CHAPTER – I

1. INTRODUCTION

Urinary tract infection (UTI) is the commonest bacterial infection prevalent to both female and male. Urinary tract infection comprises a wide variety of clinical entities which is the result of microbial invasion of tissues lining the urinary tract extending from the renal cortex to the urethral meatus. It also refers to the presence of bacteria undergoing multiplication in urine within the urinary drainage system. Infection of the adjacent structure such as prostrate and epididymis is also included in the definition of urinary tract infection. Infection may be expressed predominantly at a single site, kidney i.e. pelvis and cortex (pyelonephritis), pelvis and ureter (pyelitis), ureter (ureteritis), bladder (cystitis), prostrate (prostitis) and urethra (urethritis) but the entire urinary tract is always at a risk of invasion by bacteria, once any one of its part is infected. As urethra is the common site for urinary tract and genital tract, urethritis is also included in sexually transmitted disease especially if the infection is caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (Asscher *et al*., 1968)

UTI is more common in women than in men, at least partially because of the short female urethra and its proximity to the anus. The incidence of infection is highest in women, 20-50% of whom will suffer a clinical episode during their lifetime, however, most of the infections remains undiagnosed and undergoes spontaneous remission. The prevalence of infection correlates directly with age, increasing from about 1% to 6% between puberty and 60 years. Approximately 95% of infections are urinary re-infections, mostly occur in unhygienic individuals, and few are the cause of serious illness (Leigh *et al,* 1990).

Infection is less common in males, in whom it is especially related to abnormalities of the urinary tract or instrumental interference with it. The incidence of UTI among males is low until after age 60, prostatic hypertrophy then contributes to the development of UTI. Bacterial prostatitis is the key background factor for the problem of recurrent cystitis in males. Any anatomic barrier to free flow of urine throughout the urinary tract contributes to the development of UTI; involved are prostatic hypertrophy, neurogenic disorders, tumors, and stones. Previous infection with certain urea splitting organisms, notably Proteus sp and related species, is often associated with the formation of urinary stones, which further predisposes the patient to

infection. The introduction of a foreign body into the urinary tract, especially on that remains in place for a time (such as Foley catheter), carries a substantial risk of leading to infection, particularly if obstruction is present. As many as 20% of all hospitalized patients who receive short-term catheterization develop UTI (Baron and Finegold, 1990).

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 diabetics is hyperglycemia. Therefore diabetes mellitus 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 to so wide that diabetes is presently regard as a syndrome rather than disease entity. DM is the most frequent endocrine disease whose prevalence and incidence varies widely among study population (Micha el *et al*., 2005).

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 (William *et al*., 2002).

Microalbuminuria is traditionally defined as an increase in urinary albumin too subtle to be measured by chemistry sticks for total protein. With improved methodology, these low levels of albumin (20-200 µg/min, 30-300 mg/24 h or 20-200mg/L) can now be measured. Microalbuminuria is considered as a clinically important indicator of deteriorating renal function in diabetic and hypertensive patients. In these patients; the microalbuminuria phase is followed by progressive increase in urinary protein excretion and declining glomerular filtration rate. This results in chemistry stick–positive proteinuria, known as overt nephropathy or macroproteinuria. Without

treatment, the patient will develop uremia and require referral to end–stage renal-failure treatment such as dialysis or transplantation. Microalbuminuria has also proved to be a strong independent predictor for atherosclerotic disease, cardiovascular mortality and overall mortality. Increase in the transcapillary escape rate of albumin and is therefore increase in urinary albumin excretion is a marker of microvascular disease (Orion Diagnostica, 2000).

Urinary tract infection (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 diabetes than non-diabetic patients to UTI. Suggested mechanisms are decreased antimicrobial activity due to sweet urine, defects in neutrophil function and increased bacterial adherence to epithelial cells. When diabetic patients get infected, they are more severe as they are compromised host (Ruggenenti *et al*., 2000).

Several studies had been revealed the greater risk of UTI in diabetic micro-albuminuric patients. Basically, histological changes of the kidney can often be observed in poorly stabilized diabetics and hypertensive patients. As a consequence of damages to the basal membrane (e.g. due to glucose depositions), an increased amount of protein passes through the membrane and is excreted with the urine. The presence of albumin can be diagnosed and monitored very early with the aid of an immunological test strip. The diagnosis can make suitable therapeutic measures to support the cure of renal damages (nephropathy) (Orion Diagnostica, 2000).

The urine secreted in the kidney is sterile unless the kidney as well as other organ of tracts is infected. The urethra, however, contains a normal microbial flora, which contaminates urine in passage, so that voided urine may contain small numbers of bacteria in the absence of urinary tract infection. Because it is necessary to distinguish contaminating from etiologically important organisms, only quantitative urine examination can yield meaningful results (Jawetz *et al*, 1980).

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

The bacterial count of 10^3 CFU/ml to 10^4 CFU/ml may be considered as contaminants but it is always not true. Patients with true UTI whose urine may yield fewer number of bacteria than the classic 10^5 CFU/ml include infants and children, pregnant females, males , catheterized patients, patients who have received antibacterial agents previously, patients who consume large amount of liquid and thus dilute bladder urine, symptomatic patients(usually with pyuria), patients with urinary obstruction that may prevent organisms from being excreted, and patients with pyelonephritis acquired from haematogenous spread (particularly yeasts, *Staphylococcus aureus* and *Mycobacterium tuberculosis* infections). The concept of significant bacteria is based on the bacteria that mostly belongs to Enterobacteriaceae family which reach a count of 10^5 CFU /ml. or more on overnight incubation in the bladder urine. But these criteria may not be true for those organisms which grow in urine at a slower rate and also for anaerobic organisms causing infection. So it is suggested that low count significant bacteriuria should also be considered (Baronand Fine gold, 1990).

Gram negative bacilli and *Enterococc*i 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 (Mims *et al*., 2003).

Antimicrobial resistancy is a global public health problem and multiple drug resistant (MDR) bacterial isolates have been frequently reported from different parts of the world as an emergence of treatment problem. Multi-drug resistance has been defined as resistance to ≥two classes of antibiotics (WHO, 2004).

In our country, this is fact that different types of infections are found because most of the people do not have access to proper hygiene and good sanitation practices. Study on the frequency of UTI in Nepalese people has been carried out frequently but there is un-picturize condition about the frequency of UTI in diabetic micro-albuminuric patients which has a threat to the Nepalese people. Hence this study was aimed to investigate the prevalence of UTI among diabetic patients.

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To determine the prevalence of urinary tract infection in diabetic micro-albuminuric patients visiting B &B Hospital.

2.2 SPECIFIC OBJECTIVES

i. To screen diabetic microalbuminuric and non-diabetic non-micro-albuminuric patients on the basis of sugar and micro-albumin levels.

ii. To categorize the types of diabetes mellitus and micro-albumin in diabetic microalbuminuric patients.

iii. To isolate and identify the bacteria associated with urinary tract infection.

iv. To analyze the antibiotic susceptibility pattern of the isolated organisms.

v. To find out the prevalence of Multi-Drug Resistant organisms among the total isolates.

vi. To evaluate the urine culture test for the detection of UTI on the basis of urinary sugar and micro-albumin level.

CHAPTER – III

3. LITERATURE REVIEW

3.1 GLUCOSE HOMEOSTASIS

Glucose is a monosaccharide with a postprandial blood concentration of 5 -6.5 mmol/ L. It serves as an indispensable energy supply for cellular function. The glucose catabolism takes place via the glycolysis as the first step, followed by the citric acid cycle and oxidative phosphorylation. In the fasting state, glucose turnover in a 70 kg individual is approximately 2mg/ kg / min (200 g / 24 hr). The plasma glucose concentration reflects the balance between intake (glucose absorption from the gut), tissue utilization (glycolysis, pentose phosphate pathway, tricarboxylic acid cycle, glycogen synthesis) and endogenous production (glucogenolysis and gluconeogenesis). Glucose homeostasis is controlled primarily by the anabolic hormone insulin and also by several insulin–like growth factors. Several catabolic hormones (glucagons, catecholamine, cortisol and growth hormone) oppose the action of the insulin, they are known as counter regulatory hormones.

3.2 DIABETES

Diabetes is a disease caused by a failure of glucose homeostasis. The term diabetes mellitus describes a metabolic disorder of multiple etiology, which is characterized by hyperglycemia (fasting plasma glucose, FPG>7.0 mmol/L or 126 mg/dL), fasting is defined as no caloric intake for at least 8 hours or two hour post 75 gm oral glucose load plasma glucose >11.1 mmol/L (200 mg/dL), on two or more occasions], with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. In the absence of unequivocal hyperglycemia with acute metabolic decompensation, these criteria should be confirmed by repeat testing on a different day. The oral glucose tolerance test (OGTT) is not recommended for clinical use, but may be required in the evaluation of patients with intermediate fasting glucose level (IFG) or when diabetes is still suspected despite a normal FPG. Once regarded as a single disease entity, diabetes is now seen as a heterogeneous group of diseases, characterized by state of chronic hyperglycemia, resulting from a diversity of etiologies, environmental and genetic, acting jointly(WHO,1980). Characteristically, diabetes is a long term disease with variable clinical manifestations and progression. Chronic hyperglycemia, from whatever cause, leads to a number of complications- cardiovascular, renal, neurological, and ocular and others such as intercurrent infections.

3.2.1 CLASSIFICATION

An international expert committee released a report with new recommendations for the classification 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).

Diabetes is a common disorder and its frequency is dramatically rising around the world. The global increase in diabetes will occur because of population ageing and growth, and because of increasing trends towards obesity, unhealthy diets and sedentary lifestyles.

Clinical classification of diabetes mellitus adopted by WHO (1985), Techn. Rep. is given in Table 1

Table 1: Classification of Diabetes

Class	Name
1	Diabetes mellitus (DM)
	a. Insulin-dependent diabetes mellitus (IDDM, Type 1)
	b. Non-insulin dependent diabetes mellitus (NIDDM, Type 2)
	c. Malnutrition-related diabetes mellitus (MRDM)
	d. Other types (Secondary to pancreatic, hormonal , drug-induced , genetic and other abnormalities)
2	Impaired glucose tolerance (IGT)
3	Gestational diabetes mellitus (GDM)

The type of diabetes for which neither an etiology nor a pathogenesis is known (idiopathic) is named **Type 1 diabetes** and encompasses the majority of cases, primarily due to pancreatic islet beta-cell destruction, which are prone to ketoacidosis, and includes those cases attributable to an autoimmune process.

Type 1 diabetes is usually characterized by the presence of autoantibodies against the signaling mechanism in the islet cells. Its onset is typically abrupt and is usually seen in individuals less than 30 years of age (In some subjects with this clinical form of diabetes, no evidence of an autoimmune is demonstrable and these are classified as “Type 1 idiopathic”). It is lethal unless promptly diagnosed and treated. This form of diabetes is immune-mediated in over 90 per cent of cases and idiopathic in less than 10 per cent cases.Type 1 diabetic is usually associated with ketosis in its untreated state. It occurs mostly in children, the incidence is highest among 10 -14 year old group, but occasionally occurs in aduluts. It is catabolic disorder in which circulating insulin is virtually absent, plasma glucagons is elevated, and the pancreatic β cells fail to respond to all insulinogenic

stimuli. Exogenous insulin is therefore required to reverse the catabolic state, prevent ketosis, reduce the hyperglucagonemia, and reduce blood glucose.

Type 2 diabetes includes the common major forms of diabetes which results from defect(s) in insulin secretion, almost always with a major contribution from insulin resistance and much more common than Type 1 .It is often discovered by chance. It is typically gradual in onset and occurs mainly in the middle-aged and elderly, frequently mild, slow to ketosis and is compatible with long survival if given adequate treatment. About 90.0% of persons (life time risk is 5 -7 %) 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 and hyperglycemia. The etiology of Type 2 diabetes mellitus is multi-factorial and probably genetically based, but also has strong behavioral components.

Impaired glucose tolerance (IGT) describes a state intermediate – "at risk" group –between diabetes mellitus and normality. It can only be defined by the oral glucose tolerance test.

Gestational diabetes mellitus (GDM) is carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or during pregnancy. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy.

3.2.2 ETIOLOGY OF DIABETES MELLITUS

Several pathogenesis processes are involved in the development of diabetes. Diabetes results from deficient insulin secretion, decreased insulin action or both. Many processes can be involved, ranging from

1. Autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency.
2. Incomplete understood abnormalities that result in resistance to insulin action.
3. Defects in the formation of insulin e.g. synthesis of an abnormal, biologically inactive insulin molecule.
4. Genetic factors are involved in both type 1 and type 2 mechanisms.

In type 1 diabetes, there is an absolute deficiency of insulin whereas in type 2 diabetes, insulin resistance and an inability of the pancreas to compensate are involved. The abnormalities of carbohydrate, fat and protein metabolism are due to the deficient action of insulin on target tissue. This can result from the insensitivity for insulin or lack of insulin.

Hyperglycemia, sufficient to cause tissue damage can be preventing without clinical symptoms for many years before diagnosis.

3.2.3 RISK FACTORS OF DIABETES MELLITUS

Diabetes may occur at any age, sex that may be due to the genetic factors, obesity, sedentary life styles, diet, malnutrition and certain chemical reagents as well as social factors. Stress (Surgery, trauma) also associated with diabetes.

Surveys indicate that prevalence rises steeply with age. Type 2 diabetes mellitus usually comes to light in the middle years of life and thereafter begins to rise in frequency. Malnutrition related diabetes affects large number of young people. In some countries (eg U.K.) overall male-female ratio is about equal. In South–East Asia, an excess of male diabetes has been observed. The genetic nature of diabetes is undisputed. It was showed that in identical twins that developed Type 2 diabetes mellitus, concordance was 90 per cent, thus demonstrating a strong genetic component. In Type 1 diabetes mellitus, the concordance was only about 50 per cent indicating that this type of diabetes is not totally a genetic entity.

Obesity particularly central adiposity has long been accepted as a risk factor for Type 2 diabetes and the risk is related to both the duration and degree of obesity. The association has been repeatedly demonstrated in longitudinal studies in different populations, with a striking gradient of risk apparent with increasing level of basal metabolic index (BMI), adult weight gain, waist circumference or waist to hip ratio. Indeed waist circumference or waist to hip ratio (reflecting abdominal or visceral adiposity) are more powerful risk factor of subsequent risk of type 2 diabetes than BMI. Central obesity is also an important risk factor of insulin resistance, the underlying abnormality in most cases of type 2 diabetes.

Sedentary life style appears to be an important risk factor for the development of type 2 diabetes. Lack of exercise may alter the interaction between insulin and its receptors and subsequently lead to type 2 diabetic

Offsprings of diabetic pregnancies including gestational diabetes are often large and heavy at birth, tend to develop obesity in childhood and are at high risk of developing type 2 diabetes at an early age. Those born to mothers after they have developed diabetes have a three-fold higher risk of developing diabetes than those born before.

A high saturated fat intake has been associated with a higher risk of impaired glucose tolerance, and higher fasting glucose and insulin levels. Higher proportions of saturated fatty acids in serum lipid or muscle phospholipids have been associated with higher fasting insulin, lower insulin sensitivity and a higher risk of type 2 diabetes.

Malnutrition in early infancy and childhood may result in partial failure of β-cell function. Damage to beta cells may well explain the associated impaired carbohydrate tolerance in kwashiorkor. Excessive intake of alcohol can increase the risk of diabetes by damaging the pancreas and liver and by promoting obesity. A number of chemical agents are known to be toxic to beta cells e.g., alloxan, streptozotocin, the rodenticide VALCOR, cyanide producing foods (e.g. cassava and certain beans).

High rates of diabetes have been associated with a number of social factors such as occupation, marital status, religion, economic status, education, urbanization, changes in life style etc.

3.2.4 DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS

In the part, the commonest approach to diabetes screening was a preliminary, semi-quantitative test for glucose in a urine sample, followed by an oral glucose tolerance test for those found to have glycosuria. Previously recommended oral glucose tolerance test 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 gm (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) and is much more reproducible than the oral glucose tolerance test or the 2 hr PPG test and easier to perform in a clinical setting.

