6 Bacteriological examination of urine

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

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

) Standard Loop Method

An inoculating loop of standard dimensions is used to take up a small, approximately fixed and known volume of mixed uncentrifuged urine and inoculate on to an agar culture medium. The plate is incubated, the number of colonies is counted and this

number is used to calculate the number of viable bacteria per ml of urine. Thus, if a 0.002ml loopful of urine yields 25 colonies, then the approximate number of cfu per ml of urine will be 25 x 500 = 12,500. Such a count should be reported as 10^4-10^5 colonies/ml (Collee *et al*, 1999).

3.11 URINARY ANTISEPTICS

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

- **Sulphonamides:** The sulphonamides are extremely useful for the treatment of uncomplicated urinary tract infection caused by *E. coli* in domiciliary practice. *S. pneumoniae*, ß-hemolytic streptococci and *P. mirabilis* are almost always sensitive to the sulphonamide; those almost always resistant include *E. faecalis*, *P. aeruginosa*, indole-positive *Proteus* spp. and *Klebsiella* spp. Trimethoprim-sulfomethaxole (TMP-SMX) has been the standard therapy for UTI, however, *E. coli* is becoming increasingly resistant to medication. Pregnant women should avoid TMP-SMX because of fetal hepatotoxicity (Simon *et al*, 2000).

- **Nitrofuran compounds:** Nitrofurantoin is active against most members of *Enterobacteriaceae*, but not against *Pseudomonas* spp. It may cause nausea and gastrointestinal distress. It is most active at acid pH.

- **4-quinolone antibacterials:** Fluoroquinolones have become popular treatments for patients with uncomplicated UTI because of *E. coli*'s emerging resistance to other common medications. Nalidixic acid is active against several different types of Gram negative bacteria, whereas Gram-positive organisms are resistant. However, the new Fluoroquinolone derivatives show superior activity against *Enterobacteriaceae* and *P. aeruginosa*, and their spectrum includes *staphylococci* but not *streptococci.* Extensive studies with Norfloxacin have demonstrated as a drug with a promising future in the treatment of urinary infections. The Infectious Diseases Society of

America (IDSA) guidelines recommend the use of Fluoroquinolones (e.g. Ciprofloxacin, Norfloxacin and Ofloxacin) as first-line agents in communities with greater than 10.0% to 20.0% resistance rates to TMP-SMX (Naber, 2000).

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

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

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

3.11.1 Bacterial resistance to antimicrobial agents

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

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 the six commonly prescribed drugs (Tuladhar *et al*, 2001).

The need for treatment of asymptomatic bacteriuria remains controversial in diabetic patients. Because of the frequent (asymptomatic) upper tract involvement and the possible serious complications, many experts recommend a 7 to14 day oral antibacterial regimen for bacterial cystitis in these patients, with an antibacterial agent that achieves high concentrations both in the urine and in urinary tract tissues (Meiland *et al*, 2003).

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

In recent years resistance to first-line antibiotics such as Ampicillin, Tetracyclines, Chloramphenicol and Sulphonamides has been increasing.Most drug resistance in enteric bacteria is attributable to the widespread transmission of resistance plasmids. The abundant use of antimicrobial drugs favors the persistence and growth of drug-resistant bacteria, including *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp. *and Serratia* spp. (Brooks *et al*, 2004).

Emergence of Methicillin resistance in *S. aureus* appears to be due to the presence of a penicillin binding protein termed PBP2a or PBP2' which has a reduced affinity for

Methicillin, the gene responsible is the mecA gene. Today MRSA is resistant to all ß-lactam antibiotics and some strains are resistant to Erythromycin, Fusidic acid, Tetracycline, Streptomycin, Sulphonamides and other disinfectants.

All bacterial resistance strategies are encoded by one or more genes, and these resistance genes are readily shared between strains of the same species, between species of different genera, and even between more distantly related bacteria. Therefore, resistance may spread to a wide variety of bacteria, and any single organism can acquire multiple genes and become resistant to the full spectrum of available antimicrobial agents (Hugo and Russell, 1993).

3.11.2 Predisposing factors

The predisposing factors for the development of bacterial resistance to antimicrobial agents include:

- Wrong selection of drugs and inappropriate use of antimicrobial when the drugs are taken in wrong dosages and for an insufficient length of time.
- Inadequate control measures in the use of chemotherapeutic agents in hospitals inadvertently give rise to the development of resistance to various drugs by bacteria.
- Frequent prophylactic use of antimicrobials may also enhance the development of bacterial drug resistance. This is not right for the broad-spectrum antibiotics (Hugo and Russell, 1993).

3.11.3 Types of drug resistance

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

Natural drug resistance is an innate property of the bacterium and is unrelated to the previous exposure to the drug. An entire bacterial species may be resistant to an antibiotic even before the introduction of the drug. For e.g. *S. pyogenes* is resistant to Polymyxins and *P. mirabilis* is resistant to Colistin because of lack of penetration of the drug through the cell wall, lack of suitable cell wall or other target receptors that may have existed before the introduction of the drug which may be lethal to the drug (Hugo and Russell, 1993).

Acquired drug resistance may be attained by bacteria by two ways

Mutation: Bacterial resistance may develop as a result of spontaneous mutation. Resistance of *M. tuberculosis* to Streptomycin develops by mutation (Hugo and Russell, 1993).

Gene transfer: Transfer of genetic material from one organism to another is a cause of resistance. This resistance is transferred from one bacterium to another of the same species or between different species, and sometimes, even between related genera (Hugo and Russell, 1993).

Most of the acquired drug resistance of *S. aureus* and Gram negative bacilli are R factor or plasmid mediated and achieved by conjugation. Plasmid mediated resistance in *S. aureus* is achieved by transduction. Secondly, the plasmids in *S. aureus* usually code for resistance to one, two or more antibiotics at a time (Hugo and Russell, 1993).

Genetic transfer of antimicrobial resistance can also take place through transposons. For e.g.TEM-1 controls the production of ß-lactamase in some strains of *E. coli, Klebsiella* spp., *H. influenzae* and *N. gonorrhoeae* (Hugo and Russell, 1993).

3.11.4 Mechanisms of antimicrobial resistance

(i) Bacteria alter the permeability of their cell membrane, so that it is difficult for an antimicrobial to enter the bacterial cell. For example as in *P. aeruginosa.*

(ii) Bacteria change the proteins and other components which are used by antimicrobials as binding sites and sometimes divert the biochemical pathway to avoid the action of antibiotic. For e.g. Resistance to Erythromycin, Sulphonamides and Amino glycosides.