Criteria of defining as diabetes mellitus according to the 1998 WHO – 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 / dl (180 mg/ dl) .

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 to126 mg/dl (6.1 to 7 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 2 diabetes mellitus (Mayfield, 1998).

Table No 2: Diagnostic values for the oral glucose tolerance test (WHO, 1985)

	Whole blood		Plasma	
Diabetes mellitus	Venous	Capillary	Venous	Capillary
a. Fasting value	≥120	≥120	≥120	≥120
b.2 hrs after glucose load	≥180	≥200	≥200	≥200
Impaired glucose tolerance	Venous	Capillary	Venous	Capillary
a. Fasting value	110-126	110-126	110-126	110-126
b.2 hrs after glucose load	140-200	140-200	140-200	140-200

The glucose tolerance test gives many false positives results because the test itself can be stress inducing, causing epinephrine release. This hormone decreases the release of insulin from the β-cells, and thus impairs the response to a glucose load. As a result, the glucose tolerance test is usually used only in situation in which diagnosis is uncertain or as a test for gestational diabetes. For general population, a fasting blood glucose test is the more commonly used diagnostic tool. Broadly type 1 diabetes is differentiated from type 2 diabetes on the basis of ketone bodies tests especially acetone. Acetone test is generally positive in fasting urine sample of type 1 diabetes.

3.2.5 COMPLICATIONS ASSOCIATED WITH DIABETES MELLITUS

The long-standing elevation of blood glucose causes the chronic complications of diabetes-premature atherosclerosis, retinopathy, nephropathy and neuropathy. Intensive treatment with insulin delays the onset and slows the progression of these long-term complications. For example, the incidence of retinopathy decreases as control of blood glucose improves and HbA_{1C} levels decrease. The benefits of tight control of blood glucose outweigh the increased risk of severe hypoglycemia. How hyperglycemia causes the chronic complications of diabetes is unclear (Champe C .P *et al,* 2005)

Long term complications

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

Short term complications

Short term complications included glycosuria, hyperglycemia, ketoacidosis, hypertriacylglycerolemia etc.

Infectious complications

Diabetic patients are susceptible to various types of infection. Several aspects of immunity are altered in patients with diabetes. Poly-morphonuclear leucocytes function is depressed, particularly when acidosis present. Leucocytes adherence, chemotaxis and phagocytosis may be affected. 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. So the diabetic individuals are not only predisposed to infections but that infection also complicates the control of the diabetes (Herbal *et al.*2000*)*

3.2.6 FREQUENTLY ENCOUNTERED INFECTIONS IN DIABETES

Patients with diabetes mellitus are more predisposed to infections. This predisposition is due to a combination of anginopathy, neuropathy , and hyperglycemia (Golden *et al.*,1999*)*

Foot infections

Diabetic patients are particularly susceptible to foot inections. These infections are leading cause of limb loss in the United States and are responsible for the majority of hospitalizations of diabetic patients (Gibbons and Habershaw, 1991).

Respiratory infections

Diabetic patients may be more predisposed to lower respiratory tract infections (Fajans *et al*., 2001)

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

Upper soft tissue infections

The incidence of hand upper extremity infections is higher among diabetic patient (Archibald *et al*, 1997). 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 (Golden *et al*., 1999).

H. pylori infection

A higher frequency of Helicobacter pylori infection among dyspeptic diabetic patients than among non-diabetic patients was reported (Stephen *et al*., 2005)).

Urinary tract infection

Urinary tract infection is common in diabetic patients due to decreased resistance to infection. Diabetes predisposes the patient to renal abscesses which increase the risk of

UTI. 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).
Autopsy study in 1940 showed that 18% of patients with diabetes mellitus had a serious infection of urinary tract (Robert *et al*., 2001).

Anatomic and functional abnormalities of the urinary tract are also associated with diabetes. Women with diabetes are at a particularly increased risk of urinary tract infection (Stapleton, 2002).

3.3 MICROALBUMINURIA

Albumin is normally found in the blood and filtered by the kidneys. When the kidneys are working properly, albumin is not present in the urine. However, when the kidneys are damaged, small amount of albumin leaks into the urine. This condition is called micro-albuminuria (Healthwise, 2006).
Micro-albuminuria is defined as the condition in which albumin is excreted (24 hr urine or time day collection) at a rate between 20 and 200 µg/min or 30 – 300 mg/24 hr .A normal albumin excretion rate (AER) should amount to 2 to 20 µg /min or 3 to 30 mg/24 hr ,while a clinical albuminuria or micro-albuminuria is present at an excretion rate above 200 µg/min or >300mg/24 hr. (Orion Diagnostica, 2000).
Definition criteria of micro-albuminuria is given in Table3
Table No3.Definition of micro-albuminuria (Mogensen CE *et al*., 1995)

Nature of Urine sample	Criteria
Timed collected urine	Albumin excretion 20-200µg/min
Urine collection for 24 hr	Albumin excretion 30-300mg/24hr
Spot urine sample (preferably first morning urine)	Albumin concentration 20-200mg/l

3.3.1 ETIOLOGY OF MICROALBUMINURIA

Micro-albuminuria is most frequently caused by kidney damage from diabetes. However, many other conditions can lead to kidney damage, such as high blood pressure, heart failure, cirrhosis, or systemic lupus erythematosus (SLE). If early kidney damage is not treated, larger amounts of albumin and protein may leak into the urine. This condition is called macro-albuminuria or proteinuria. When the kidneys spill protein, it can mean serious kidney damage is present. This can lead to chronic kidney disease

The development of micro-albuminuria probably involves both metabolic and haemodynamic factors affecting renal microcirculation. On the other hand, sustained hypertension is known to cause transcapillary escape of proteins, such as albumin, by increasing the intra-glomerular pressure. On the other hand, metabolic disorders directly affect the glomerular basement membranes and their permeability, thereby altering glomerular function and ultimately causing glomerular sclerosis (Orion Diagnostica, 2007).

Haemodynamic aspects

Glomerular hydrostatic pressure is normally regulated by the relative vasoconstriction-vasodilation of the blood vessels leading to and from the glomerulus. Defects of this autoregulatory function may lead to increased glomerular hydrostatic pressure and increased urinary albumin excretion.

Metabolic aspects

Microalbuminuria could also be due to a loss of the anionic charge of the glomerular basement membrane. This has been observed in diabetic patients, in whom advanced glycosylation end products (AGE products) may bind to and neutralize the anionic proteins of the basement membrane, with concurrent increase in the trans-membrane passage of albumin. In type 1 diabetes, urinary albumin excretion correlates with the deposits of AGE products. The risk of micro-albuminuria has been found to increase with the amount of glycosylated hemoglobin, $HbA_{1c,}$ in blood.

Metabolic syndrome

Microalbuminuria often occurs together with the metabolic syndrome consisting of hyperinsulinaemia and insulin resistance, increased triglyceride and decreased high-density lipoprotein (HDL) levels, hyperglycemia and hypertension.

3.3.2 DIAGNOSTIC CRITERIA FOR MICROALBUMINURIA

Urinary albumin excretion has a high intra-individual variability, even when individuals refrain from physical exercise on the day before the collection of urine, and when overnight collections are used to avoid acute variations from dietary protein. Therefore multiple measurements are usually required to make a diagnosis, and repeated collections over time are required to establish the progression of albumin excretion over time .A result is considered to be diagnostic when two out of three urine specimens are positive within a 3-month period (Orion Diagnostica, 2000).

Sometimes it is more practical to test early morning urine samples and to measure the albumin/creatine ration.Microalbuminuria is considered to be present when the ratio of

albumin/Creatinine in urine reaches a level greater than 30mg/g. This method may theoretically avoid falsely negative results due to polyuria and spurious reductions in urinary albumin concentration (Orion Diagnostica, 2000).

Generally, screening for microlabuminuria can be performed by three methods:

a. Measurement of albumin concentration in first-void morning urine or measurement of the albumin to Creatinine ratio in a random spot

b. 24-hour urine collection with Creatinine measurement, allowing simultaneous determination of Creatinine clearance

c. Timed (e.g. 4-hour to overnight) urine collection.

The first method is often found to be the easiest to carry out and generally provides accurate information. General criteria for abnormalities in albumin excretion are shown in table no 3.4 (Orion Diagnostica, 2000).

Table No 4 Definitions of abnormalities in albumin excretion

Category	**24-h collection (mg/24 h)**	**Timed collection (µg/ min)**	**Spot collection(µg /mg creatinine)**
Normal	<30	<20	<30
Microalbuminuria	30-299	20-199	30-299
Clinical albuminuria	>300	>200	>300

3.3.3 COMPLICATIONS ASSOCIATED WITH MICROALBUMINURIA

Microalbuminuria is a reliable indicator of a risk of progressive renal and cardiovascular disorders. In 80 % of people with type 1 diabetes and microalbuminuria, urinary albumin excretion increases at a rate of 10-20 % per year, with development of clinical proteinuria (> 300mg albumin/day) in 10-15 years. After development of clinical grade proteinuria, most (> 80 %) patients go to develop decreased glomerular filtration rate (GFR) which lead to end-stage renal disease (Rudberg *et al*. 1997).

Some important consequences associated with micro-albuminuria are follows:

1. Increased risk of cardiovascular disease.
2. Increased risk of early mortality in patients with acute myocardial infarction.
3. Pregnancy complications and kidney dysfunction in hypertensive patients

3.3.4 MICROALBUMINURIA IN DIABETES

Diabetic micro-albuminuria is defined as the clinical diabetes with the diagnosed microalbumin in urine. All patients with type 1 or type 2 diabetes should be annually screened for micro-albuminuria. If a positive result for it is obtained in a type 1 diabetic, the

patient's treatment should be intensified to diminish urinary albumin excretion and to halt further deterioration of renal function (Gaede *et al*., 1999).

In type 2 diabetics, microalbuminuria is a significant indicator of increased cardiovascular risk. The level of micro-albuminuria should be measured every two to three months until a plateau is reached. The action required after the detection of microalbuminuria in a diabetic patient consists of four approaches.

a. The effective management of blood glucose concentration.
b. Hypertension should be optimally treated.
c. The patient should be encouraged to give up smoking.
d. The intake of dietary protein should be restricted.

In type-2 diabetes, increased urinary albumin excretion is a strong independent predictor of progressive renal disease, atherosclerotic disease, cardiovascular mortality and overall mortality. Conversely, insulin resistance has been suggested to predict an elevation in urinary albumin excretion and to precede microalbuminuria (Alzaid A, 1996).

3.4 URINARY TRACT INFECTION

Urine secreted in the kidney is sterile unless the kidney is infected. Uncontaminated bladder urine is also normally sterile. The urethra, however, contains a normal microbial flora which contaminates urine in passage, so that void urine may contain small numbers of bacteria in absence of urinary tract infection (Jawetz *et al*, 1980).

Urethra colonizes a resident microflora on its transitional epithelium. These resident microflora include coagulase negative *staphylococci*, viridans and non-haemolytic *streptococci*, lactobacilli, diphtheroids (*Corynebacterium* sps), non pathogenic *Neisseria* sps, transient gram negative aerobic bacilli (including enterobacteriaceae), anaerobic cocci, *Propionibacterium* sps, anaerobic gram-negative cocci and bacilli, commensal *Mycobacterium* sps, commensal *Mycoplasma* sps and occasional yeasts. All areas of the urinary tract above the urethra in healthy human are sterile
(Baron and Binegold, 1990).

Infection of urinary tract occurs when bacteria capable of proliferating in urine get access into the tract because human urine contains no humoral or cellular defenses against bacterial growth (Fowler, 1990). Normal urine, unlike other body fluids, does not contain significant quantities of lysozyme or immunoglobulin, and any complement present is inactivated. Increased concentrations of I_gA and I_gG(Jodal *et al*., 1997) and, according to some author but not others I_gM (Kaufman *et al*., 1970) are found in adults and children with urinary tract infection but this is probably only a result of proteinuria and leakage of

inflammatory exudates (Burdon,1973). Phagocytosis of bacteria is impaired both by the absence of opsonins and the wide range of osmolarity in urine (Chernew *et al*., 1962).

Components that support bacterial growth are urinary pH, osmolality, and chemical constituents such as glucose and amino acids etc. Normal urine usually contains sufficient glucose to supports maximum growth rates and any lowering of the P^H is prevented by its buffering capacity. The activity of neutrophils, T-lymphocytes and natural killer cells is decreased due to higher glucose concentration resulting higher risk of UTI (Forsblom *et al*., 1999).

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).

UTI is defined as the detection of both bacteriuria (10^5 CFU/ml) and pyuria (10 leucocytes / HPF) (Kass, 1957).

The term urinary tract infection (UTI) refers to the invasion of the urinary tract by a non resident infectious organisms. 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 including in the definition (Pokhrel, 2004).

Glucose provides source of energy for the growth of urinary pathogenic bacteria so the number of bacteria in the urine of diabetes micro-albuminuric patients was significantly higher than in that of non-diabetic controls (Mogensen, 2000).

In diabetes micro-albuminuric patients, cellular immunity will be decreased due to hyperglycemia, higher osmolality.

The definition of UTI in diabetic microalbuminuria is also similar with classical definition of UTI along with clinical diagnosed condition of diabetes and microalbuminuria. Susceptible bacteria are more responsible for urinary tract infection in diabetes micro-albuminuric patients.

3.4.1 Classification of urinary tract infection

Based on clinical presentation, degree of tissue invasion, epidemiologic settings, and requirements for antibiotic therapy, there are four major types of UTI, urethritis, cystitis, the urethral syndrome and pyelonephritis

Table No 5: Classification of UTI (Norrby, 1990).

Classified by	Groups	Definition
Symptoms	Symptomatic	UTI symptoms during the preceding two weeks
	Asymptomatic	No symptoms during preceding two weeks
Level	Lower (Cystitis)	Bacteriuria limited to the bladder.
	Upper (Pyelonephritis)	Bacteriuria involving the kidneys.
Complications	Uncomplicated	No identified anatomical defects, foreign Bodies or tumours.
	Complicated	Identify anatomical defects, foreign Bodies or tumors.
Recurrences	Sporadic	<2 episodes or UTI in the preceding 6 months And < 3 episodes in the preceding year.
	Recurrent	>2 episodes of UTI in the preceding 6 months or >3 episodes in the preceding year.

Sometimes UTIs are classified as uncomplicated or complicated. Uncomplicated infections occur primarily in otherwise healthy females and occasionally in male infants and adolescent and adult males. Most uncomplicated infections respond readily to antibiotic agents to which the etiologic agent is susceptible. Complicated infections occur in both sexes (Farrel *et al*., 2003).

Based on the interrelated parameters of frequency, bacteriology, response to treatment of sequential infections, site of infections, and impact in morbidity; categorization of UTI can be done as

a. Urinary tract infection in children and infants

UTI 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 (Hooton *et al*., 19901).

b. 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).

c. 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 (Emerson, 1996).

d. Complicated urinary tract infection

Complicated UTI occurs in a patient who has functionally, metabolically or anatomically abnormal urinary tract or those caused by pathogens that are resistant to antibiotics (Schaffer, 1998).

e. Asymptomatic bacteriuria

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).

f. 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).

g. Catheter-associated urinary tract infection

Cather–associated UTIs account for 40.0% of nosocomial infection and are the most common source of Gram. Negative bacteriuria in hospitalized patients (Warren, 1997).

h. Complications of urinary tract infection in diabetes and micro-albuminuria

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

3.4.2 Risk factors for the development of UTI

Many different factors predispose to the development of urinary tract infection (Leigh, 1990).