(iii) Bacteria produce enzymes which inactivate the drug. The genes are carried on plasmids, e.g., *S. aureus* and *H. influenzae* producing ß-lactamase to destroy Penicillin.

3.12 ANTIMICROBIAL SUSCEPTIBILITY TESTING

The primary goal of antimicrobial susceptibility testing is to determine whether the bacterial etiology of concern is capable of expressing resistance to the antimicrobial agents that are potential choices as therapeutic agents for managing the infection. According to Greenwood (2000), since therapy of infection begins before laboratory results are available, antibiotic susceptibility testing primarily plays a supplementary role in confirming that the organism is susceptible to the agent that is being used.

Antimicrobial susceptibility testing is an *in vitro* method for estimating the activity of drugs which will assist clinician in selecting an antimicrobial effective in inhibiting the growth of an infecting microorganism in vivo. As antibiotics are concentrated in urine to higher levels than are found in the tissues, high-content test discs should be used.

WHO recommended modified Kirby-Bauer disc diffusion technique is used by most laboratories to test routinely for antimicrobial susceptibility. Using this test, antimicrobial resistance is detected by allowing the antibiotics to diffuse from a point source, commonly in the form of an impregnated filter paper disc, into an agar medium that has been seeded with the test organism. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level for the test strain (Collee *et al,* 1999). Following incubation, the diameter of the zone of inhibition around each disc is measured in millimeters.

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 MATERIALS

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

4.2 METHODS

The study was conducted in "OM" Hospital and Research Center, Chabahil, Kathmandu from March 24, 2006 to June 13, 2006 in joint collaboration with Central Department of Microbiology, Tribhuvan University, Kirtipur.

One hundred blood and mid-stream urine samples each from diabetic patients and same number of samples from nondiabetic clinically UTI suspected patients were examined by biochemical test of blood sample and routine examination, culture and antibiotic susceptibility tests of urine sample.

4.2.1 Blood sample

4.2.1.1 Specimen collection

Blood was collected twice from same patient, one sample for estimation of fasting blood sugar level and the other for post prandial blood sugar level.

- Using a tourniquet, a suitable vein was located in the arm.
- Wearing gloves, the venepuncture site was thoroughly disinfected using 70% ethanol; the area about 5 cm diameters was cleansed and allowed to dry.
- Using a sterile syringe and needle, about 1-2 ml of blood was withdrawn from a patient. The blood was kept in test tube labeled with the name and number of the patient and the date and time of collection.
- The tube was allowed to stand in water bath at 37°C for 10 minutes which caused blood to clot.
- The clotted blood was centrifuged at 4500 rpm for 10 minutes for separation of serum.

4.2.1.2 Detection of blood sugar

Blood sugar was detected by enzymatic method using standard kit and standard protocol as provided by the manufacturer. The detailed procedure is mentioned in Appendix V

4.2.2 Urine sample

4.2.2.1 Specimen Collection

The patient was given a sterile, dry, wide-necked leak-proof container for collection of 10-20 ml of first morning clean-catch mid-stream urine. The patient was given instructions for the collection of CC-MSU. The detailed procedure is mentioned in Appendix IV. The container labeled with date, name and number of the patient and the time of collection was delivered to the laboratory along with the request form as soon as possible.

4.2.2.2 Macroscopic examination

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

4.2.2.3 Microscopic examination

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

4.2.2.4 Chemical examination

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

4.2.2.5 Culture of specimen

The urine sample was cultured onto the MacConkey agar and Blood agar medium by the semi-quantitative culture technique using a standard loop. The method used for loop standardization is given in the Appendix III.

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

The bacterial count was reported as

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

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

4.2.2.6 Identification of the isolates

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

Each of the organisms was isolated in pure form before performing biochemical and other tests. Gram staining of an isolated colony was done from primary culture. For

gram negative organism a speck of single isolated colony from 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 tests and antibiotic susceptibility test.

The Gram- staining procedure is mentioned in the appendix III.

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

Gram-positive organisms were identified primarily on the basis of their response to gram's staining, catalase, 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 test are mentioned in the appendix II. The procedure for performing biochemical tests are mentioned in appendix VI.

4.2.3 Antibiotic susceptibility testing

The antibiotic sensitivity testing was performed according to the recommended Kirby-Bauer sensitivity testing method (NCCLS,1999).

- Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer.
- The pH of the medium 7.2-7.4 and the depth of the medium at 4 mm (about 25 ml per plate) were maintained in petri dish.

- Using a sterile wire loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into a nutrient broth tube and was incubated for 2-4 hrs.
- After incubation in a good light source, the turbidity of the suspension was matched with the turbidity standard of MacFarland 0.5.
- Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture technique.
- Using sterile forceps, appropriate antimicrobial discs (6 mm diameter) was placed, evenly distributed on the inoculated plates, not more than 6 discs were placed on a 90 mm diameter Petri plate.
- Within 30 minutes of applying the discs, the plates were taken for incubation at 35^0C for 16-18 hrs.
- After overnight incubation, the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured and results interpreted.

4.2.4 Purity plate

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

4.2.5 Quality control for test

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

CHAPTER -V

5. RESULTS

5.1 CLINICAL PATTERN OF RESULTS

5.1.1 Pattern of patient requesting for urine culture

Out of 100 diabetic patients, 52 were female, while 48 were male. Where as out of 100 non diabetic patients, 77 were female and 23 were male. The results are shown in table 1 and figure 2.

Table 1 Pattern of patients requesting for urine culture

	Male		**Female**		**Total**
	No.	**%**	**No.**	**%**	
Diabetic patient	48	48.0	52	52.0	100
Nondiabetic patient	23	23.0	77	77.0	100
Total	71		129		200

5.1.2 Age and gender wise distribution of patients requesting for urine culture

In case of diabetic patients age group 51-60 years had the maximum number of request i.e. 32 (32.0%) for urine culture followed by age group 41-50 with 26 requests. Age group of below 10 years requested the least with only 1 sample. Similarly in case of non diabetic patients age group 31-40 years had the maximum request of 27 (27.0%) for urine culture, while age group 21-30 followed with 20 requests. only 1 request was from age group of above 80 years. In the age groups mentioned above female requests were more than male. The results are shown in table 2 and figure 3.

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

Age group	Diabetic Patients			Nondiabetic Patients			TOTAL (%)
	Male	Female	Significant growth (%)	Male	Female	Significant growth (%)	
0-10	0	0	0 (0.0)	1	2	1(8.3)	3 (1.5)
11-20	0	1	1 (4.5)	3	4	0(0.0)	8 (4.0)
21-30	0	5	1 (4.5)	2	18	2(16.6)	25 (12.5)
31-40	8	6	2 (9.0)	4	23	2(16.6)	41 (20.5)
41-50	12	14	4 (18.1)	3	16	1(8.3)	45 (22.5)
51-60	14	18	7 (31.8)	6	9	3(25.0)	47 (23.5)
61-70	7	7	6 (27.2)	2	2	0(0.0)	18 (9.0)
71-80	6	1	1 (4.5)	2	1	1(8.3)	10 (5.0)
81-90	1	0	0 (0.0)	0	2	2(16.6)	3 (1.5)
TOTAL	**48**	**52**	**22**	**23**	**77**	**12**	**200**

5.1.3 Growth pattern of bacteria in urine samples

Out of 100 diabetic samples, 22 samples showed significant growth, giving 22 bacterial isolates, whereas majority of samples i.e. 66 (66.0%) showed no growth, 8 showed no significant growth and out of total samples 4 showed mixed growths. Similarly among 100 nondiabetic samples 12 samples showed significant growth, where as 85 samples showed no growth, 2 samples showed no significant growth and 1 sample showed mixed growth. The results are shown in table 3 and figure 4.

Table 3 Growth pattern of bacteria in urine sample

Specimen	Total no. of samples		Significant growth		No significant growth		Mixed growth		No growth	
			No.	%	No.	%	No.	%	No.	%
Urine	Diabetic patient	100	22	22.0	8	8.0	4	4.0	66	66.0
	Non diabetic patient	100	12	12.0	2	2.0	1	1.0	85	85.0

5.1.4 Pattern of genderwise significant bacterial growth from urine samples

In case of diabetic patients among total of 22 isolates, 13 (59.5%) were from females and 9 (40.9%) were from males. Where as from control patients, among 12 isolates, 10 (83.3%) were from female and 2 (16.6%) from male. The results are shown in table 4.

Table 4 Pattern of genderwise significant bacterial growth from urine sample

Sample	Male	Female	Total
	Isolates (%)	Isolates (%)	
Diabetic patient	9 (40.9)	13 (59.5)	22
Non diabetic patients	2 (16.6)	10 (83.3)	12

5.2 BIOCHEMICAL PATTERN OF THE RESULTS

5.2.1 Blood sugar level verses significant growth pattern of the isolates in urine samples of diabetic patients

In case of diabetic patients the fasting blood sugar level ranged form 6.6 mmol/L to 16.5 mmol/L and the post prandial blood sugar level was found from 10.1 mmol/L to 20.5 mmol/L in culture positive patients. The results are shown in table 5.

Table 5 Blood sugar level (mmol/L) verses significant growth pattern of isolates in urine samples of diabetic patients

Fasting blood sugar level (mmol/L)	Total no. of patients (N= 100)	Sugar in urine	No. of Positive growth
6.0-6.9	21	N=18, T=1, ≥+=2	2
7.0-7.9	18	N=10, T=3, ≥+=5	2
8.0-8.9	18	N=6, T=0, ≥+=12	5
9.0-9.9	11	N=3, T=4, ≥+=4	4
10.0-10.9	14	N=5, T=0, ≥+=9	8
11.0-11.9	6	N=0, T=1, ≥+=5	0
12.0-12.9	1	N=0, T=0, ≥+=1	0
13.0-13.9	4	N=0, T=0, ≥+=4	1
14.0-14.9	3	N=0, T=0, ≥+=3	0
15.0-15.9	3	N=0, T=0, ≥+=3	0
16.0-17.0	1	N=0, T=0, ≥+=1	0

The result showed that with increased fasting blood sugar level the number of positive growth in urine increased up to 10.0 – 10.9 mmol/L then the number of positive growth in urine decreased sharply even after increased blood sugar level.

5.2.2 Correlation of albuminuria with culture result

Out of total samples, 21.0% (21/100) samples were positive for albumin test, among them 61.9% (13/21) showed significant growth on culture. While 79.0% of samples showed negative albumin test, and among them, 11.3% (9/79) gave positive culture result in diabetic patients. Similarly 15.0% (15/100) samples were positive for albumin test, among them 53.3% (8/15) showed significant growth on culture. While 85.0% of samples showed negative albumin test, and among them, 4.7% (4/85) gave positive culture result in non diabetic patients. The results are shown in table 6 and figure 5.

Table 6 Correlation of albuminuria with culture result in diabetic patients

Albumin test	Culture positive (%)	Culture negative (%)	Total (%)
Positive (≥1+)	13 (61.9)	8 (38.0)	21 (21.0)
Negative (<1+)	9 (11.3)	70 (88.6)	79 (79.0)
Total	**22 (22.0)**	**78 (78.0)**	**100 (100.0)**

5.2.3 Correlation of glycosuria with culture result

Out of total 100 samples, 57.0% (21/100) samples were positive for sugar test, among them 29.2% (17/57) showed significant growth on culture. While 43.0% of samples showed negative sugar test, and among them, 11.6% (5/43) gave positive culture result in diabetic patients. All the samples gave negative sugar test in case of nondiabetic patients. The results are shown in table 7.

Table 7 Correlation of glycosuria with culture result in diabetic patients

Sugar test	Culture positive (%)	Culture negative (%)	Total (%)
Positive (≥1+)	17 (29.8)	40(70.1)	57(57.0)
Negative (<1+)	5 (11.6)	38(88.3)	43 (43.0)
Total	**22 (22.0)**	**78 (78.0)**	**100 (100.0)**

5.3 MICROSCOPIC OBSERVATION OF URINE

5.3.1 Microscopic observation of pus cells against the culture result

Among the total 100 diabetic samples, 18 (18.0%) showed significant pyuria (>5 WBC/hpf), and among these 72.2% (13/18) gave positive culture results. Similarly 82 (82.0%) of total samples showed insignificant or no pyuria, and among these 10.9% (9/82) samples gave positive culture results and 89.0% (73/82) showed culture result negative. Similarly among the total 100 nondiabetic samples, 11 (11.0%) showed significant pyuria and among these 72.7% (8/11) gave positive culture results. Similarly 89 (89.0%) of total samples showed insignificant or no pyuria, and among these 4.4% (4/89) samples gave positive culture results and 95.5% (85/89) showed culture result negative. The results are shown in table 8 and figure 6.