Table No. 6 Possible risk factors in the development of UTI

Factors	Features
Anatomical	Congenital abnormalities; prostate hypertrophy;cystcele, uterine prolapse
Pathological	Surgical operations on the urogenital tract; tumors of bladder and prostate. Urethral catheterization,atropic vaginitis;neurological disorder of bladder
Infective	Vulvovaginitis; vaginal discharge
Social	Sexual intercourse; menstruation, sanitary pad; intrauterine coil, diaphragm, contraceptive pill
Environmental	Exposure to cold; swimming; nylon underwear; tight clothes; sedentary occupation; long distance driving

Source of table: Godkar, 1994

The incidence of urinary tract infection is greatly influenced by age and sex and by factors that impair the defense mechanisms that maintain the sterility of urinary tract (Leigh, 1990).

The incidence of UTI is highest in women than in men, at least partially because of short female urethra and its proximity to the anus. The relative frequency of urinary tract infection in men may be attributable to the length of male urethra and the bactericidal activity of the prostatic fluid (Baron and Finegold, 1990).

UTI in males is most common during early childhood and old age. The prevalence of infection in women correlates directly with age, increasing from about 1% to 6% between puberty and 60 years. Approximately 95 % of infections are urinary reinfections, most occur in individuals who are healthy, and few are the cause of serious illness (Fowler, 1990). Sexual activity serves to increase the chances of bacterial contamination of the female urethra which finally lead to predispose o infection of bladder e.g. ‘honeymoon

cystitis' being a well recognized clinical entity in females (Leigh, 1990)The use of diaphragm for contraception ,however ,clearly promotes introital colonization by gram negative bacilli and increases the risks of bacteriuria (Chow *et al.*, 1986).

Pregnancy causes anatomic and hormonal changes that favor development of UTIs. Pregnancy predispose to upper urinary tract infection due to

1. Dilation of ureter and renal pelvis.
2. Stasis in right ureter and to some extent in left ureter up to the brim of bony pelvis.
3. Atony-reduce tone in ureteric musculature during pregnancy results from inhibitory effect of progesterone.
4. Temporal incompetence of vesico-urethral valves. (Chakraborty, 1995)

Any anatomic barrier to free flow of urine through the urinary tract contributes to the development of UTI; involved are prostatic hypertrophy ,stone tumor, neurogenic disorders like spinal cord injury, multiple sclerosis etc.(Baron and Finegold,1990).

UTIs are very often encountered in patients with diabetes mellitus due to reasons that

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

UTIs are also encountered more frequently in micro-albuminuria or proteinuria patients due to sufficient availability of albumin in urine for bacterial multiplication (Hoepelman, 1994).

3.5 Pathogenesis

a. Routes of infection

Bacteria can invade and cause a UTI via two major routes: ascending and hematogenous pathways. Although the ascending route is the most common route of infection in females, ascent in association with instrumentation (e.g., urinary catheterization, cystoscopy) is the most common cause of hospital-acquired UTIs in both sexes. For UTIs to occur by the ascending pathway, enteric gram-negative bacteria and other microorganism that originate in the gastrointestinal tract must be able to colonize the periurethral area. Once these

organisms gain access to the bladder, they may multiply and then pass up the ureter to the kidneys. Hematogenous spread usually occurs as a result of bacteremia. Any systemic infection can lead to seeding of the kidney, but certain organisms, such as Staphylococcus aureus or Salmonella sp, are particularly invasive. Although most infections involving the kidneys are acquired by the ascending route, yeast(usually Candida albicans), Mycobacterum tuberculosis, Salmonella sp, Leptospira sp, or Staphylococcus aureus in the urine often indicate pyelonephritis acquired via hematogenous spread accounting > 5% of UTI (Stamm WE, 1988).

b. The Host - parasite relationship

Whether an organism is able to colonize and then cause a UTI is determined in large part by a complex interplay of host and microbial factors. In the majority of cases, the host defense mechanisms are able to eliminate the organisms .Urine itself is inhibitory to some of the urethral flora such as anaerobes. In addition, if urine has a low P^H, high or low osmolality, high urea concentration or high organic acid content, even organisms that can grow in urine may be inhibited. If bacteria do gain access to the bladder, the constant flushing of contaminated urine from the body either eliminates bacteria or maintains their numbers at low levels. Any interference with the act of normal voiding, such as mechanical obstruction resulting from kidney stones or strictures, will promote the development of UTI (Farrel *et al*., 2003).

Many microorganisms can cause UTIs, the majority is caused by only a few organisms like *E. coli.* Strains of it that because UTIs possess certain virulence factors that enhance their ability to colinize and invade the urinary tract as follows

1. The adhesion that has been most closely associated with uropathogenic E. *coli* is the P fimbriae. It binds not only to red cells but also to a specific galactose disaccharide that is found on the surfaces of uroepithelial cells in approximately 99.0% of the population.

2. Uropathogenic strains of E.coli are resistant to the complement dependent bactericidal effect of serum.

3. They can also produce siderophores that play an essential role in iron acquisition for the bacteria during or after colonization.

4. They also produce α-haemolysin, which are cytotoxic due to formation of Tran membranous pores in host cells.

5. The K antigens of *E. coli* 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.

Proteus strains 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 P^H that is directly toxic to kidney cells and also stimulates the formation of kidney stones. Similar findings have been made with *Klebsiella* sp. and *S. saprophyticus* also adheres better to uroepithelial cells than do *S. aureus* and *S. epidermidis*.

Suggested mechanisms of an increased susceptibility to UTI in diabetes microalbuminuric patients are

1. Decreased antibacterial activity due to 'sweet urine'

2. Increased multiplication of pathogens in urine due to enough protein.

3. Increased adherence to uro-epithelial cells.

Hyperglycemia by itself does not predictably increase bacterial rates of multiplication, however infection is established by the impaired neutrophil function in the presence of higher urinary or tissue glucose concentration (Geerlings *et al*., 1999).

Tamm- Horsfall protein is an important defense as it traps type 1 fimbriated *E.coli* in uromucoid present on epithelial cells and prevents adherence and cell entry. This protein is markedly reduced in patients with diabetes (Todar, 2002).

There is no study till now, to compare bacteremic strains in patients with and without diabetes /microalbuminuria, and there is no evidence yet to prove that uropathogens are indeed different between these populations. So well defined pathogenesis of uropathogens in diabetes micro-albuminuric patients could be explaining on basis of impaired transport system of metabolic end products and their consequences (Foggensteiner, 2000).

3.6 DIAGNOSIS OF URINARY TRACT INFECTION

All urine specimens brought to the microbiology laboratory should be examined at once, or placed in a refrigerator at 4^0C until they can be examined. 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. The examination procedure includes –

a. Specimen collection and transport.

b. Screening test

c. Bacteriological examination by the definitive culture.

d. Antibiotic susceptibility tests on clinically significant bacterial isolates.

a. Urine sample collection, transport and analysis

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

Prevention of contamination by normal vaginal, perineal, and anterior urethral flora is the most important consideration for collection of a clinically relevant urine specimen (Farrel *et al*., 2003).

Different methods adopted for collection of urine are –

a. Clean-catch, midstream urine

b. Straight catheterized urine specimen

c. Suprapubic bladder aspiration

d. Indwelling catheter

b. Specimen transport

Urine is an excellent supportive medium for growth of most bacteria, so it must be immediately refrigerated or preserved. Bacterial counts in refrigerated (40C) urine remain constant for as long as 24 hours. Urine can be preserved in tube containing boric acid, glycerol and sodium formate for as long as 24 hours when greater than 10^5 CFU/ml is present in initial urine specimen. For populations of patients from whom colony counts or organisms of less than 100,000/ml might be clinically significant, plating within 2 hours of collection is recommended (Collins *et al*., 1994).

c. Screening procedures

More than 50% 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. Many screening methods have been advocated for use in detecting bacteriuria and /or pyuria. These include microscopic methods, colorimetric filtration, bioluminescence, electrical impedance, enzymatic methods, photometric detection of growth, and enzyme immunoassay.

d. Macroscopic examination

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

e. Microscopic examination

It includes,

Erythrocyte count

Erythrocytes are found in small numbers in normal urine as in normal male and female as 0-2/hpf or 3-12 /µL. Increased number of erythrocytes in the urine may be present in renal diseases, acute appendicitis, urinary schistosomiasis, leptospirosis, infective endocarditis, malignancy of urinary tract and hemorrhagic conditions.

Leukocyte count

Normal urine contains 2-3 pus cells/hpf. Pyuria is usually regarded as significant when moderate or many pus cells are present i.e.> 10 WBC/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 etc. (Godkar, 2001). Pyuria is significant if more than or equal to 5 WBCs are seen under high power field in the sediment (Abyad, 1991; Block, 1990; Chakraborty, 2001; Steward *et al*, 1985).

Epithelial count

It is normal to find a few epithelial cells in urine. When seen in large numbers, however they usually indicate inflammation of the urinary tract or vaginal contamination of the specimen (Cheesbrough, 1984).> or equal to 5 squamous epithelial cells per high power field is considered as abnormal (Wargotz *et al*, 1999)

Gram stain of urine

Urinary Gram stain appears to be more reliable than urinalysis in detecting urinary tract infection .Gram stain had higher sensitivity than overall urinalysis (94 % versus 67%), higher specificity (92% versus 79%) and higher predictive value (53 % versus 23 %) (Lockhart *et al*., 1995).

The presence of at least two bacteria per oil immersion field of the Gram stained smear signifies bacteriuria of 100,000 or more colonies per ml (Pollock, 1983).

Enzymatic examination of urine

Frequently, screening tests detect bacteriuria or pyuria by examining for the presence of bacterial enzymes and /or PMN enzymes rather than the organisms or PMNs themselves.

Nitrate reductase (Greiss) test

It looks for the presence of urinary nitrite, an indicator of UTI. Nitrate-reducing enzymes that are produced by the most common urinary tract pathogens reduce nitrate to nitrite.

β-Glucuronidase test

This test is for rapid identification of urinary *E. coli*. The test determines the ability of an organism to produce the enzyme β-glucuronidase. The enzyme hydrolyses the 4-nitropheny-β-D-glucopyranosiduronic acid (PGUA) reagent to glucuronic acid and p-nitrophenol.

Leucocyte esterase test

Presence of pus cells in the urine is the evidence of host response against UTI. This inflammatory cells produce leukocyte esterase, so this test measures this enzyme in the urine. The nitrate reductase and leukocyte esterase tests have been incorporated into a paper strip for better screening but this combination screening test is not great enough to recommend its use as a stand-alone test in most circumstances(Fuselier P A et al., 2002).

Catalase test

This is rapid screening test, based on the detection of catalase present in somatic cells and in most bacterial species commonly causing UTIs except for S*treptococci* and *Enterococci* etc.

Chemical examination of urine

The chemical examination of urine for protein and glucose is an important part in the diagnosis of bacterial infection but routine testing of urine specimens for glucose and protein in the laboratory is not indicated (Cheesbrough, 2000).

Bacteriological examination of urine by culture

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, aymptomatic bacteriuria is common condition. Methods employed for the bacteriological culture may be quantitative or semi-quantitative.

Quantitative technique

Pour plate or surface viable count method

It is time consuming and expensive in the use of materials, so most laboratories do not practice this method.

Semi-quantitative technique

This technique is choice of most laboratories and more economical for processing large numbers of urine specimens.

Calibrated loop method

An inoculating loop of standard dimensions is used to take up a small, approximately fixed and known volume of mixed un-centrifuged 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 the 2 µl loopful of urine yields 25 colonies, then the approximate number will be 25 × 500 = 12,500 cfu/ml (Collee *et al*., 1999).

The recommended procedure uses a calibrated plastic or metal loop to transfer 1µl of urine to the culture medium (MacConkey agar with crystal violet and non-selective blood agar (WHO, 2004).

Filter-paper dip-strip method

It is based on the absorption and subsequent transfer of a fixed amount of urine to a suitable plating agar medium. It should be compared with the calibrated loop technique before

adopting the routine investigations. Strips are available commercially (Leigh and Williams, 1994).

Urine Dip Slide mehod

Urine Dip Slide is a commercially prepared special plastic device used for the bacteriological examination of urine samples. It contains MacConkey agar on one side and Cystine lactose electrolyte deficient (CLED) on the other side with grid surface under the CLED medium. UTI is signifies by count range from 200 to over 1000 (Faiers *et al*., 1991).

Urine Dip Spoon method

It is less convenient than other so, less practice in most of laboratories.

Antibiotic susceptibility test

The major concern of antibiotic susceptibility testing is to determine whether the bacterial etiology of concern is capable of expressing resistance to the antibiotics that are potential choices as therapeutic agents for managing the infection. So this test measures the ability of an antibiotic to inhibit bacterial growth in vitro. This ability may be estimated by either the dilution method or the diffusion method.

WHO recommended modified Kirby-Bauer disc diffusion technique, is used by the most laboratories to test routinely for antibiotic susceptibility. Using this test, antibiotic 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 organisms. 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).

3.7 Diagnostic criteria for the urinary tract infection

Confirmation of UTI based on finding appreciable numbers of pathogenic in the bladder urine (White *et al*., 1997). For many years, only the presence of at least 10^5cfu/ml in a clean-catch midstream urine specimen was considered clinically relevant for a diagnosis of urinary tract infection. This assumption has been challenged; some experts feel that 10^4cfu or even fewer may indicate infection. Others believe that the presence of polymorphonuclear leukocytes plays an important role in the pathology and clinical manifestations of UTI. It is not possible to define precisely the minimum number of bacteria per milliliter of urine that is definitely associated with UTI.

Table No 7, Recommendations for reporting UTI (WHO, 2004)

Category	**Count** (cfu/ml)	**Report**	**Remark**
1	$< 10^4$	Probable absence of UTI	Exceptions[1]
2	10^4-10^5	a. Asymptomatic	a. Request second urine sample
		b. Symptomatic UTI	b. Report the identification and drug sensitivity test
3	$>10^5$	UTI with count	c. Report the identification and drug sensitivity test

1. If less than 10^4 cfu /ml are present in urine taken directly from the bladder by suprapubic puncture or cystoscopy, symptomatic women or in the presence of leukocyturia – report the identification and susceptibility test.

If more than two species of bacteria are present in urine samples in categories 2 and 3, report as “Probably contaminated” and request to a fresh, clean catch specimen (WHO, 2004).

In order to confirm UTI with reasonable confidence, the criteria of clinical feature, bacteriuria and pyuria must be met. Significant bacteriuria is defined as bacteria which are 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;

1.Less than 10^4 cfu/ ml indicate contamination.

2.Equal to or more than 10^5cfu/ml indicate significant bacteriuria.

3.10^4- 10^5 cfu/ ml indicate low count significant bacteriuria.

Low count significant bacteriuria subject to the following conditions-

1. Patient under treatment and with obstruction in the ureter.

2. Urine was collected before the organisms reached to log phase of growth after the entry of bacteria into the urinary tract.

3. Some times in younger female, the count is low such as honeymoon cystitis.

4. Patient with certain endocrine disorder e.g. diabetes.

5. Chronic kidney infection where concentration power of kidney is low.

6. Infection with relatively slow growing organisms e.g. *S. saprophyticus*, Streptococci other than *Enterococci, Haemophilus influenzae* etc.

3.8 Etiological agents of urinary tract infection

Numerous micro-organisms (including bacteria, fungi and parasites) listed below are the possible pathogens most likely to cause urinary tract infection.

Table no 8, Possible pathogens in urine sample

Bacteria	**Possible Pathogens**
Gram Positive	*Staphylococcus aureus*, Coagulase negative *Staphylococcus*, *Streptococcus faecalis.*
Gram Negative	*E. aerogen,s Escherichia coli, Klebsiella pneumonia, K.oxytoca* *Proteus mirabilis, P.vulgaris, Enterobacter Cloacae, Providencia rettgeri,* *P. stuartii, Morganella morganii,* *Citrobacter fruendii, C.diversus, Serratia marcescen,* *Pseudomonas aeruginosa, Occasionally Salmonella typhi,* *Salmonella paratyphi and Neisseria gonorrhoeae*
b. Fungi	*Candida albicans*
c. Parasites	*Schistosoma haematobium and Trichomonas vaginalis*

Also, Mycobacterium tuberculosis, Leptospira interrogans, Chlamydia and Mycoplasma sp are also considered as possible pathogens of urinary tract infection (Cheesbrough, 1984).

Among the microorganisms causing UTI, *E. coli* is responsible for 74.6%, *Proteus* sp. responsible for 8.0%, Klebsiella sp. Responsible for 2.0%, Pseudomonas sp. is responsible for 2.0 % and other Gram negative organisms are responsible for13.3 % of the total cases of UTI (Mims *et al*., 1993).

E. coli is the most common infecting organisms in patients with uncomplicated UTI. 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* sp., *Enterobacter* sp., *Proteus vulgaris* and *Citrobacter* sp. are also frequent(Johnson,1991).*Candida* infection, *Gardnerella vaginalis* infection may occur in diabetic and immuno-compromised patients (Collins *et al*.,1986).

3.9 MICROBIOLOGY OF UTI IN DIABETES MICROALBUMINURIC PATIENTS

The bacteria causing UTI in diabetes microalbuminuric patients are the same as in complicated UTI in non-diabetes non-microalbuminuric patients. Bacteria and yeasts are the major pathogens. Enteric bacteria are common pathogens especially *E. coli* and *Klebsiella sp* (Wahl *et a*l., 1989).

P. aeruginosa is found in patients with previous experience of antimicrobial therapy. Among diabetic individuals with UTI, Proteus sp and S. aureus account for the remaining infections (Holt *et al*., 1984).

Urinary pathogens and Multidrug resistivity

The defining criterion for Multi-drug Resistant (MDR) is the resistance to $\geq$2 of the antimicrobial agents belonging to different structural classes (WHO, 2004).

For epidemiologic purposes, Multidrug Resistant organisms (MDROs) are defined as microorganisms, predominantly bacteria that are resistant to one or more classes of antimicrobial agents. Although the names of certain MDROs describe resistance to only one agent (e.g., MRSA, VRE), these pathogens are frequently resistant to most available

antimicrobial agents. These highly resistant organisms deserve special attention in healthcare facilities In addition to MRSA and VRE, certain GNB, including those producing extended spectrum beta-lactamases (ESBLs) and others that are resistant to multiple classes of antimicrobial agents, are of particular concern (Shrestha D, 2005).

The first report of bacterial drug resistance was in 1887. A scientist called Kossiakoff described the acclimatization of *Bacillus subtilis* to mercuric chloride and boric acid when the organism was grown in a medium containing these chemicals.

While working on penicillin, Florey and Chain in Oxford noted that some strains of *E. coli* produced a 'penicillinase' enzyme that inactivated the drug. This 'penicillinase', quickly renamed 'ß-lactamase' was soon to spread to MDR-strains other organisms.

Today, MRSA is resistant to all β-lactam antibiotics and in addition some strains have been reported to resistant to erythromycin, fusidic acid, tetracycline, monocycline, streptomycin, sulphonamides, disinfectants and toxic metals (mercury, cadmium etc).

Automated and Semi-automated Systems

Automated and Semi-automated screening systems offer the promise of a large throughput with minimal labor and a rapid turn-around time compared with conventional cultures. These methods works by examining images of un-centrifuged urine samples using a video camera, the yellow IRIS system is able to recognize many cellular structures, including leucocytes and bacteria. Due to substantial cost for the instrumentation, these methods are less practice in the developing countries like Nepal.

CHAPTER-IV

4. MATERIALS AND METHODS

4.1 MATERIALS

i. Equipments

ii. Biochemistry Analyser

iii. Waterbath

iv. Centrifuge

v. Micro-pipettes

vi. Microscope

vii. Autoclave

viii. Hot air oven and incubator

ix. Refrigerator

x. Glass wares; Petri plates, Culture tubes, Glass slides

xi. Media; Blood agar,Chocolate agar, MacConkey agar, Muller Hinton agar and Biochemical media, Hugh and Leifson Media, Hugh and Leifson Media Triple Sugar Iron Agar, Urea Agar and Simmon's Citrate agar

xii. **Reagents**

Reagent kits for sugar determination in blood and microalbumin determination in urine and Urine strip for qualitative estimation of urine sugar and urine albumin

Catalase reagent (3% H_2O_2), Barritt's reagent (40% KOH, 5% α-naphthol in a ratio of 1:3), Barium chloride, Sulfuric acid Kovac's reagent, Oxidase reagent (1%Tetramethyl*p*-phenylenediamine dihydrochloride.

Blood Plasma/serum/whole blood and urine sample

4.2 METHODOLOGY

The study was conducted in B & B Hospital, Gwarko, kathmandu from April 10, 2007 to August 20, 2007 in joint collaboration with Central Department of Microbiology, Tribhuvan Universtiy, Kirtipur.

A. Blood sample collection and determination

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.

1. Using a tourniquet, a suitable vein was located in the arm.
2. Wearing gloves, the venepuncture site as thoroughly disinfected using 70% ethanol; the area about 5 cm diameters was cleansed and allowed to dry.
3. 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.
4. The tube was allowed to stand in water bath at 37^0C for 10 minutes which causes blood to clot.
5. The clotted blood was centrifuged at 4500 rpm for 10 minutes for separation of serum.

Detection of blood sugar

Blood sugar was detected by enzymatic method using standard kit and standard protocol as provided by the manufacturer.

Method: Glucose oxidase method

Principle:

The aldehyde group of glucose is oxidized by glucose to give gluconic acid and peroxide

H_2O_2 ---------Peroxidase--------$H_2O + O_2$

The oxygen reacts with 4- Aminophenazone in the presence of phenol to form a pink colored compound and intensity of which can be determined at 530 nm in colorimeter or analyzer.

Normal value: Fasting; 75-115m

2Hrs PP; 80-140 mg/dl

Sample: Fluoride plasma or serum collected within 30 minutes of blood collection

Reagents: Glucose reagents kit mainely contains Buffer-Enzymes (Glucose Oxidase, Peroxidase, 4-Amino phenazone and Azide); Phenol Reagent and Glucose Standard. Stability of reagents at 2-8°C for 6 months.

Preparation of Glucose Reagent

2 parts of buffer/enzymes reagent and 1 part of phenol reagent are mixed to get reagent. It is preferable to prepare the reagent fresh daily. Otherwise it is stable at 2-8°C in an amber colored bottle for 2 weeks. A pink coloration of reagent due to aging does not affect the results if a standard & blank is put up with each batch of determination. All reagents are brought to room temperature before use.

Procedure

Pipette in the tubes labeled as follows

Table no 9, Procedure for glucose estimation in blood

	Blank	Standard	Test
1) Glucose reagent, ml	1.0	1.0	1.0
2) Dstilled water, ml	0.01	-	-
3) Glucose standard (100mg/ml), ml	-	0.01	-
4) Plasma/serum,ml	-	-	0.01

All tubes are mixed properly and kept at 37°C for 15 minutes or at room temperature for 30 minutes. The intensities of the color produced in all tubes are measured at 530 nm (green filter).

Calculation;

$$\text{Plasma (or serum) glucose, mg/dl} = \frac{\text{O.D. of Test} \times 100}{\text{O.D. of Standard}}$$

Programme can be designed for any specific automated analyzer.

B. Urine specimen collection, transport and analysis

The patient was given a sterile, dry, wide-necked leak-proof container and requested a 10-20ml of first morning mid-stream urine, explaining the need to collect the urine with as little contamination as possible i.e. a clean-catch specimen. The patient was instructed to cleanse the area around the urethral opening with clean water, dry the area and then begin to void and collect the mid-stream urine sample. The container labeled with the date, the name and the number of the patient, the time of collection was delivered to the laboratory along with the request form as soon as possible. When immediate delivery was not possible, the specimen was refrigerated at 4-6^{0}C, and when a delay in delivery of more than 2 hours was anticipated, boric acid (1.8 % w/v) was added as preservative to the urine.

1. Macroscopic examination

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

2. Chemical examination

a. Urine sugar and albumin detection

The detection of albumin and sugar was performed by using uristrix. 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. 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.

b. Acetone detection in urine

Diabetes mellitus is the most important disorder in which ketonuria occurs. Detection of ketonuria in a patient with diabetes mellitus is of great significance since a change in an insulin dosage or other management is often indicated. Detection of acetone in urine is also significant since it also classifies the diabetes mellitus in two categories; in type 1 diabetes, there is usually present of Acetone in urine where as in type 2, there is not detection of acetone in urine. Acetone is detected by Rothera's method where Nitroprusside in alkaline medium reacts with acetone to form a purple colored compound. Intensity of color is directly proportional to the concentration of acetone in urine.

c. Microalbumin detection in urine

Requirements

Micro-albumin reagent kits, racks, plastic disposable tubes, biochemistry analyzer, urine sample, micropipettes.

Principle

Detection of microalbumin depends on the immuno-agglutination method. Microalbumin presents in urine binds with anti- human immunoglobulin coated with latex particles from goat serum to form visible agglutinates which can be measured by analyzer in 430 nm filter. More the agglutinates, there will be more optical densities hence thereby more albumin in given urine samples.

Procedure

After careful urine sample collection, method is followed as below

Table no 10, Procedure for microalbumin determination in urine

	Blank	Standard	Test
Buffer solution, ml	0.5	0.5	0.5
Distilled water,µl	10	-	-
Standard (20mg/ml), µl	-	10	-
Urine sample, µl	-	-	10

After mixing properly and incubated at 37°C for 3 minutes, anti-human antiserum 20µl to standard and test except blank and incubated at 37°C for 5 minutes and optical densities will be taken at 430 nm in either colorimeter or biochemistry analyzer.

Calculation

Microalbumin, mg/dl== O/D. of test/O.D. of standard x 20

Results

A microalbumin test evaluates urine for the presence of a protein called albumin.Microalbumin is the most frequently caused by kidney damage from diabetes. Normal results may very depending on;

- The frequency
- The type (random verses timed) of urine sample collected.
- The time of the day of sample.
- Whether you are male or female.
- Whether you are rest or able to move about normally.

	Microalbumin	Albumin
Normal	< 2.1 mg/ml	< 30 mg/dl

Abnormal Results

You may need more than one test to find out how well your kidneys are working

- When your kidneys do not work well and leak between 165 and 300 mg of albumin in 24 hours, your doctor may check your urine more often to watch for kidney damage.
- If kidneys leak 300 mg or more of albumin in 24 hours (Microalbuminuria), we may have chronic kidney disease. If we have 2 or 3 high results in a 3 to 6 months period and you have diabetes, your doctor may find kidney damage (diabetic nephropathy) Even though diabetes is the most common resin for high results, there are many other kidney problems that can high results.

Pregnant women with diabetes may have their urine checked to watch for high amounts of albumin.

The test can be affected by

- High blood sugar levels, urinary tract infections, high blood pressure, heart failure or a high fever during an infection
- Recent exercise
- Medicaions such as aspirin, corticosteroids and some antibiotics suchas amoxicillin
- Menstrual bleeding and vaginal discharge, which may dilute the urine.

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 (> 10cells/ml) of urine will indicate significant pyuria .One WBC/LPF correspond to 3 cells/µL (Cheesbrough, 2000). Other tests in microscopic examination are RBCs, casts, crystals, epithelial cells and bacteria by making smear of deposit after centrifugation at 3500 rpm for 5 -10 minutes under high power microscope.

C. 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.

i. After mixing the urine sample in the container thoroughly, 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.
ii. Without flaming the loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies.
iii. The plates were incubated aerobically at 35-37^{0}C overnight.
iv. The approximate number of colonies was counted and the number of bacteria i.e. Colony forming unit (CFU) per ml urine 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.

Reporting of culture positivity;

1. Less than 10^4 /ml organisms: **not significant**
2. 10^4 -10^5 /ml organisms: **doubtful significance** (suggest repeat specimen)
3. More than 10^5 /ml organisms: **Significant bacteriuria**

However, if the culture indicated the appearance of ≥ three organisms types with no predominating organism, this was interpreted as due to possible contamination of the specimen and asked for another specimen (Forbes *et al.*, 2002).

i. 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 and Scott's, 1990; Cheesbrough, 1984; Mackie and McCartney, 1998).

The Gram negative isolates were identified by standard diagnostic procedure as:

- For lactose fermenters, media inoculation for motility, insole production and citrate utilization tests were carried out and incubated overnight.

- Individual colonies of clinically significant, lactose non-fermenters were inoculated into 2 ml of urea broth and incubated for 4 hr. at 37^0 C. Any urease positive culture was then plated to check for purity. The 4 hr. suspension would serve as the inoculum for biochemical tests for strains of other genera, and a purity check on MacConkey agar.

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

ii. Antibiotic susceptibility testing

Antibiotic susceptibility testing of the isolates was performed according to the guidelines given by the NCCLS recommended Kirby-Bauer susceptibility testing method. (NCCLS, 1999).

1. Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer.
2. The p^H 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.
3. 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.
4. After incubation in a good light source, the turbidity of the suspension was matched with the turbidity standard of MacFarland0.5.
5. Using a sterile swab, a plate of MHA was inoculated with the bacterial suspension using carpet culture technique.
6. 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 a90mm diameter Petri plate.
7. Within 30 minutes of applying the discs, the plates were taken for incubation at35^0C for 16-18 hrs.
8. 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

iii. 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 sub-

cultured in respective medium and incubated. The media was then checked for the appearance of pure growth of organism.

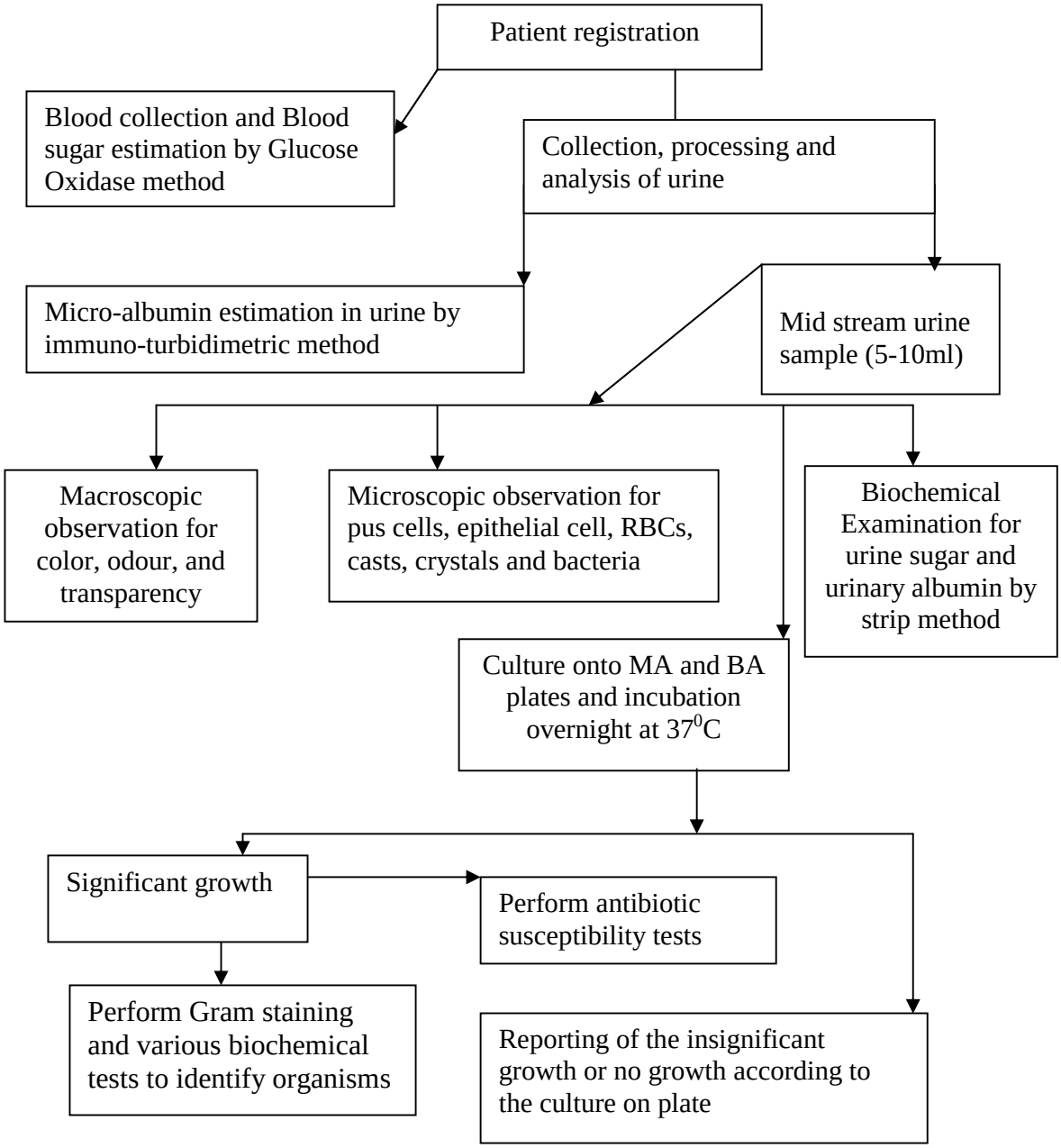

Figure 1: Flow diagram for processing blood and urine sample
(Source: Cheesbrough, 2000)