Table 8 Microscopic observation of pus cells against the culture result in diabetic patients

Pyuria	Culture positive (%)	Culture negative (%)	Total (%)
Significant (>5/hpf)	13 (72.2)	5 (27.7)	18 (18.0)
Insignificant (<5/hpf)	9 (10.9)	73 (89.0)	82(82.0)
Total	**22 (22.0)**	**78 (78.0)**	**100(100.0)**

5.3.2 Microscopic observation of RBC against the culture result

Among the 100 diabetic samples, 2.0% of samples showed significant haematuria (>3RBC/hpf), however among these 0.0% of samples gave positive culture results. Similarly 98.0% of total samples showed insignificant haematuria and among these 22.4% samples gave positive culture results. Similarly in case of 100 nondiabetic samples 3.0% of samples showed significant haematuria (>3RBC/hpf), however among these 66.6% of samples gave positive culture results. Similarly 97.0% of total samples showed insignificant haematuria and among these, 10.3% samples gave positive culture results. The results are shown in table 9 and figure 7.

Table 9 Microscopic observation of RBC against the culture result in diabetic patients

Haematuria	Culture positive (%)	Culture negative (%)	Total (%)
Significant (>3/hpf)	0 (0.0)	2 (100.0)	2 (2.0)
Insignificant (<3/hpf)	22 (22.4)	76 (77.5)	98 (98.0)
Total	**22 (22.0)**	**78 (78.0)**	**100 (100.0)**

5.3.2 Microscopic observation of epithelial cells against the culture result

Out of the total 100 diabetic samples, 89.0% (89/100) samples showed insignificant epithelial cells, of which 19.1% (17/89) samples showed positive culture. Similarly out of total, 11.0% (11/100) samples showed significant epithelial cells (≥5 epithelial cells/hpf) and among them 45.4% (5/100) samples showed positive culture results. Similarly out of 100 nondiabetic samples 85 (85.0%) samples showed insignificant epithelial cells, among them 11.7% (10/85) samples showed positive culture. Similarly out of total, 15.0% samples showed significant epithelial cells with 13.3% (2/100) samples positive culture results. The results are shown in table 10.

Table 10 Microscopic observation of epithelial cells against the culture result in non diabetic patients

Epithelial cells	**Culture positive (%)**	**Culture negative (%)**	**Total (%)**
Significant (≥5/hpf)	2 (13.3)	13 (86.6)	15 (15.0)
Insignificant (≤5/hpf)	10 (11.7)	75 (88.2)	85 (85.0)
Total	**12 (12.0)**	**88(88.0)**	**100 (100.0)**

5.4 MICROBIOLOGICAL PATTERN OF RESULTS

5.4.1 Pattern of bacteria isolated from culture positive urine samples according to Gram's stain

Out of the total 22 isolates in diabetic patients, maximum isolates 18 (81.8%) were found to be Gram negative bacilli and the remaining 4 (18.1%) were found to be Gram positive cocci. Similarly in case of nondiabetic patients all the isolates were found to be Gram

negative bacilli and none of the isolates were Gram positive. The results are shown in table 11 and figure 8.

Table 11 Pattern of bacteria isolated from culture positive urine samples according to Gram's stain

Isolated organism	Diabetic patients		Nondiabetic patients	
	No. of isolates	**% of isolates**	**No. of isolates**	**% of isolates**
Gram negative bacteria	18	81.8	12	100.0
Gram positive bacteria	4	18.1	0	0.0
Total	22	100.0	12	100.0

5.4.2 Pattern of microbial isolates from urine sample

A total of 22 organisms were isolated from growth positive urine samples of diabetic patients and 12 organisms were isolated from nondiabetic control.Among the isolates Gram negative organisms were predominant in both the cases. In nondiabetic patients Gram positive organisms were not isolated and in diabetic patients *S. aureus* was the only Gram positive species isolated.

Among the isolates, *E. coli* was found to be the most predominant in both groups i.e. 9 (40.9%) in diabetic and 10 (83.3%) in nondiabetic patients. The results are shown in tables 12 and figures 9 and 10.

Table 12 Pattern of different species of bacteria isolated from infected urine of diabetic and nondiabetic patients

Types of organism	**Diabetic patients**		**Nondiabetic patients**	
Gram negative bacteria	**No. of organism**	**% of organism**	**No. of organism**	**% of organism**
E.coli	9	40.9	10	83.3
C. freundii	6	27.2	-	-
K. pneumoniae	1	4.5	1	8.3
P. vulgaris	1	4.5	1	8.3
P. aerogenosa	1	4.5	-	-
Gram positive bacteria				
S. aureus	4	18.1	-	-
Total	**22**	**100.0**	**12**	**100.0**

5.5 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE ISOLATES

5.5.1 Antibiotic susceptibility pattern of Gram positive isolates

Among the 4 Gram positive isolates from diabetic patients, most of them i.e. 3 (75.0%) were susceptible to Erythromycin followed by Ofloxacin, Norfloxacin and Cotrimoxazole with the susceptibility of 50.0% for all three drugs. Amoxycillin, Ceftriazone and Cloxacillin were found to be the least effective as only 1 (25.0%) isolates were sensitive to the drugs. None of the gram positive organisms were isolated from nondiabetic patients. The results are shown in table 13 and figure 11.

Table 13 Antibiotic susceptibility pattern of gram positive bacteria isolated from urine samples of diabetic patients.

Antibiotic used	Sensitive		Moderately sensitive		Resistant		Total
	n	%	n	%	n	%	
Amoxycillin	1	25.0	0	0.0	3	75.0	4
Ceftriazone	1	25.0	1	0.0	2	50.0	4
Cotrimoxazole	2	50.0	0	0.0	2	50.0	4
Cloxacillin	1	25.0	0	0.0	3	75.0	4
Erythromycin	**3**	**75.0**	0	0.0	1	25.0	4
Ofloxacin	2	50.0	0	0.0	2	50.0	4
Norfloxacin	2	50.0	0	0.0	2	50.0	4

5.5.2 Antibiotic susceptibility pattern of Gram negative isolates

Among the common antibiotics used against all Gram negative isolates, Nitrofurantoin was the drug of choice as 11 (61.1%) isolates from diabetic patients and 10 (83.3%) from nondiabetic patients were found to be susceptible to the drug followed by Ofloxacin with a susceptibility of 50.0% and 41.6% in diabetic and non diabetic patients respectively. Most of the Gram negative isolates, i.e. 14 (77.7%) in diabetic and 10 (83.3%) in nondiabetic patients were resistant to Nalidixic acid respectively. The results are shown in tables 14 and 15 and figure 12.