CHAPTER-V

5. RESULTS

5.1 Pattern of Blood sugar level in patients of different age group

Blood samples were collected from different age group of total 300 patients ranging from < 10 years of age to >90 years of age. Mostly normal blood sugar was found in patients of age < 30 years and generally higher blood sugar was found in age group > 30 years. The result is shown below,

Table no.11 Pattern of Blood sugar level in patients of different age group

Patients age group (years)	Blood sugar level, mg/dl						Total
	<100	100-126	>126-150	>150-180	>180-200	>200	
0-10	12	02	00	01	00	00	**15**
11-20	13	08	01	03	00	00	**25**
21-30	17	05	03	05	12	08	**50**
31-40	33	07	07	10	15	07	**79**
41-50	08	05	08	05	10	10	**46**
51-60	11	07	02	03	05	11	**39**
61-70	03	04	01	01	04	07	**20**
71-80	06	02	00	00	02	04	**14**
81-90	03	02	00	00	01	03	**09**
>90	01	01	00	00	00	01	**03**
Total	**107**	**43**	**22**	**28**	**49**	**51**	**300**

5.2 Pattern of Micro-albumin in patients of different age group

All 300 patients were requested to give the urine sample along with blood samples. Micro-albumin was determined both in diabetic and non-diabetic patients. Normal micro-albumin level was found in mostly in below 20 years of age. Increasing in this level was found in above 40 years of age. Among them 150 samples had normal micro-albumin and remaining samples showed varying grades of micro-albumin as shown below in table no 12.

150 samples were considered as control samples because their microalbumin level is within normal level (< 2.0 mg/dl) and remaining 150 samples were considered as cases as their microalbumin levels were higher than normal level.

Table no. 12, Pattern of Micro-albumin in patients of different age group

Patients age group (years)	Micro-albumin level in urine, mg/dl					Total
	≤2.0	2.1-5.0	5.1-10	10.1-20.0	>20	
0-10	07	02	00	00	00	**09**
11-20	13	04	02	00	00	**19**
21-30	37	36	03	01	02	**79**
31-40	43	42	07	02	01	**95**
41-50	22	20	08	01	00	**51**
51-60	13	07	02	00	00	**22**
61-70	03	03	01	00	00	**07**
71-80	05	02	01	00	00	**08**
81-90	05	01	00	00	01	**07**
>90	02	01	00	00	00	**03**
Total	**150**	**118**	**24**	**04**	**04**	**300**

5.3 Pattern of Micro-albumin in gender

Abnormal micro-albumin level or micro-albuminuria was found more in female patients as compared to male patients. Table no 10 shows that female patients have high concentration of micro-albumin up to the concentration 10 mg/ml while above 10 mg/ml male patients and female patients were equal in no. as shown below table.

Table no. 13, Pattern of Micro-albumin in gender

Micro-albumin level in urine	**Male**	**Female**	**Total**
	No.	**No.**	**No.**
2.1-5.0 mg/dl	44	74	118
5.1-10.0 mg/dl	06	18	24
10.1-20.0 mg/dl	02	02	04
>20 mg/dl	02	02	04
≤2.0 mg/dl	37	113	150
Total	91	209	300

5.4 Categorization of diabetes mellitus into type 1 and type 2 on the basis of acetone test in urine sample and its pattern on gender

Acetone test was performed in all 300 patients in diabetic patients to categorize the patient into type 1 and type 2 diabetes. Among 150 diabetic patients, maximum no. of diabetes patients had shown acetone positive in urine sample (type 1 diabetes) as shown in table no 14 below.

Table no.14, Categorization of diabetes mellitus into type 1 and type 2 on the basis of acetone test in urine sample and its pattern on gender

Acetone test				Diabetes mellitus				Non-diabetes		Total
Positive		Negative		Type 1		Type 2		negative acetone test		no. of patients
male	female	male	female	male	female	male	female	male	female	
38	72	-	-	38	72	-	-	-	-	110
-	-	16	24	-	-	16	24	-	-	40
-	-	-	-	-	-	-	-	37	113	150
										300

5.5 Pattern of patient requesting for urine culture

Out of 300 patients, 209 (30.4%) were female, while 91 (69.6%) were male. Of the total samples, 150 (50.0%) were from diabetic micro-albuminuric patients, among whom 96 (64.0%) were female and 54 (36.0%) were male. The results are shown in table 15.

Table no.15, Pattern of patient requesting for urine culture

	Male		**Female**		**Total**
	No.	**%**	**No.**	**%**	
Diabetes micro-albuminuric patient	54	36	96	64	150
Non-diabetes non-micro-albuminuric patient	37	24.67	113	75.33	150
Total	91	30.4	209	69.6	300

5.5.1 Significant growth of pathogens from urine samples of requesting patients.

In the case of diabetic micro-albuminuric patients the age group of 31-40 had the maximum requests of 54(36.0%) for urine culture followed by age group 21-30 of 48 (32.0%).Age group of >90 years requested the least with only 1 sample. Similarly, in case of non-diabetic non-micro-albuminuric patients age group 21-30 had the maximum requests of 48(32.0%) for urine culture followed by age group 61-70 of 23 (15.4%) . Age group of >90 years requested the least with only 1 sample. In the age group mentioned above female requests were more than male. The results are shown in table 16.

Table no 16, Significant growths of pathogens from urine samples of requesting patients.

Age Group	Diabetes micro-albuminuric patients			Non-diabetes non-micro-albuminuric patients			Total (%)
	Male	Female	Significant growth (%)	Male	Female	Significant growth (%)	
0-10	0	0	0 (0.0)	1	3	1 (2.6)	4 (1.3)
11-20	2	7	4 (5.2)	6	7	1 (2.6)	22(7.3)
21-30	16	32	28 (36.4)	6	42	11 (28.9)	96 (32.0)
31-40	20	34	30 (39.0)	3	9	3 (7.9)	66 (22.0)
41-50	4	10	8 (10.4)	3	18	4 (10.5)	35 (11.7)
51-60	3	8	5 (6.5)	5	13	6 (15.8)	29 (9.7)
61-70	5	3	0 (0.0)	7	16	9 (23.7)	31 (10.3)
71-80	2	0	1 (1.3)	2	5	2 (5.3)	9 (3.0)
81-90	2	1	0 (0.0)	3	0	1 (2.6)	6 (2.0)
>90	0	1	1 (1.3)	1	0	0 (0.0)	2 (0.7)
Total	**54**	**96**	**77**	**37**	**113**	**38**	**300**

5.5.2 Growth pattern of bacteria in urine sample

Out of 300 urine samples received, 115 (38.3%) samples showed significant growth, giving 115 bacterial isolates, and whereas. 106 (35.3%) showed no growth and 79(26.4%) showed no significant growth. Out of the total diabetic micro-albuminuric samples, majority ones i.e. 77 (54.1%) showed significant growth, 37(24.6%) showed no growth, and 36(24%) showed no significant growth Out of the total non-diabetic non-micro-albuminuric samples, 38 (25.4.1%) showed significant growth, majority ones i.e. 69(46.0%) showed no growth, and 43 (28.6%) showed no significant growth The results are shown in table 17

Table no.17, Growth pattern of bacteria in urine sample

Type of patient	Significant growth		No significant growth		No growth		Total
	No.	%	No.	%	No.	%	
Diabetes micro--albuminuric patient	77	51.4	36	24.0	37	24.6	**150**
Non-diabetes non-micro-albuminuric patient	38	25.4	43	28.6	69	46.0	**150**
Total	**115**	**38.3**	**79**	**26.4**	**106**	**35.3**	**300**

5.5.3 Pattern of Gender and Significant bacterial growth from types of diabetic patients

Among the total of 115 isolates in different categories of diabetes mellitus, type1, type2 and non-diabetes showed 61 (5.5%),16(40.0%) and 38(25.4%) significant bacterial growth respectively. In all cases the no of samples as well as isolate % were higher in female than male patients. The results are shown in table 18.

Table no.18, Pattern of Gender and Significant bacterial growth from types of diabetic patients

Type of patient	Male		Female		Total	
	No.	Isolate (%)	No.	Isolate (%)	No.	Isolate (%)
Type1	38	19 (17.3)	72	42 (38.2)	110	61 (55.5)
Type2	16	4 (10.0)	24	12 (30.0)	40	16(40.0)
Non-diabetes	37	8 (5.4)	113	30 (20.0)	150	38 (25.4)
Total	91	31 (10.4)	209	84 (28.0)	300	115 (38.4)

5.5.4 Correlation of diabetes with micro-albuminuria and significant growth

Among the total 61 isolates of type 1 diabetes ,highest significant growth was observed in the samples from 10.1-20mg/dl albumin as 3(100.0%),followed by 11(73.4%) in the samples from 5.1-10.0mg/dl albumin. Similarly, among the type2 diabetes highest significant growth was observed in the samples from 2.1-5.0mg/dl albumin as 14(46.7%), followed by 2(22.3%) in the samples from 5.1-10.0mg/dl albumin. The results are shown in table 19

Table no. 19, Correlation of diabetes with microalbuminuria and significant growth

Microalbumin level in urine	Type1 Diabetes		Type2 Diabetes		Non-diabetes		Total
	No. (%)	Isolate (%)	No. (%)	Isolate(%)	No. (%)	Isolate(%)	
2.1-5.0 mg/dl	**92(75.4)**	**47(51.0)**	30(24.6)	**14(46.7)**	0(0.0)	0(0.0)	**122**
5.1-10.0 mg/dl	15(62.5)	11(73.4)	**9(37.5)**	2(22.3)	0(0.0)	0(0.0)	24
10.1-20.0 mg/dl	3(75.0)	3(100.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	4
≤2.0 mg/dl	0(0.0)	0(0.0)	0(0.0)	0(0.0)	150(100.0)	38(25.4)	150
Total	110	61(55.5)	40	16(40.0)	150	38(25.4)	300

5.5.5 Pattern of significant bacterial growth according to Gram's stain

Among the total 115 isolates, maximum isolates 111(96.5%) were found to be Gram negative bacilli (GNB) and the remaining 4(3.5%) were found to be Gram positive cocci (GPC). In the case of 77isolates from diabetic micro-albuminuric patients, 74(96.1%) were found to be GNB whereas 38 isolates from non-diabetic non-micro-albuminuric patients 37(97.1%) were found to be GNB**.** The results are shown in table 20.

Table no.20, **Pattern of significant bacterial growth according to Gram's stain**

Isolated organisms	Diabetic micro-albuminuric patients		Non-diabetic non-microalbuminuric patients		Total
	No.	%	No.	%	
Gram negative bacteria	74	66.7	37	33.3	111
Gram positive bacteria	3	75.0	1	25.0	4
Total	77		38		115

5.5.6 Pattern of microbial isolates from the urine sample

A total of 115 bacterial uropathogens were isolated from growth positive urine samples of diabetic micro-albuminuric and non-diabetic non-micro-albuminuric patients. Among both cases GNB are predominant. Among the isolates, *E .coli* was found to be most predominant

in the both groups ie 42(54.5%) diabetic micro-albuminuric and 35(92.2%) non-micro-albuminuric patients.This followed by *Klebsiella pneumoniae* as16(20.9%) in diabetic microalbuminuric. Similarly, among non-microalbuminuric patients by *Enterobacter aerogenes Citrobacter freundii* and *S .epidermidis* as 1(2.6%) each. The results are shown in the table 21.

Among the total of 115 isolates, highest no of isolates i.e. 39 belonged from the patients of age-group 21-30, this was followed by 33 isolates from the age group of 31-40. *Escherichia coli* the most predominant of the species, was isolated chiefly from patients of age-group 21-30 as 39, this was followed by the 31-40 as 33. One *Escherichia coli* was isolated from patient of age-group >90.

Table no.21, Pattern of microbial isolates from the urine sample

Types of bacteria	**Diabetic microalbuminuric Patients**		**Non-diabetic non-Microalbuminuric patients**		**Total**
Gram negative bacteria	**No. of isolate**	**% of the isolate**	**No. of isolate**	**% of the isolate**	
Escherichia coli	42	54.5	35	92.2	77
Klebsiella pneumoniae	16	20.9	0	0.0	16
Pseudomonas aeruginosa	7	9.0	0	0.0	7
Enterobacter aerogenes	3	3.9	1	2.6	4
Proteus mirabilis	3	3.9	0	0.0	2
Citrobacter freundii	3	3.9	1	2.6	4
Gram positive bacteria					
Alpha *Streptococci*	1	1.3	0	0.0	1
Staphylococcus aureus	2	2.6	0	0.0	2
S.epidermidis	0	0.0	1	2.6	1
Total	77	100.0	38	100.0	115

5.5.7 Antibiotic susceptibility pattern of gram negative bacteria isolated from diabetic micro-albuminuric patients

Among the common antibiotics used against all Gram-negative isolates, Imipenem was the drug of choice with a susceptibility of 56 (91.9%). Imipenem followed Meropenem with susceptibility of 85.3%. Most of the Gram negative isolates, i.e.55 (90.1%) were resistant to Amoxycillin. The results are shown in table 22.

Table no. 22, Antibiotic susceptibility pattern of gram negative bacteria isolated from diabetic micro-albuminuric patients

Antibiotic used	Sensitive		Intermediate		Resistant		Total
	No. of Isolates	%	No. of Isolates	%	No. of Isolates	%	
Amoxycillin	6	9.9	0	0.0	**55**	**90.1**	61
Amikacin	42	68.9	0	0.0	19	31.1	61
Chloramphenicol	37	60.7	2	3.3	22	36.0	61
Cefepime	11	18.0	3	5.0	47	76.0	61
Ciprofloxacin	41	67.3	0	0.0	20	32.7	61
Co-trimoxazole	8	13.2	1	1.7	52	85.1	61
Imipenem	**56**	**91.9**	1	1.7	4	6.4	61
Gentamycin	35	57.4	1	1.7	25	40.9	61
Kanamycin	9	14.8	0	0.0	52	85.2	61
Ofloxacin	41	67.3	0	0.0	20	32.7	61
Meropenem	52	85.3	2	3.3	7	11.4	61
Nalidixic acid	6	9.9	1	1.7	54	88.4	61
Nitrofurantoin	25	42.4	3	5.0	31	52.6	59
Tobramycin	7	11.5	2	3.3	52	85.2	61

5.5.8 Antibiotic susceptibility pattern of gram positive bacteria isolated from UTI patients

Among the Gram positive isolates, 3(100.0%) of them were susceptible to tetracycline is the drug of choice. Tobramycin, this is followed by the co-trimoxazole and ciprofloxacin sensitive as 75 % each. Nalidixic acid, Penicillin-G was found to be the least effective as only 0 (0.0%) isolates were sensitive. The results are shown in table 23.