Table 14 Antibiotic sensitivity pattern against gram negative bacteria isolated from urine samples of diabetic patients

Antibiotic used	Sensitive		Moderately sensitive		Resistant		Total
	n	%	n	%	n	%	
Amoxycillin	5	27.7	3	16.6	10	55.5	18
Ceftriazone	6	33.3	2	11.1	10	55.5	18
Cotrimoxazole	4	22.2	3	16.6	11	61.1	18
Ofloxacin	9	50.0	1	5.5	8	44.4	18
Nalidixic Acid	4	22.2	0	0.0	14	77.7	18
Nitrofurantoin	**11**	**61.1**	2	11.1	5	27.7	18
Norfloxacin	3	16.6	0	0.0	15	40.2	18

Table 15 Antibiotic susceptibility pattern against gram negative bacteria isolated from urine samples of nondiabetic patients

Antibiotic used	Sensitive		Moderately sensitive		Resistant		Total
	n	%	n	%	n	%	
Amoxycillin	3	25.0	1	8.3	8	66.6	12
Ceftriazone	4	33.3	2	16.6	6	50.0	12
Cotrimoxazole	3	25.0	1	8.3	8	66.6	12
Ofloxacin	5	41.6	2	16.6	5	41.6	12
Nalidixic Acid	2	16.6	0	0.0	10	83.3	12
Nitrofurantoin	**10**	**83.3**	2	16.6	0	0.0	12
Norfloxacin	3	25.0	0	0.0	9	75.0	12

5.6 ANTIBIOTIC RESISTANCE PATTERN OF THE ISOLATES

5.6.1 Distribution of MDR pathogens in diabetic and nondiabetic patients

Among the 19 *E. coli* isolates, 9 (47.3%) were from diabetic patients, of which 8 (88.8%) isolates were MDR-strains 10 (52.6%) were from nondiabetic patients, of which 7 (70.0%) isolates were MDR- strains. Similarly out of 100.0% of *C. freundii* and *P.*

aeruginosa all were the MDR-strains and isolated form diabetic patients. Out of 4 (100.0%) isolates of *S. aureus* came from diabetic patients and among them 75.0% were the MDR-strains. The results are shown in table 16 and figures 13.

Table 16 Distribution of MDR pathogens in diabetic and nondiabetic patients

Organisms	**Cases from diabetic patients**		**Cases from non diabetic patients**		**Total**
	Frequency	**%**	**Frequency**	**%**	
E.coli	9 (8)	88.88	10 (7)	70.00	19 (15)
C. freundii	6 (6)	100.00	0 (0)	0.00	6 (6)
K. pneumoniae	1 (1)	100.00	1 (1)	100.00	2 (2)
P. vulgaris	1 (1)	100.00	1 (1)	100.00	2 (2)
P. aeruginosa	1 (1)	100.00	0 (0)	0.00	1 (1)
S. aureus	4 (3)	75.00	0 (0)	0.00	4 (3)
Total	**22 (20)**	**90.90**	**12 (9)**	**75.00**	**34(29)**

Note Figures in parentheses indicate the number of MDR-strains isolated for that species.

5.6.2 Antibiotic resistance pattern of the isolates from urine sample

Out of the 22 isolates from diabetic samples, 20 (90.9%) isolates were resistant to >3 drugs. 88.8 % *E. coli* isolated and 75.0% of *S. aureus* isolated were found to be MDR strains. All the isolates (100%) of C. *freundii*, *K. pneumoniae, P. aeruginosa* and *P. vulgaris* were found to be MDR-strains. Similarly Out of the 12 isolates from non diabetic samples, 9 (75.0%) isolates were resistant to >3 drugs. 70.0 % *E. coli* isolated were found to be MDR strains. The results are shown in tables 19 and 20.

Table 19 Antibiotic resistance pattern of isolates from urine samples of diabetic patients

Organisms isolated	Total isolate	Resistance to					
					MDR Strains		
		0 Drug	1 Drug	2 Drug	3 Drugs	>3 Drugs	Total (%)
E. coli	9	0	1	0	1	7	8(88.8)
C. freundii	6	0	0	0	0	6	6(100.0)
K. pneumoniae	1	0	0	0	0	1	1(100.0)
P. vulgaris	1	0	0	0	0	1	1(100.0)
P. aerogenosa	1	0	0	0	0	1	1(100.0)
S. aureus	4	0	1	0	1	2	3(75.0)
Total	**22**	**0**	**2**	**0**	**2**	**18**	**20(90.9)**

Table 20 Antibiotic resistance pattern of the isolates from urine sample of nondiabetic patients

Organism isolated	Total isolate	Resistance to					
					MDR Strains		
		0 Drug	1 Drug	2 Drugs	3 Drugs	>3 Drugs	Total (%)
E. coli	10	2	0	1	0	7	7(70.0)
K. pneumoniae	1	0	0	0	0	1	1(100.0)
P. vulgaris	1	0	0	0	0	1	1(100.0)
Total	**12**	**2**	**0**	**1**	**0**	**9**	**9(75.0)**

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6. 1 DISCUSSION

This study was conducted among diabetic and non diabetic patients suspected of urinary tract infection, attending "OM" Hospital and Research Centre, Kathmandu, Nepal. Two hundred blood samples and two hundred mid stream urine samples were collected and subjected to biochemical analysis and routine examination and then processed for culture and sensitivity from patients visiting the hospital. The results obtained were tabulated in the previous chapter. In this chapter, the results are discussed and compared with the findings of other investigators.

Altogether, 200 samples from outdoor patients were included in this work, of which 100 were from diabetic patients taken as test sample and remaining 100 were from non diabetic patients taken as control samples. The fasting glucose level ranged from 6.6 mmol/L to 16.5 mmol/L(Normal range 3.5-6.5mmol/L, WHO, 2002) and the post prandial blood sugar level was found from 10.1mmol/L to 26.7mmol/L(Normal range <10.1 mmol/L, WHO, 2002). Where as the random blood sugar was found to ranged from 3.5 to 9.0mmol/L in case of control (Normal range <10.1 mmol/L, WHO, 2002). Out of 100 test samples, 22 samples showed significant growth, giving 22 bacterial isolates, whereas majority of samples i.e. 66 (66.0%) showed no growth, 8 showed no significant growth and out of total samples 4 showed mixed growths. Similarly among 100 control samples, 12 samples showed significant growth, where as 85 samples showed no growth, 2 samples showed no significant growth and 1 sample showed mixed growth.