Table no.23, Antibiotic susceptibility pattern of gram positive bacteria isolated from UTI patients

Antibiotic used	Sensitive		Intermediate		Resistant		Total
	Isolate no.	%	Isolate no.	%	Isolate no.	%	
Amoxycillin	2	50.0	0	0.0	2	50.0	4
Tetracycline	**3**	**100.0**	0	0.0	0	0.0	3
Co-trimoxazole	3	75.0	0	0.0	1	25.0	4
Ciprofloxacin	3	75,0	0	0.0	1	25.0	4
Cefepime	1	33.4	0	0.0	2	66..6	3
Cephlexin	1	25.0	0	0.0	3	75.0	4
Kanamycin	0	0.0	0	0.0	2	100.0	2
Tobramycin	0	0.0	0	0.0	**2**	**100.0**	2
Erythromycin	1	25.0	0	0.0	3	75.0	4
Nalidixic acid	0	0.0	0	0.0	1	**100.0**	1
Penicillin-G	0	0.0	0	0.0	2	**100.0**	2
Cloxacillin	2	50.0	0	0.0	2	50.0	4
Ofloxacin	1	50.0	0	0.0	1	50.0	2

5.5.9 Antibiotic susceptibility pattern of gram negative bacteria isolated from Non-diabetic non-micro-albuminuric patients

Among the common antibiotics used against all Gram-negative isolates, Imipenem was the drug of choice with a susceptibility of 9 (90.0%). Imipenem followed by Amikacin with susceptibility of 12(83.4%). The least effective drug for Gram negative isolates is Gentamycin as susceptibility.12 (100.0%). The results are shown in table 24.

Table no.24, Antibiotic susceptibility pattern of gram negative bacteria isolated from non-diabetic non-microalbuminuric patients.

Antibiotic used	Sensitive		Moderately sensitive		Resistant		Total
	Isolate no.	%	Isolate no.	%	Isolate no.	%	
Amoxycillin	27	71.0	0	0.0	11	29.0	38
Amikacin	10	83.4	0	0.0	2	16.7	12
Chloramphenicol	9	75.0	0	0.0	3	25.0	12
Ciprofloxacin	21	55.3	0	0.0	17	44.7	38
Co-trimoxazole	19	50.0	0	0.0	19	50.0	38
Imipenem	**9**	**90.0**	0	0.0	1	10.0	10
Gentamycin	0	0.0	0	0.0	**12**	**100.0**	12
Ofloxacin	22	59.5	0	0.0	15	40.5	37
Meropenem	8	80.0	0	0.0	2	20.0	10
Nitrofurantoin	32	86.5	0	0.0	5	13.5	37

5.6 Antibiotic susceptibility pattern of *E .coli* isolated from urine sample

The antibiotic susceptibility pattern of *Escherichia coli* showed that Nitrofurantoin was the drug of choice as 47 (94.4%) of isolates were sensitive towards this drug. Meropenem was the second most effective drug with 42 (84.0%) of the isolates sensitive. Nalidixic acid was found to be the least effective with only 2(5.0 %) isolates sensitive. The results are shown in table 25.

Table no.25, Antibiotic susceptibility pattern of *E. coli* isolated from urine sample

Antibiotic used	Sensitive		Intermediate		Resistant		Total
	Isolate no.	%	Isolate no.	%	Isolate no.	%	
Amoxycillin	12	16.0	0	0.0	63	84.0	75
Amikacin	38	73.1	0	0.0	14	26.9	52
Chloramphenicol	34	65.4	1	1.9	17	32.7	52
Cefepime	9	22.5	1	2.5	30	75.0	40
Ciprofloxacin	45	60.0	0	0.0	30	40.0	75
Co-trimoxazole	19	25.7	0	0.0	55	74.3	74
Imipenem	**47**	**94.0**	1	2.0	2	4.0	50
Gentamycin	22	42.3	0	0.0	30	57.7	52
Kanamycin	8	20.0	0	0.0	32	80.0	40
Ofloxacin	46	61.3	0	0.0	29	38.7	75
Meropenem	42	84.0	1	2.0	7	14.0	50
Nalidixic acid	2	5.0	0	0.0	**38**	**95.0**	40
Nitrofurantoin	44	58.7	1	1.3	30	40.0	75
Tobramycin	4	10.0	1	2.5	35	87.5	40

5.6.1 Antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from urine sample

The antibiotic susceptibility pattern of Staphylococcus aureus showed that tetracycline was the drug of choice as 2 (100.0%) of isolates were sensitive towards this drug. Cotrimoxazole, Ciprofloxacin, Ofloxacin and Cloxacin were the second most effective drug with 50 % of the isolates sensitive .Cefepime, Kanamycin,Cephalexin, Tobramycin, Erythromycin, Nalidixic acid and penicillin G were found to be the least effective with only 0(0.0 %) isolates sensitive. The results are shown in table 26.

Table no.26, Antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from urine sample

Antibiotic used	Sensitive		Intermediate		Resistant		Total
	Isolate no.	%	Isolate no.	%	Isolate no.	%	
Amoxycillin	0	0.0	0	0.0	**2**	**100.0**	2
Tetracycline	**2**	**100.0**	0	0.0	0	0.0	2
Co-trimoxazole	1	50.0	0	0.0	1	50.0	2
Ciprofloxacin	1	50.0	0	0.0	1	50.0	2
Cefepime	0	0.0	0	0.0	**2**	**100.0**	2
Cephalexin	0	0.0	0	0.0	**2**	**100.0**	2
Kanamycin	0	0.0	0	0.0	**2**	**100.0**	2
Tobramycin	0	0.0	0	0.0	**2**	**100.0**	2
Erythromycin	0	0.0	0	0.0	**2**	**100.0**	2
Nalidixic acid	0	0.0	0	0.0	**1**	**100.0**	1
Penicillin-G	0	0.0	0	0.0	**2**	**100.0**	2
Cloxacillin	1	50.0	0	0.0	1	50.0	2
Ofloxacin	1	50.0	0	0.0	1	50.0	2

5.6.2 Pattern of MDR pathogens in diabetic micro-albuminuric and non-diabetic non-micro-albumunuric patients

Among the total isolates of 111 exhibiting drug resistance, 71 isolates from both test and control samples showed multi-drug resistance pattern. Total 8 different bacterial isolates are involved in the drug resistance. Among these isolates, 100.0% multi-drug resistance was shown by *S. aureus* which is followed by *E. coli* as 68.9%.Higher rate of multi-drug resistance was found on diabetic micro-albuminuric patients as 67.2% while in control cases it was found as 57.9%. The results are shown in table 27.

Table no.27, Pattern of MDR pathogens in diabetic micro-albuminuric and non-diabetic non-micro-albumunuric patients

Organisms	diabetic Microalbuminuric Patients			non-dibetic Microalbuminuric patients			Total
	Isolate (MDR)		%	Isolate (MDR)		%	
Escherichia coli	42	(33)	78.6	35	(20)	57.2	**77 (53)**
Klebsiella pneumoniae	16	(8)	50.0	0	(0)	0.0	**16 (8)**
P. aeruginosa	7	(4)	57.2	0	(0)	0.0	**7 (4)**
Enterobacter aerogens	0	(0)	0.0	1	(1)	100.0	**1 (1)**
Staphylococcus aureus	2	(2)	100.0	0	(0)	0.0	**2 (2)**
S.epidermidis	0	(0)	0.0	1	(1)	100.0	**1 (1)**
Proteus mirabilis	3	(1)	33.4	0	(0)	0.0	**3 (1)**
Citrobacter fruendii	3	(1)	33.4	1	(0)	0.0	**4 (1)**
Total	**73**	**(49)**	**67.2**	**38**	**(22)**	**57.9**	**111(71)**

5.6.3 STATISTICAL PATTERN OF THE RESULTS

The statistical analysis of different results obtained above is scrutinized and test with appropriate statistical methods. We tested different parameters (i.e. independent variables) with the culture positivity like diabetic micro-albuminuria, gender, types of diabetes mellitus and level of albumin in urine to find the significant association exists or not. Results of these tests are tabulated in the table 24 .Details are given in the appendix**.**

The sensitivity and specificity of urine culture test to detect UTI on the basis of sugar and albumin level in the urine sample was found 67% and 60.55 respectively. This result is compatible with the finding got by Sandberg *et al*. (1985).He reported significant albuminuria (measured by immunoassay) in 8 of 15 non-pregnant women with cystitis. Amongst 100 consecutive hospitalized patients with culture-confirmed UTI, a reagent-strip result trace for protein showed a sensitivity for UTI of 63%[.In contrast, another type of result was got by Van Nostrand *et al*.(2000) He used a stepwise b**i**nary logistic regression analysis to test the ability of various urinalyses to predict infection amongst 225 urine samples, 33 of which were culture positive. Proteinuria did not have a statistically significant independent relationship with presence of UTI (odds ratio 1.29, $P = 0.504$).

Photograph 2 Antibiotic susceptibility test of *Escherichia coli* showing inhibition zones on Mueller-Hinton agar.

(Am- Amoxycillin; Nf- Nitrofurantoin; Na-Nalidixic Acid; G- Gentamicin; Cf- Cifrofloxacin; C- Chloramphenicol)

.

Photograph 2 Multi-drug resistant Gram negative isolate (*Escherichia coli*) on Mueller-Hinton agar

Photograph 3 Biochemical tests for *Escherichia coli.*

Key: TSI-Triple sugar iron: y/y no H_2S but gas Citrate test: Negative
SIM-Simmon's Indole motility: Positive MR (methyl red): Positive
VP (Voges-proskaur test): Negative Urease test: Negative
O/F (oxidative/Fermentative): Positive Nitrate reduction test: Positive

Photograph 4 Researcher performing work at lab

CHAPTER-VI

6.1 DISCUSSION

The present study was conducted in B & B Hospital, Gwarko, and Kathmandu from April 10, 2007 to August 20, 2007 among diabetic micro-albuminuric and non-diabetic non-micro-albuminuric patients suspected of urinary tract infection. 300 blood samples and 300 mid stream urine samples were collected from patients visiting in out patient department at B & B hospital and all samples were subjected to biochemical and routine examination. All 300 mid stream urine samples were processed for culture and antibiotic sensitivity.

Out of 300 samples, 150 samples were from diabetic micro-albuminuric patients and remaining 150 samples were from non-diabetic non-micro-albuminuric patients as control. Establishment of diabetic micro-albuminuria and non-diabetic non-micro-albuminuria were accomplished by blood sugar/ urine sugar and micro-albumin level in urine samples. Out of 300 urine samples processes, 91(30.4) were from male, while 209(69.6) were from female. Age group of 21-30 years had the maximum requests of 96 (32.0%). Age group of 31-40 years had maximum request of 36%for urine culture among diabetic micro-albuminuric patients whereas age group 21-30 years had maximum request of 32% for urine culture among non-diabetic non-micro-albuminuric patients. This variation is based on fact that diabetes and its consequences like micro-albuminuria are normally associated with higher age. Although, maximum request for urine culture was from age group of 21-30 but highest growth positivity was found from age group of 61-70 among control cases. There are various supporting facts behind this occurrence. The patients from age group of 21-31 have more chances of exposure to uropathogens, more sexually active, higher cases of pregnancies and high consciousness to health .All these factors compel to request for urine culture but patients of elder age have been associated with other complications like diabetes, nephropathy, micro-albuminuria/proteinuria, decreased immune response etc. in addition to factors mentioned above to establish UTI.

Out of total samples, 115 (38.4%) showed significant growth, among which 73% were from female whereas 27% from male. A similar study carried out by Dhakal *et al*. (1999) showed growth positivity of 25.16% and in their study, among the total requests for urine culture; 53.46% were from female patients. Among highest growth positivity in diabetic micro-albuminuria was found in age group of >90 years as 100.0%. This indicates that diabetic microalbuminuria and bacteriuria are quite common in later stage of life. This finding is

compatible with similar type of research carried out by Kosakai *et al* (1990); Rajbhandari and Shrestha (2002).

Out of 150 diabetic micro-albuminuric patients 77 (51.4%) showed significant growth, among which 70.2% were from female whereas 29.8% from male. Similarly, 150 non-diabetic non-microalbuminuric control samples, 38 (25.4%) showed significant growth, among which 79.0% were from female whereas 21.0% from male patients. The positive growth in diabetic micro-albuminuria was found higher, when compared to that in control. This finding showed significant difference between positive growth in diabetic microalbuminuric and non-diabetic non-microalbuminuric patients ($p<0.05$). A study carried out by Puri *et al.* (2006) showed that growth isolates of UTI in diabetes mellitus was higher, when compared to that in controls (22.0% vs 12.0%, $p>0.05$) Similar study carried out by Gautam *et al.*(2003) showed compatible findings between diabetic and non diabetic patients($p<0.05$). Moreover, this result pattern was also similar by findings of Goswami *et al.* (2001), Ronald *et al* (2001) and Mendoza *et al.* (2002) on similar type of research. Increased occurrence of UTI among diabetic micro-albuminuric patients than non-diabetic non-micro-albuminuric patients in our study was due to many reasons but major ones are – decreased antibacterial activity due to swine urine, defects in neutriphil function, enough availability of protein and increased adherence to uro-epithelial cells.

The higher number of requests and higher growth positivity seen in females was found to be statistically insignificant ($p>0.05$). The reasons behind obtaining such result in our study is due to lack of coherency in different age groups among both test and control cases and insufficient sample size, if sample size had been increased then a significant association could have been established.

Among the total isolates, 96.5% were Gram negative bacteria. In a similar study performed by Blomberg *et al.* in Tanzania in 1996, out of 107 urinary isolates, 66.36% constituted Gram negative isolates. Among the total 77 isolates from the test, 96.2% were from Gram negative bacteria whereas 97.4% were from Gram negative bacteria in control. In a similar study carried out by Puri *et al.* (2006) showed 81.8% and 100.0% Gram negative bacterial isolates were found among diabetic and non-diabetic urine samples respectively.

Altogether, seven species and 3 species of total nine genuses of bacteria were isolated from test and control in this study. Among them, *E .coli* was the most predominant isolate accounting 67.0% in total isolates and followed by *K. pneumoniae* as 14.0% among total

isolates in diabetic micro-albuminuric and non-diabetic non-microalbuminuric patients, *E. coli* accounting 54.5% and 92.2% respectively. This was followed by *Klebsiella pneumoniae* 20.9% among diabetic micro-albuminuric and *C. freundii, S. epidermidis and Enterobacter aerogenes* as 2.6% among non-diabetic non-micro-albuminuric patients. This result is supported by similar findings by Puri *et al.* (2006) and Goswami *et al.* (2001). Higher prevalence of *E. coli* seen in this study was compatible with the study done by various other workers viz: Akbar *et al.*(2001); Chhetri *et al.* (2001); Dhakal *et al.* (2002); Kosakai *et al.* (1990); Jha and Yadav (1992); Manandhar *et al.* (1995); Mendoza *et al.* (2002); Mohammad *et al.* (2005); Rajbhandari and Shrestha (2002); *K. pneumoniae* was second principal isolate among total isolate and similar result of second predominant bacteria among Gram negative bacteria was obtained by Bomjan *et al.* (2005) and Dhakal *et al.* (1999).

But Bais *et al.* (1980) in India found *Pseudomonas aeruginosa* as the predominant organism followed by *E. coli* and *Klebsiella* species. The reason for predominant *E. coli* isolation is that it can bind to the glycoconjugate receptor of the epithelial cells of human urinary tract (Bock *et al*., 1985) so it can initiate infection itself. *E. coli* is the most predominant organism to colonize the urethral meatus (Schaeffer and Chmiel, 1983) and perineum (Leigh, 1990) before ascending to the bladder. This ability of *E .coli* is the important reason to be the most frequent organism to cause UTI in both sexes all over the world.

E. coli infection is high in female as compared to male, in both diabetic microalbuminuric and non-diabetic non-microalbuminuric patients. In our study, also 76.6% E. coli infection was confined in female as compared to male in both test and control cases. Similar type of result was found by Dhakal *et al.* (1999); Manandhar *et al*. (1995); Gautam *et al.* (1998); Obi *et al.* (1996).

Seven *E. coli,* one *Pseudomonas aeruginosa,* one *Staphylococcus aureus, one Proteus mirabilis* and one *Citrobacter freundii* were isolated from age group 41-50 in total isolates. Among these one *Staphylococcus aureus* and one *Pseudomonas aeruginosa* were isolated from diabetic patients of micro-albumin level 5.1-10 mg/dL. These uropathogens are involved in bladder carcinogenesis and this result is harmony with the fact that these are primary pathogen in compromised host (Koskai *et al*., 1990) .The incidence of *S. aureus* in older age reflects pyelonepritis acquired via haematogenous spread. So it unwise to assess

the higher albumin level from this bacteria infected patient was due to diabetes mellitus (Baron and Finegold, 1990).

Type1 microalbuminuric patients had higher percentage of isolate then type2 micro-albuminuric patients as 55.5% and 40.0% respectively. This result is harmony with the findings from Geerlings *et al*. (2000). He demonstrated among Dutch women with type1 diabetes high prevalence of asymptomatic bacteriuria. Our result could get more validity in these findings if we could go through the clinical data of patients, pus cell count etc. However our result of higher proportion of occurrence of UTI in type 1 micro-albuminuric patients is not statistically significant ($p>0.05$). The authors postulate that asymptomatic bacteriuria and other diabetic complications including nephropathy may have their origins in common pathophysiological processes (e.g. endothelial dysfunction and oxidative stress).

Phanichphant and Boonpucknavig (1986) provide further evidence that female glomerulopathic patients with proteinuria exceeding 1g/day are more likely to harbor asymptomatic UTI than equivalent patients with lower levels of protein excretion. Similarly, Rai *et al*. (2001) have demonstrated that among patients with nephrotic syndrome the concomitant presence of asymptomatic bacteriuria was associated with higher urinary protein excretion ($P< 0.05$). These authors mention that these studies provide some evidence that patients with more advanced nephropathy, defined in terms of urinary protein excretion, may be more susceptible to UTI, but they neither refute nor support the existence of a causative relationship between UTI and proteinuria. But our studies gave the result of significant association of UTI to the increase level of micro-albumin in urine ($p<0.05$) (See appendix)

The sensitivity and specificity of urine culture test on the basis of urine sugar and albumin level was found 67% and 60.5 % among diabetic micro-albuminuric patients respectively. Similarly, Positive predictive value and negative predictive value were found as 51.4% and 74.6% respectively. A similar study carried out by Puri *et al.*(2006) showed sensitivity 59.0% and specificity 89.7% .Higher value of these predictors in our study for urine culture test are compatible with many findings and reviews worldwide. 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.

Johnson *et al*. (1974) reported that of the 1684 girls screened, 10 had 0.3 g/l protein of which three had coexistent bacteriuria. Most cases asymptomatic UTI exists in the absence of proteinuria /microalbuminuria and *vice versa*. It is also unwise to mention that micro-albuminuria does not appear to be a useful screening test for identifying asymptomatic bacteriuria in all cases. However, none of these studies set out to formally test whether a causal relationship exists, and none provided evidence on the effect of antibiotic treatment in bacteriuric individuals and other confounding factors on micro-albuminuria. In a small study of eight children with relapsed nephrotic syndrome and coexistent bacteriuria (mostly asymptomatic), treatment of the infection with antibiotics was found to have an additional effect on reducing protienuria (estimated 'semi-quantitatively') in addition to the treatment with steroids alone (Kumar *et.et al*, 1977). This could be interpreted as evidence that UTI contributed to proteinuria /microalbuminuria although it is difficult to establish whether the UTI was the cause of the relapse or the result of it.

Among the common antibiotics used against all Gram negative isolates of diabetic micro-albuminuric patients, Imipenem was the drug of choice, followed by Meropenem with susceptibility 91.9% and 85.3% respectively. Similarly, Imipenem again was the drug of choice against all Gram negative isolates of control patients with susceptibility as 90.0%, followed by Nitrofurantoin with susceptibility 86.5%. Our finding of Nitrofurantoin as major drug of choice for Gram negative uro-pathogens was compatible with the result pattern of many researchers (Puri *et al.*, 2006; Dhaka *et al.* 1999; Gautam *et al*. 1998; Jha and Yadav, 1992). Among all Gram positive isolates tetracycline was found drug of choice with susceptibility 100.0% and this is followed by Co-trimoxazole and ciprofloxacin with susceptibility 75.0% each. Tobramycin, Nalidixic acid were found least effective drug for Gram negative isolate with sensitivity 0.0% each. Our result of co-trimoxazole as one of the drug of choice is harmony with the finding of Puri *et al.* (2006). But Maartens and Oliver (1994) suggested that co-trimoxazole should no longer be prescribed for urinary tract infection unless susceptible isolate has been cultured. So it seems clear that we should go pathogen directed therapy rather than empirical therapy for proper treatment of UTI even though of clear findings in past.

Most frequently isolated bacteria was *E. coli* in both test and control patients. Imipenem showed highest drug sensitive against *E. coli* with susceptibility 94.0% followed by Meropenem and Amikacin with susceptibility 84.0% and 74.1% respectively. Nalidixic acid was found least effective drug with susceptibility as 5%. Similar finding was obtained by Dhakal *et al.* (1999). Similarly, most frequent Gram positive uropathogen *S. aureus* showed

that tetracycline is drug of choice with susceptibility 100% whereas Amoxycillin, Nalidixic acid, Tobramycin, Kanamycin, Erythromycin, Cefepime and Penicillin G were least effective drug with susceptibility 0.0% each.

Out of 100.0% isolates of *S. aureus*,and *S. epidermidis* all were the MDR-strains, and isolated from diabetic micro-albuminuric patients and control patients respectively. There is no significant association of MDR and non-MDR strains among diabetic micro-albuminuric and Non diabetic non-microalbuminuric patients ($p>0.05$).Higher frequency of drug resistant by *E. coli* among common drugs like Amoxicillin, Nalidaxic acid ,Ciprofloxacin, Kanamycin, Co-trimoxazole and Cefepime with resistance 84.0%, 40%, 95.0%, 80.0%, 74.3% and 80.0% respectively

Resistance towards β-lactam drug in Gram negative enteric uropathogens is mediated primarily by β-lactamase, which can hydrolyze the β-lactam ring and thus inactivates the antibiotic. The classical TEM-1, TEM-2 and SHV-1 enzymes are the predominant plasmid-mediated β-lactamases of Gram negative rods (Livermore, 1995). In addition MDR efflux pump system also contributes the emergence of multi-drug resistance primarily in *E. coli* (Sulaik *et al.,* 2001). Moreover, other newer β-lactamase are now increasing concern towards the emergence of MDR strains in Gram negative rods, mainly extended- spectrum β-lactamase, Hyper AmpC β-lactamase producers , Inhibitor resistant β-lactamase, broad spectrum β-lactamase, metallo β-lactamase etc. Till now, there is no established fact about the association of these MDR predictors of uropathogens with level of sugar and albumin in urine.

6.2 CONCLUSION

Hence, the study of urinary tract infection in the patients of diabetic micro-albuminuria visiting B & B hospital was accomplished. There is strong association of culture positivity of the urine samples from diabetic micro-albuminuric patients but statistically insignificant among gender. The sensitivity and specificity of urine culture test on the basis of urine sugar and micro-albumin level was found high which can be exploiting to diagnose UTI in diabetic micro-albuminuric patients.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

SUMMARY

1. Altogether, 300 samples were analyzed. Out of total 150 samples were screened for diabetic micro-albuminuria whereas remaining 150 samples were screened for non-diabetic non-microalbuminuria .
2. Out of the 150 mid stream urine samples processed from diabetic micro-albuminuric patients , 77(51.4%) samples showed significant growth, among them 49 (63.7%) were MDR-strains. Out of the 150 mid stream urine samples processed from non-diabetic non-microalbuminuric patients, 38(25.4%) samples showed significant growth, among them 22 (57.9%) were MDR-strains. Out of total isolates 61.7% were MDR.
3. Frequency of positive growth of isolates was found to be higher in diabetic microalbuminuric patients (51.4%) in comparison to non-diabetic non-microalbuminuric patients(25.4%) which is found statistically significant ($p<0.05$).
4. Rate of urinary tract infection was found to be higher in female, as 40.2%of the positive culture results (84/209) were obtained from female patients but the result was statistically insignificant.
5. The predominant bacteria causing UTI were the Gram negatives which constituted 96.5% (111/115) as majority ones from diabetic micro-albuminuric patients (74/111). Gram positive bacteria constituted only 3.5% (4/115) and of them majority ones were from diabetic micro-albuminuric patients 3/ 4).
6. Altogether 9 different species of bacteria were isolated from the growth positive cultures. *Escherichia coli* (N=77) was found to be the most predominant isolate (67.0%). Among Gram positives, *Staphylococcus aureus* (N=2) was the most predominant with 1.7 % of total isolates.
7. Urine culture test on the basis of urinary sugar and albumin showed the sensitivity and specificity 67.0% and 60.55% respectively. The positive predictive value and negative predictive value were 51.4 % and 74.6 % respectively.
8. Imipenem was found to be the most effective drug with a susceptibility of 91.9% against the Gram negative bacteria among diabetic micro-albuminuric patients whereas susceptibility of 90.0% against non-diabetic non-microalbuminuric patients. Similarly, Tetracycline was found to be the most effective agent against Gram positive bacteria

with susceptibility of 100%. These drugs were also the most effective against the MDR-strains among the isolates

9. Out of the total *E. coli* isolates 78.6% and 57.2% were found to be MDR strains among diabetic microalbuminuric and nondiabetic non-microalbuminuric patients. Similarly out of 2 isolates 100.0% were MDR strains among diabetic micro-albuminuric patients and no isolates and MDR strains were found from non-diabetic non-microalbuminuric patients.

RECOMMENDATIONS

1. Detailed clinical history of diabetic micro-albuminuric patients as well as control should be taken to draw valid conclusion whether diabetes micro-albuminuria is real factor for urinary tract infection or other complications because in that groups of patients other complications like glomerulonephritis, neuropathy and bladder dysfunction are common problem.
2. Type1 diabetes is highly associated with the asymptomatic UTI so it will be better to diagnose UTI whether symptomatic, asymptomatic or complicated.
3. Detection of protein in the urine is also important for the diagnosis of UTI and the patients with albuminuria should be advised for urine culture.
4. Systemic bacterimia may be the cause of UTI so it is advisable to early diagnose of causative pathogens and treatment.
5. Microorganisms involved to cause UTI in diabetic micro-albuminuric patients express different virulence factors like subunit –A,G-adhesin I,II,III ,aerobactin etc. so characterization of these factors among different organisms are important .
6. Genotypic characterization of MDR strains should be carried out in order to establish the location of drug resistance genes.
7. Determination of microalbumin in urine in UTI patients should be carried out to prevent the kidneys from further bacterial invasion and damage.

CHAPTER-VIII

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CHAPTER-IX

9. APPENDICES

APPENDIX-I

QUESTIONNAIRE

CLINICAL AND MICROBIOLOGICAL PROFILE OF PATIENT

Clinical Profile:

Name: … … … … … … … … … … … … … … … … Lab No: … … … …

Age / Sex: … … … … … … … … …… … … … … Date: … … … …

…

Address: … … … … … … … … … … … … … … Patient: OPD / IN

Ward: … … … …

…

Bed No: … … … …

Brief Clinical History:

Patients with history of diabetes mellitus Yes No

Duration of disease:……..

Chronic kidney disease Yes No

If yes, Duration of disease:……

Patient taking medication Yes No

Patient on antibiotics: Yes No

If Yes, Antibiotic(s) taken: 1) … … … … … … … … 2) … … … …… …

… …

Duration of treatment: … … … …… … … …… … … …

Microbiological Profile:

Day 1 (… … / … … / … … … …)

Specimen: … … … … … … … …

Time of sample collection: … … … … … … … …

Mode of Collection: … … … … … … … … Receiving time at the laboratory:

… … … …

Direct Microscopic observation (if necessary):

1) … … … … … … … …

Incubation: 1) Aerobic 2) Anaerobic 3) Microaerophilic

Incubation temperature: … … … … … … … … Incubation time: … … … … … … … …

Culture on: 1) … … … … 2) … … … … … 3) … … … … …

Day 2 (… … / … … / … … … …)

Reading of Culture Plates:

Media used	*Feature*	*Shape*	*Size*	*Color*	*Texture*	*Opacity*	*Consistency*

Gram staining results: … … … … … … … … … … … … … … … … …

Catalase: … … … … … … … … … Oxidase: … … … … … … … … …

Coagulase: … … … … … … … … … Others: … … … … … … … … …

Provisional Identification of Organism: … … … … … … … … … … …

Inoculation on: 1) … … … … … … 2) … … … … … … 3) … … … … … …

Day 3 (… … / … … / … … … …)

BIOCHEMICAL TESTS

TSI: … … … … … … … … … SIM: … … … … … … … … …

Citrate: … … … … … … … … … Urea: … … … … … … … … …

Others: … … … … … … … … …

Organism Identified as: …

Antibiotic sensitivity test method: **Kirby-Bauer Method**

Antibiotics used	***Zone of inhibition (mm)***	***Interpretation***

Note:… … … … … … … … … … … … … … … … … … … …

Performed by Checked by

APPENDIX-II

I. Composition and Preparation of Different Culture Media

The culture media used were from two companies
A.Hi-Media Laboratories Pvt. Limited, Bombay, India.
B Oxoid Unipath Ltd. Basingstoke, Hampshire, England

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

1. Blood agar (BA)

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

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

Final pH (at 25^0C) 7.3±0.2

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

2. Chocolate agar (CA)

The sterilized blood agar was poured in Petri plates and was allowed to solidify and was heated at 75^0C in an oven for 30 minutes. By this time, the color changes to chocolate brown.

3. MacConkey Agar (MA)

(Without sodium taurocholate, without salt and crystal violet)

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

Final pH (at 25^0C) 7.4±0.2

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

4. Mueller Hinton Agar (MHA)

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

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

5. Nutrient Agar (NA)

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

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

6. Nutrient Broth (NB)

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

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

II. Biochemical Test Media

1. MR-VP Medium

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

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

2. Hugh and Leifson's Medium

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

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

3. Sulphide Indole Motility (SIM) medium

Ingredients	gm/litre
Beef Extract	3.0
Peptone	30.0
Peptonized Iron	0.2
Sodium Thiosulphate	0.025
Agar	3.0
Final pH (at 25^0C)	7.3±0.2

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

4. Simmon Citrate Agar

Ingredients	gm/litre
Magnesium Sulfate	0.2
Mono-ammonium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Agar	15.0
Bromothymol Blue	0.08
Final pH (at 25^0C)	6.8±0.2

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

5. Triple Sugar Iron (TSI) Agar

Ingredients	gm/litre
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0
Final pH (at 25^0C)	7.4±0.2

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

6. Christensen Urea Agar

Ingredients	gm/litre
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Dipotassium Phosphate	1.2
Mono-potassium Phosphate	0.8
Phenol Red	0.012
Agar	15.0
Final pH (at 25^0C)	7.4±0.2

24 grams of the medium was suspended in 950 ml distilled water and sterilized by autoclaving at 121^0C for 15 minutes. After cooling to about 45^0C, 50 ml of 40% urea was added and mixed well. Then 5 ml was dispensed in test tube and set at slant position.

III. Staining and Test Reagents

1. For Gram's Stain

(a) Crystal Violet solution

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

Distilled Water (D/W) to make 1 litre

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

(b) Lugol's Iodine

Potassium Iodide	20.0 g
Iodine	10.0 g
Distilled Water	1000 ml

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

(c) Acetone-Alcohol Decoloriser

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

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

(d) Safranin (Counter Stain)

Safranin	10.0 g
Distilled Water	1000 ml

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

3. Normal saline

Sodium Cholride	0.85 g
Distilled Water	100 ml

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

4. Test Reagents

a. For Catalase test

Catalase Reagent (3% H_2O_2)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

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

b. For Oxidase Test

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

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

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

c. For Indole Test

Kovac's Indole Reagent

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

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

d. For Methyl Red Test

Methyl Red Solution

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

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

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

Solution A

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

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

Solution B

Potassium hydroxide	40.0 g
Distilled Water	1000 ml

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

f. Bile Salt Solution

Commercially available sodium deoxycholate	10 g
Distilled Water	100 ml

Preparation: A 10% solution of sodium deoxycholate was prepared by adding 10 gm sodium deoxycholate powder in 100 ml distilled water and transferred in a clean brown bottle and was autoclaved.

g. Name of Sputasol

"Dithiothreitol"

CODE-SR 89

Oxoid Limited, Basingstoke

Hampshire, England.