The prevalence of UTI in diabetes mellitus was higher, when compared to that in controls but the result showed no significant difference between positive growth in diabetic and nondiabetic patients (22.0% vs 12.0%, P>0.05). But similar study carried out by Gautam *et al* (2003) showed significant growth (19.0% vs 5.0%, p<0.05) between diabetic and nondiabetic patients. Similar type of result, 9.0% growth in diabetic patients vs 0.7% in

nondiabetic patients, P=0.005 was obtained in study done by Goswami *et al* (2001) and Ronald *et al* (2001). In a study by Mendoza *et al* (2002) microbial growth was in 40% of samples from diabetic women and 6% of samples from controls ($p < 0.01$). The reason behind obtaining such result in my study may be due to the fact that only 100 samples were included for each group; if sample size had been increased then a significant association could have been established between the two groups. The next reason may be that the patients visiting "OM" Hospital and Research Centre were aware of the possibility of getting infection in case of diabetes and they had improved their sanitary condition. There is a significant association between the hygiene status and urinary tract infection. This result gets its support from the similar result obtained by Bonadio *et al* (1999). In his study he found no significant differences in epidemiological, clinical and microbiological evaluated features of diabetics and nondiabetics, except for the higher frequency of bladder catheterization of diabetics than nondiabetics.

In this study 59.5% (13/22) and 83.3% (10/12) females with diabetes had asymptomatic bacteriuria in test and control respectively, whereas it was found to be 40.9% (9/22) and 16.6% (2/12) in males in test and control respectively. This higher number of requests and higher growth positivity seen in females was found to be statistically significant ($P<0.05$) and is attributed to their anatomical structure (short urethra and proximity to anal orifice) leading to easy access for coliform bacilli. This is comparable with studies by Boroumand *et al* (2005) (10.9%); Kayima *et al* (1996) (11.2%); Zhanel *et al* (1995) (7.9%). But prevalence of ASB in diabetic women was reported 26.0% in Geerlings *et al*'s study (2000) and 26.6% in Alebiosu *et al*'s report (2003). This result also confirms and expands the previous findings of Akbar, (2001); Chhetri *et al* (2001); Jha and Yadav (1992); Mendoza *et al* (2002); Patterson *et al* (1995); Rajbhandari and Shrestha (2002); Steensberg *et al* (1969).

The majority of the patients belonged to age group 51-60 in case of diabetic patients and 31-40 in case of non diabetic patients, and represented 32.0% and 27.0% respectively of the total requests. The high-infected females also belonged to the same group. The study is since focused on diabetic patients; the majority of patients being included in this work

are of higher age group because diabetes is mostly seen in person above 40 years of age. Among males highest growth positivity was found among age group of 51-80 years. This indicated that diabetes and bacteriuria are quite common in later stage of life. This finding is in agreement with the results of studies done by Kosakai *et al,* (1990); Rajbhandari and Shrestha (2002).

Among the total 22 bacterial isolates from test, 18 (81.8%) were Gram negative rods and the remaining 4 (18.1%) were found to be Gram positive cocci. Similarly among the total 12 isolates form control all the isolates were Gram negative rods and none were Gram positive cocci In a study by Okada *et al* (1994) 70.2% of the isolates were found to be Gram negative rods and 29.8% of the isolates were Gram positive cocci.

Altogether 6 species vs 3 species of bacteria were isolated from test and control in this study. Among the culture positive cases, *E. coli* was the most predominant isolate accounting for 40.90% (9/22) vs 83.3% (9/22) of total bacterial isolates. In test *E.coli* were followed by *C. freundii* and *S. aureus* both accounting for 27.2% (6/22) and 18.1% (4/22) of total isolates respectively. Other bacterial isolates constituted *P. aeruginosa, P. vulgaris and K. pneumoniae accounting* for 4.5% of total isolates. All the species isolated from control in this study were gram negative species and none of the isolates were gram positive. *E.coli* was followed by *P. aeruginosa* and *P. vulgaris* accounting for 8.3% of total isolates. This result is supported by similar findings by Goswami *et al* (2001).

Higher prevalence of *E. coli* seen in this study resembled the study done by various other workers viz: Akbar (2001); Chhetri *et al* (2001); Dhakal *et al* (2002) Kosakai *et al* (1990); Jha and Yadav (1992); Manandhar (1995); Mendoza *et al* (2002); Mohammad *et al* (2005); Rajbhandari and Shrestha (2002); Steensberg *et al* (1969); Tuladhar *et al* (1989) . *E coli* was the most frequently isolated strain, in 55.0% of patients and 100.0% of controls. *K. pneumoniae* was isolated in 10.0% of diabetics, coagulase negative *Staphylococcus* in 10%, *Enterococcus* spp. in 10.0% and *P. aeruginosa* in 5.0% in a study by Mendoza *et al* (2002).

C. freundii was the second principal isolate among Gram negative bacilli constituting 27.2% of the total bacterial isolates. In a similar study carried out by Shrestha *et al*

(2004) this species represented 3.8% of the total isolates. However this finding is not well supported by other studies because *C. freundii* has been isolated as etiological agent of UTI in very few cases in other studies. In many studies carried out by various workers, it has been established that more than 80.0% of the urinary isolates belong to *Enterobacteriaceae* family, and as *C. freundii* also belongs to this family, so it would not be much surprising to isolate this organism as the second most common isolate in this study. In this study, 81.8% (18/22) of total bacterial isolates were found to belong to the *Enterobacteriaceae* family.

Two isolates each of *P. aeruginosa* were isolated in this study from both groups. Similar study by Bonadio *et al* (1999) had found 8.5% vs 17.2% of *P. aeruginosa* in diabetic and nondiabetic patients. This bacterium plays an important role in bladder carcinogenesis (Kaji, 1994), and is considered as primary pathogen in compromised hosts (Dolan *et al,* 1989), hospitalized patients (Lohr *et al,* 1989) and in complicated urinary tract infection (Kosakai *et al,* 1990).

Among the Gram positive isolates, *S. aureus* was found to be the only isolate with 18.1% of the total isolates in test patients. Presence of this organism in urine often indicates pyelonephritis acquired via hematogenous spread, so a pure culture of *S. aureus* is considered to be significant regardless of number of cfu (Forbes *et al,* 2002). A similar study by Goswami *et al* (2001) had isolated 21.4% *S. aureus*. Asymptomatic bacteriuria is several-fold more common among women and acute plyelonephritis is five to ten times more common in both sexes.

Similarly in a study conducted by Dhakal *et al* (1999) *E. coli* (47.3%) was the commonest isolate followed by *Klebsiella* spp. (13.1%), *S. aureus* (10.5%) and *P. aeruginosa* (7.8%). Tuladhar *et al* (1989) studied on urinary pathogens and their sensitivity to various antimicrobial drugs where he found *E. coli* was the most common (34.0%) isolates followed by *P. aeruginosa* and other Gram negative bacilli.

Similarly, *E. coli* was found to be the commonest bacteria accounting for 59.8% followed by *K. pneumoniae* (10.9%), *E. faecalis* (6.5%), *S. aureus* (5.4%) and others (17.4%) in a study conducted by Regmi *et al* (2003).

Shrestha *et al* (2004) conducted a study on UTI in female patients in Kathmandu and among 49.3% female patients with UTI, *E. coli* (52.9%) was the most predominant etiological agent followed by *S. epidermidis* (20.7%), *M. morganii* (6.7%), *C. freundii* (3.8%), *S. aureus* (2.9%), *P. aeruginosa* (2.5%).

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

In this study, significant pyuria was observed in 18.0% and 11.0% of requests in diabetic and nondiabetic patients respectively. This value is more or less similar to that obtained in a study by Corman *et al* (1982) where the rate was 23.0%. In this study, out of 82 and 89 cases of insignificant pyuria, 9 and 4 gave culture positive in diabetic and nondiabetic patients while remaining 73 and 85 showed culture negative results. Similarly out of 18 cases of significant pyuria, 72.2% cases were culture positive and 27.7% were culture negative. Based on this result, the sensitivity and specificity of pyuria as a screening test for urinary tract infection were calculated as 59.0% vs 66.6% and 93.5% vs 96.5% in diabetic and nondiabetic patients respectively. Thus it was seen that although the presence of WBCs were specific for significant bacteriuria, the sensitivity was relatively low. It was seen that 40.9% of the positive samples (9/22) in diabetic and 33.3% (4/12) would have been interpreted falsely as being negative, if only microscopy for pyuria were used. In a study carried out by Shrestha *et al* (2004) the sensitivity and specificity of pyuria were found to be 67.3% and 93.8% respectively. The false negative results could be due to early urinary tract infection or presence of asymptomatic UTI patients, diabetes, enteric fever or bacterial endocarditis. Where as false positive results (pyuria with negative culture result) could be due to prior use of antibiotics by the patients or presence of bacteria which were unable to grow on the routine culture media.

Similarly, out of 2 and 3 cases of significant haematuria in diabetic and non diabetic patients, none showed culture positive while all showed culture negative results in former and out of 98 cases of insignificant haematuria, 22 cases were culture positive and 76

were culture negative. In case of nondiabetic patients, out of three significant hematuria, two showed cultures positive result and one was culture negative while out of 97 non significant cases 10 were culture positive and remaining culture negative. Based on this, the sensitivity and specificity of haematuria were calculated as 0.0% vs16.6% and 97.4% vs 98.8% in diabetic and nondiabetic patients respectively. Due to a higher quantity of false negative results seen, using microscopy of RBCs as a screening test of UTI was not found to be reliable. Schumann and Schweitzer (1991) suggested that observation of 0-2 RBCs/hpf on the urinary sediment is normal.

In our study, the sensitivity of epithelial cells count was calculated as 22.7% and 16.6% and specificity was 92.3% and 85.2% in case of diabetic and nondiabetic patients respectively. The low sensitivity and predictive positive value for this test suggested that microscopy of epithelial cells is of poor significance for UTI prediction. Epithelial cells appear in urine as a result of normal exfoliation along the urinary tract (Schumann and Schweitzer, 1991). The finding of large number of squamous epithelial cells or approximately 1-2 cells/hpf in the voided specimen of a female is not uncommon if proper technique for the collection of an uncontaminated specimen are not followed (Fowler, 1990).

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

In this study, 79 out of total samples were negative for albumin test, while 2 contained trace albumin, 3 contained 1+ albumin, 10 samples contained 2+ albumin, 5 samples contained 3+ albumin and only 1 sample contained 4+ albumin in diabetic patients. While in non diabetic patients 81 out of total samples were negative for albumin test, while 3 contained trace albumin, 9 contained 1+ albumin and 3 samples contained 2+ albumin. The sensitivity of albumin test was calculated as 59.0% and 66.6% and specificity was 89.7% and 92.0% in diabetic and nondiabetic patients respectively. There are various conditions in which protein (albumin) appear in urine, UTI is one of them.

According to the North Thames Regional Guidelines for Diagnosis and Management of Urinary Tract Infection, if dipstick proteinuria is consistently more than 1+, then this may indicate UTI and a MSU specimen for culture should be taken. So, it could be concluded that detection of protein (albumin) in urine is also important for diagnosis of UTI and mid-stream urine sample should be cultured in cases when there is absence of significant pyuria.

In this study, all the non diabetic patients were negative for urinary sugar test and among diabetic patients 57 were with positive sugar test i.e. > +1 sugar while remaining 43 were negative for sugar test. Though the patients were considered to be diabetic they were negative for urinary sugar. The blood sugar even higher than upper level of normal range i.e. 120mg/dL it is not excreted in urine unless the level increases to 180 mg/dL. The threshold of kidney to excrete sugar in urine is 180 mg/dL (Murray *et al,* 2000).

Among the common antibiotics used against all Gram negative isolates, Nitrofurantoin was the drug of choice with susceptibility of 61.1% as 11 out of 18 isolates from diabetic patients and 10 out of 12 isolates (83.3%) from nondiabetic patients were found to be susceptible to the drug followed by Ofloxacin with a susceptibility of 50.0% and 41.66% in diabetic and nondiabetic patients respectively. Most of the Gram negative isolates, i.e. 77.7% (14/18) in diabetic and 83.3% (10/12) in nondiabetic patients were resistant to Nalidixic acid respectively. In a similar study carried out by Dhakal *et al* (1999), 84.2% of urinary isolates were susceptible to Nitrofurantoin. Nitrofurantoin was found to be the most effective drug against urinary pathogens also in other similar studies by Gautam *et al* (1998); Jha and Yadav (1992); Shrestha *et al* (2004).