APPENDIX-III

A. Gram-staining Procedure

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

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

APPENDIX-IV

1. BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

A. Catalase test

During aerobic respiration, in the presence of oxygen, microorganisms produce hydrogen peroxide, which is lethal to the cell itself. Catalase enzyme breaks down hydrogen peroxide into water and oxygen. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, the main exception being *Streptococcus* sp.

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

B. Oxidase test

This test is performed for the detection of cytochrome oxidase in bacteria which catalyzes the transport of electrons between electron donors. In the presence of redox dye Tetramethyl-*p*-phenylene diamine dihydrochloride, the cytochrome oxidase oxidizes it into a deep purple colored end product Indophenol which is detected in the test.

A piece of filter paper was soaked with few drops of oxidase reagent. Then the colony of the test organism was smeared on the filter paper. The positive test is indicated by the appearance of blue-purple color within 10 seconds.

C. Oxidation-Fermentation test

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

The test organism was stabbed into the bottom of two sets of tubes with Hugh and Leifson's media, bromothymol blue being the pH indicator. The inoculated medium in one of the tubes was covered with a 10 mm deep layer of sterile

paraffin oil. The tubes were then incubated at 37°C for 24 hours. After incubation the tubes were examined for carbohydrate utilization as shown by acid production. Fermentative organism utilizes the carbohydrate in both the open and sealed tubes as shown by a change in colour of the medium from green to yellow. Oxidative organisms, however, are able to use the carbohydrate only in the open tube.

D. Indole Production test

This test detects the ability of the organism to produce an enzyme: 'tryptophanase' which oxidizes tryptophan to form indolic metabolites: indole, skatole (methyl indole) and indoleacetic acid.

A smooth bacterial colony was stabbed on SIM (Sulphide Indole Motility) medium by a sterile stab wire and the inoculated media was incubated at 37°C for 24 hours. After 24 hours incubation, 0.5 ml of Kovac's reagent was added. Appearance of red color on the top of media indicates indole positive. Indole if present combines with the aldehyde present in the reagent to give a red color in the alcohol layer. The color reaction is based on the presence of the pyrrole structure present in indole.

E. Methyl Red test

This test is performed to test the ability of an organism to produce sufficient acid from the fermentation of glucose to give a red color with the indicator methyl red (denotes changes in degree of acidity by color reactions over a pH range of 4.4-6.0).

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

F. Voges Proskauer (VP) test

This test is employed to detect the production of acetyl methyl carbinol (a neutral end product) or its reduction product 2, 3-butanidiol during fermentation of carbohydrates.

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

G. Citrate Utilization test

This test is performed to detect whether an organism utilizes citrate as a sole source of carbon for metabolism with resulting alkalinity. Organisms capable of utilizing citrate as its sole carbon source also utilizes the ammonium salts present in the medium as its sole nitrogen source, the ammonium salts are broken down to ammonia with resulting alkalinity.

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

H. Motility test

The motility media used for motility test are semisolid, making motility interpretations macroscopic. Motile organisms migrate from the stabline and diffuse into the medium causing turbidity. Whereas non-motile bacteria show the growth along the stabline, and the surrounding media remains colorless and clear.

I. Triple Sugar Iron (TSI) Agar

The TSI agar is used to determine the ability of an organism to utilize specific carbohydrate incorporated in the medium (glucose, sucrose and lactose in concentrations of 0.1%, 1.0% and 1.0% respectively), with or without the production of gas (indicated by cracks in the media as well as an air gap at the bottom of the tube) along with determination of possible hydrogen sulfide production (detected by production of black color in the medium).

The test organism was streaked and stabbed on the surface of TSI and incubated at 37°C for 24 hours. Acid production limited only to the butt region of the tube is indicative of glucose utilization, while acid production in slant and butt indicates sucrose or lactose fermentation. Phenol red is the pH indicator which gives yellow reaction at acidic pH, and red reaction to indicate an alkaline surrounding.

J. Urea Hydrolysis test

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

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

K. Coagulase test

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

Slide Coagulase Test

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

For slide coagulase test, a drop of physiological saline was placed on three places of a slide, and then a colony of the test organism was emulsified in two of the drops to make thick suspensions. Later a drop of plasma was added to one of the suspensions and mixed gently. Then a clumping was observed within 10 seconds for the positive coagulase test. No plasma was added in second suspension. This

was used for the differentiation of any granular appearance of the organism from true coagulase clumping. The third drop of saline was used for a known strain of coagulase positive staphylococci.

Tube Coagulase Test

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

In the tube coagulase test, plasma was diluted 1 in 10 in physiological saline. Four small tubes were taken, one for test organism, one for positive control, one for negative control, and one to observe self clotting of plasma. Then 0.5 ml of the diluted plasma was pipetted into each tube and 0.5 ml of test organism, 0.5 ml of positive control (*Staphylococcus aureus* culture), and 0.5 ml negative control (*Staphylococcus epidermidis* culture) was added to three tubes, to the fourth tube, 0.5 ml sterile broth was added. After mixing gently, all tubes were incubated at 37^0C on a waterbath for 6 hours and observed for gel formation in every 30 minutes.

L. DNase (Deoxyribonuclease) test

This test is used to identify *Staphylococcus aureus* which produces deoxyribonuclease (DNase) enzyme. The deoxyribonuclease enzyme hydrolyses the DNA. The test organism was cultured on a medium containing DNA. After overnight incubation, the colonies were tested for DNase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolysed DNA. DNase producing colonies are therefore seen as clear areas surrounding colonies due to DNA hydrolysis.

APPENDIX-V

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

A. Equipments

1. Oven
2. Incubator
3. Autoclave
4. Refrigerator
5. Microscope
6. Centrifuge
7. Water bath
8. Weighing balance
9. Laminar Flow
10. Coagulator
11. Distillation Plant

B. Antibiotic Discs

Different antibiotics discs used for the sensitivity tests were from different companies as:

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

APPENDIX-VI

1 .Association of Culture positive and culture negative among diabetic micro-albuminuric and non-diabetic non-micro-albuminuric patients

	Culture positive	Culture negative	Total
Diabetic micro-albuminuric Patients	77	73	150
Non-diabetic nonmicroalbuminuric patients	38	112	150
Total	115	185	

Test statistics is χ^2

H_0: There is no significant association of culture positive and culture negative among diabetic micro-albuminuric and non-diabetic non-microalbuminuric patients.

H_1: There is significant association of culture positive and culture negative among diabetic micro-albuminuric and non-diabetic non-microalbuminuric patients.

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ we find from $\chi^2 = 24.44$

Thus χ^2_{cal} (24.44) > χ^2_{tab} at α =0.05(3.84) and α =0.01(6.63) as d.f. = 1

Hence, H_0 is rejected.

Result: It is meaningless to say that there is no significant association of culture positive and negative among diabetic micro-albuminuric and non-diabetic non-microalbuminuric patients i.e. the higher proportion of culture positive cases seen among diabetic microalbuminuric is statistically significant.

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

	Culture positive	Culture negative	Total
Male	31	60	91
Female	84	125	209
Total	115	185	

Test statistics is χ^2

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

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

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ We find From $\chi^2 = 0.84$

Thus χ^2_{cal} (0.84) > χ^2_{tab} at α =0.05(3.84) and α =0.01(6.63) as d.f. = 1

Hence, Ho is accepted.

Result: There is no significant association of culture Positive and negative among male and female patients i.e. the higher proportion of culture positive cases seen among female is not statistically significant

3. Association of culture positive and culture negative among Type1 and Type2 diabetic patients.

	Culture positive	Culture negative	Total
Type 1 patients	61	49	110
Type2 patients	16	24	40
Total	77	73	150

Test statistics is χ^2

H_0: There is no significant association of culture positive and culture negative among Type1 and Type 2 patients.

H_1: There is significant association of culture positive and culture negative among Type1 and Type 2 patients.

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ We find From $\chi^2 = 2.8$

Thus χ^2_{cal} (2.8) < χ^2_{tab} at α =0.05(3.84) and α =0.01(6.63) as d.f. = 1

Hence, H0 is accepted.

Result: There is no significant association of culture Positive and negative among Type1 and Type 2 patients.i.e.the higher proportion of culture positive cases seen among Type1 is not statistically significant.

4 Association of culture positive and culture negative among different micro-albumin level in urine sample

	Culture positive	Culture negative	Total
≤2.0 mg/dl	38	112	150
2.1-5.0 mg/dl	61	61	122
5.1-20.0 mg/dl	16	12	24
Total	115	185	300

Test statistics is χ^2

H_0: There is no significant association of culture positive and culture negative among different micro-albumin levels on urine sample.

H_1: There is significant association of culture positive and culture negative among different micro-albumin levels on urine sample.

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ we find from χ^2 = 23.58

Thus χ^2_{cal} (23.58) > χ^2_{tab} at α =0.05(5.99) and α =0.01(9.21) as d.f. = 2

Hence, Ho is rejected.

Result: There is no reason to say that there is no significant association of culture Positive and negative among different micro-albumin level in urine sample, suspected of UTI patients i.e. the higher the level of albumin in urine, greater the chance of acquiring the UTI.

5. Association of MDR and Non-MDR strains among Diabetic microalbuminuric and Non diabetic non-microalbuminuric patients.

	MDR strains	Non-MDR strains	Total
Diabetic microalbuminuric Patients	50	11	61
Non-diabetic non-microalbuminuric patients	22	5	27
Total	72	16	88

Test statistics is χ^2

H_0: There is no significant association of MDR and non-MDR strains among diabetic micro-albuminuric and non- diabetic non-micr-albuminuric patients.

H_1: There is significant association of MDR and non-MDR strains among diabetic micro-albuminuric and non-diabetic non-microalbuminuric patients.

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ we find from $\chi^2 = 0.003$

Thus χ^2_{cal} (0.003)< χ^2_{tab} α =0.05(3.84) and α =0.01(6.63) as d.f. = 1

Hence, H_0 is accepted.

Result: There is no significant association of MDR and non-MDR strains among diabetic micro-albuminuric and Non diabetic non-microalbuminuric patients.i.e. Higher possibility of multi-drug resistance among diabetic micro-albuminuric patient is not statistically significant.

6. Association of Types of diabetic patients and their level of micro-albumin among suspected UTI cases.

	2.1-5.0 mg/dl	5.1-20.0 mg/dl	Total
Type 1 patients	92	18	110
Type2 patients	30	10	40
Total	122	28	150

Test statistics is χ^2

H_0: There is no significant association of types of diabetic patients and their micro-albumin level.

H_1: There is no significant association of types of diabetic patients and their micro-albumin level.

From $\chi^2 = \Sigma \frac{(O - E)^2}{E}$ we find from $\chi^2 = 1.44$

Thus χ^2_{cal} (1.44) < χ^2_{tab} α =0.05(3.84) and α =0.01(6.63) as d.f. = 1

Hence, H_0 is accepted.

Result: There is no significant association of types of diabetic patients and their micro-albumin level .i.e. Level of micro-albumin in urine is depending upon types of diabetes is not statistically significant.

7. Evaluation of urine culture test to detect UTI on the basis of sugar and albumin level in the urine sample.

Screening test results	Culture positive	Culture negative	Total
Diabetic microalbuminuric Patients (Cases)	77	73	150
Non-diabetic nonmicroalbuminuric Patients (Control)	38	112	150
Total	115	185	300

a. Sensitivity = (77/115) x 100 = 67.0 %

b. Specificity = (112/185) x 100 = 60.5 %

c. Positive predictive value = (77/150) x 100 = 51.4 %

d. Negative predictive value = (112/150) x 100 = 74.6 %

APPENDIX-VII

TableNo.1Distinguishing reactions of the commoner and pathogenic Enterobacteriaceae

Species	Test/ substrate[a]											
	lac	mot	gas	ind	VP	cit	PDA	ure	lys	H_2S	inos	ONPG
Escherichia coli	+	+	+	+	-	-	-	-	+	-	-	+
Shigella groups A, B, C	-	-	-	±	-	-	-	-	-	-	-	-
Sh. sonnei	-	-	-	-	-	-	-	-	-	-	-	+
Salmonella (most serotypes)	-	+	+	-	-	+	-	-	+	+	±	-
Salmonella typhi	-	+	-	-	-	-	-	-	+	+	-	-
Salmonella paratyphi A	-	+	+	-	-	-	-	-	-	-	-	-
Citrobacter freundii	±	+	+	-	-	+	-	±	-	±	-	+
C. koseri	±	+	+	+	-	+	-	±	-	-	-	+
Klebsiella pneumoniae	+	-	++	-	+	+	-	+	+	-	+	+
K. oxytoca	+	-	++	+	+	+	-	+	+	-	+	+
Enterobacter aerogens	+	+	++	-	+	+	-	-	+	-	+	+
Ent. cloacae	+	+	+	-	+	+	-	±	-	-	-	+
Hafnia alvei	-	+	+	-	+	-	-	-	+	-	-	+
Serratia marcescens[b]	-	+	±	-	+	+	-	-	+	-	±	+
Proteus mirabilis	-	+	+	-	±	±	+	++	-	+	-	-
P. vulgaris	-	+	+	+	-	-	+	++	-	+	-	-
Morganella	-	+	+	+	-	-	+	++	-	±	-	-

morganii												
Providencia rettgeri	-	+	-	+	-	+	+	++	-	-	+	-
Prov. stuartii	-	+	-	+	-	+	+	±	-	-	+	-
Prov. alcalifaciens	-	+	+	+	-	+	+	-	-	-	-	-
Yersinia enterocolitica[c]	-	-	-	±	-	-	-	±	-	-	±	+
Y. pestis	-	-	-	-	-	-	-	-	-	-	-	±
Y. pseudotuberculosis	-	-	-	-	-	-	-	+	-	-	-	±

[a] lac, inos, fermentation of lactose, inositol; mot, motility; gas, gas from glucose; ind, indole production; VP, Voges-Proskauer; cit, Citrate utilization (Simmons'); PDA, phenylalanine deaminase; ure, urease; lys, lysine decarboxylase; H_2S, H_2S produced in TSI agar; ONPG, metabolism of *o*-nitrophenyl-β-D-galactopyranoside.
[b] Some strains of Serratia marcescens may produce a red pigment
[c] *Yersinia* are motile at 22°C.

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

(Source: Collee *et al.,* 1996)

APPENDIX-VIII

ZONE SIZE INTERPRETATIVE CHART

Antimicrobial Agent	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Amikacin	Ak	30 µg	14	15-16	17
Amoxycillin When testing Gram-negative enteric organisms When testing Staphylococci When testing *Haemophilus* sp.	A	10 µg	13 28 18	14-16 - 19-21	17 29 22
Ceftazidime	Ca	30 µg	14	15-17	18
Cephalexin	Cp	30 µg	14	15-17	18
Chloramphenicol	C	30 µg	12	13-17	18
Ciprofloxacin	Cf	5 µg	15	16-20	21
Cloxacillin	OB	5 µg	11	12-13	14
Erythromycin When testing Staphylococci When testing Streptococci	E	15 µg	13 15	14-22 16-20	23 21
Gentamicin	G	10 µg	12	13-14	15
Imipenem	I	10 µg	13	14-15	16
Nitrofurantoin	Nf	300 µg	14	15-16	17
Meropenem	Mr	10µg	13	14-15	16
Norfloxacin	Of	10 µg	12	13-16	17
Ofloxacin When testing Streptococci When testing Staphylococci	Of	5µg	12 12 14	13-15 13-15 15-17	16 16 17

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