Among the Gram positive isolates from diabetic patients, most of them i.e. 3/4 (75.0%) were susceptible to Erythromycin followed by Ofloxacin, Norfloxacin and Cotrimoxazole with the susceptibility of 50.0% for all three drugs. Amoxycillin, Ceftriazone and Cloxacillin were found to be the least effective as only 1 (25.0%) isolates were sensitive to the drugs. None of the gram positive organisms were isolated from non diabetic patients. Dhakal *et al* (1999) reported 37.5% of total Gram positive

urinary isolates resistant to Cotrimoxazole and in a study carried by Shrestha *et al* (2004) Cloxacillin was found to be susceptible against 38.8% of Gram positive isolates. Based on their findings, Maartens and Oliver (1994) suggested that Cotrimoxazole should no longer be prescribed for urinary tract infection unless susceptible isolate has been cultured. Helin and Araj (1987) found that resistivity of bacteria to Ampicillin and Cotrimoxazole were increasing and hence are regarded as less effective antibiotics.

Similarly out of 100.0% of *C. freundii and P. aeruginosa* all were the MDR-strains and isolated form diabetic patients. Out of 4 (100.0%) isolates of *S. aureus* came from diabetic patients and among them 3 (75.0%) were the MDR-strains. Similarly *K. pneumoniae* isolated from diabetic patient and *P. vulgaris* isolated for diabetic and nondiabetic patients all were MDR-strains. The only isolate of *P. aeruginosa* was tested against Amikacin and it was found to be sensitive to that drug.

Among the19 *E. coli* isolates, 9 (47.3%) were from diabetic patients, of which 8 (88.8%) isolates were MDR-strains and 10 (52.6%) were from non diabetic patients, of which 7 (70.0%) isolates were MDR-strains. Nitrofurantoin was most effective against this bacterium with susceptibility of 68.6% followed by Ofloxacin (47.3%) and Ceftriaxone (36.8%). Nalidixic acid, Cotrimoxazole and Norfloxacin were the least sensitive drug with the susceptibility of 21.0%. Eom *et al* (2002) reported that the incidence of quinolone-resistant *E. coli* (QREC) increased steadily from 14.4% to 21.3% during 5 years from 1996 to 2000 in Korea. In their study they found that the multi-drug resistance rate of QREC was much higher (38.3%) than those of Quinolone susceptible isolates (18.8%).

The rates of antibiotic resistance of *E. coli* in diabetic vs nondiabetic patients were Ampicillin 29.0% vs 30.6%, Cotrimoxazole 19.2% vs 17.4%, Ciprofloxacin 11.6% vs 6.6%, and Nitrofurantoin 8.4% vs 6.9%. (Bonadio *et al*, 2006)

Aminoglycoside and Ciprofloxacin can be used empirically to treat UTI in diabetics and nondiabetics (Akbar, 2001).

Among the 4 S. *aureus* isolates, 3 isolates were found to be MDR strains and among the isolates 75.0% were sensitive to Erythromycin and 3 isolates were resistant to Cloxacillin.

The prevalence of urinary tract infection (UTI) is high in patients with diabetes mellitus. They run a distinctly greater risk of complications than non-diabetics. Systematic antibiotic treatment is mandatory (Guillausseau *et al*, 2003).

6. 2 CONCLUSION

Hence, the incidence of urinary tract infection in diabetic patients attending "OM" Hospital and Research Centre and the prevalence of multi-drug resistant strains among the bacterial pathogens was studied. The study revealed that there is no significant difference in the positive growth of bacteria between diabetic and nondiabetic patients.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. Among 100 diabetic patients studied, the fasting blood sugar level ranged from 6.6 mmol/L to 16.5 mmol/L and post prandial blood sugar level ranged from 10.1 mmol/L to 26.7 mmol/L.
2. Out of 100 MSU samples processed from diabetic patients, in 22.0% sample significant growth was observed while 4.0% samples with mixed growth and 8.0% samples with non significant growth were observed. Out of 100 nondiabetic urine samples, in 12.0% significant growth was observed while 1 sample with mixed growth and 2.0% sample with non significant growth were observed.
3. Frequency of positive growth of isolates was found to be higher in diabetic patients (22.0%) in comparison to nondiabetic patients (12.0%) but this was found to be statistically non significant ($P>0.05$).
4. Rate of urinary tract infection was found to be higher in female, as 67.6% of the positive culture results (23/34) were obtained from female patients.
5. The predominant bacteria causing UTI among diabetic patients were found to be the Gram negatives (N=18) which constituted 81.8% and among them 94.9% (N=17) were MDR-strains. Gram positive bacteria (N=4) constituted only 18.1% and of them 75.0% (N=3) were MDR-strains. Whereas in nondiabetic patients all the isolates (N=12) were found as Gram negative organism.
6. Altogether 6 different species of bacteria were isolated from the growth positive cultures. *E. coli* (9/18) and (10/12) was found to be the most predominant isolate (40.9% and 83.3%) in diabetic and nondiabetic patients respectively. Among Gram positives, *S. aureus* was the only isolate.
7. Microscopy of pyuria showed the sensitivity and specificity 59.0% vs 66.6% and 93.5% vs 96.5% in diabetic and nondiabetic patients respectively. The positive

predictive values were 72.2% and 72.7% and negative predictive values were found to be 89.6% and 95.5% respectively in both cases.

8. Nitrofurantoin was found to be the most effective drug with a susceptibility of 61.1% and 83.3% against the Gram negative bacteria in diabetic and non diabetic patients respectively. Erythromycin was found to be the most effective agent against Gram positive bacteria with a susceptibility of 75.0%.
9. Of total *E. coli* isolates, 88.8% and 70.0% were found to be MDR-strains in diabetic and non diabetic patients, while all the isolates of *C. freundii* (6/6), *P. aeruginosa* (2/2), *K. pneumoniae* (1/1) and *P. vulgaris* (2/2) were found to be MDR-strains.
10. Of total four S. *aureus*, three (75.0%) were found to be MDR-strains.

7.2 RECOMMENDATIONS

1. As this study was confined to OM Hospital and Research Centre, Kathmandu, it doesn't reveal the picture of whole country, therefore this type of study should be conducted in other parts of the country.
2. Study of other infections including respiratory tract, foot, skin, oral etc should also be done. The anaerobes, fungi and parasites related to UTI should also be diagnosed.
3. If more than 5 WBCs/hpf are seen in urinary sediment, the patient should be advised for urine culture and susceptibility test.
4. Detection of protein in urine is also important for diagnosis of UTI and the patients with albuminuria should be advised for urine culture.
5. Genotypic characterization of MDR strains should be carried out in order to ascertain the location of drug resistance genes.
6. The sample size must be increased as far as possible to get the significant difference in growth positive organisms between the diabetic patients and nondiabetic patients.

CHAPTER-VIII

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