

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



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**Determination of Bacteriological profile of Antimicrobial  
Susceptibility Testing of Microbs cause UTI in Diabetic  
Patients In Shendi Hospitals.**

A thesis Submitted for partial fulfillment of the Msc Degree in Medical Laboratory  
Sciences in ( Microbiology )

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# الآية

بسم الله الرحمن الرحيم

قال تعالى :

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ﴾ ﴿١﴾ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ﴿٢﴾ ﴿اقْرَأْ  
وَرَبُّكَ الْأَكْرَمُ﴾ ﴿٣﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿٤﴾ عَلَّمَ الْإِنْسَانَ مَا لَمْ  
يَعْلَمُ ﴿٥﴾

صدق الله العظيم

سورة العلق : الآيات (١ - ٥)



## *Dedication*

*To my wonderful parents who strongly supported  
me all throughout.*

*To my beloved sister and adorable brother.*

*To all those whom I always love, care and respect.*

## **Acknowledgments**

*All thanks to Allah from start to end. The authors would like to thank the staff at clinical laboratory.*

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## Abbreviations

<b>Abbreviation</b>	<b>Mean</b>
IDDM	Insulin Dependent Diabetes Mellitus.
NIDDM	Non Insulin Dependent Diabetes Mellitus.
UTI	Urinary tract infection
WHO	World Health Organization

## Abstract

**Background:** The urinary tract infection is common microbial disease and second most infection in women due to anatomy of women and type2 diabetic is high risk to urinary tract infection .

**Objectives:** To determine the causative agent of urinary tract infection in diabetic patient and to determine the suitable antibiotic and evaluate which type of diabetes associated with urinary tract infection.

**Methodology:** a descriptive study in Shendi locality in different ages .hundred sample is med stream urine collection from Shendi hospitals from May to August 2018 and interpretation of culture growth, and made gram stain and biochemical test. And anti microbial susceptibility testing.

**Results:** The study result found of *E.coli* is the most prevelant bacteri aabout (24,6%) followed the *Staphylococcu saureus* and *Saprophiticus* in precentage about (16%) and *K.pneumonia* and *C.albican* about (14.4%) and follow with *E.feacilus* about (5.2%) and *pseudomonas* about (3.8%), *Protus vulgaris* about (2.6%) made antbiotic sensitivity that the gentamicin is high precentage about (36%) and cefatazdime has high percentage of resistant about (35%) ceftrixone and cloxacillin are resistance about (52%)

**Conclusion:** The causative bacteria detected in diabetic patient is *E.coli* and most of isolated we sensitive to gentamicin and resistance to cefatazdime.

## المستخلص:

**مقدمة :** عدوى الجهاز البولي هي أمراض ميكروبية شائعة وثاني أكثر عدوي للأثنى نتيجة إلى

تشريح الأثنى والسكري من النوع الثاني هو أكثر خطر للإصابة بعدوى الجهاز البولي .

**الهدف :** لتحديد العوامل المسببة لعدوى الجهاز البولي في مرضى السكري وتحديد المضاد

الحيوي المناسب وتقييم أي نوع من أنواع السكري مرتبط بعدوى الجهاز البولي .

**المنهجية :** هي دراسة وصفية في مدينة شندي في أعمار مختلفة تم جمع مائة عينة من منتصف

التدفق البولي في مستشفيات شندي في الفترة من مارس إلى أغسطس ٢٠١٨ وتوزيع وتفسير نمو

التوزيع وعمل صبغة الغرام واختبارات البيو كيميائية واختبار الحساسية للمضادات الحيوية.

**النتائج :** الدراسة نتجت وجود اشريكية قولونية منتشرة بصورة كبيرة حوالي ٢٧,٦% تبع

بوجودالعنقودية الذهبية و المكورات العنقودية المترمة بنسبة حوالي ١٦% كلبسيلا والفطريات

بنسبة حوالي ١٤,٤% ويتبع لوجود انتيروا كوكس فيكيلس بحوالي ٥,٢% والزائفة بحوالي

٣,٨% والمتقلبة الاعتيادية بحوالي ٢,٦% وتم عمل الحساسية للمضادات الحيوية وجد جنتاميسين

وهو اكثر حساسية بنسبة حوالي ٣٦% وسيفتازيديم هو أكثر نسبة مقاومة حوالي ٥٣%

.سيفتراكزون وكلوازسليين مقاومة بحوالي ٥٢%.

**الخلاصة:** أكثر البكيريا انتشاراً عند مرضى السكري الاشريكية القولونية حساسة للمضاد

الحيوي جينيامايسين ومقاومة إلى سيفرايازيديم.

# Chapter One

**Introduction**

**Rationale**

**Objectives**

## 1.1 Introduction

Urinary tract infection is very often encountered in patient they may present themselves as asymptomatic bacteruria with diabetic patient. The urinary tract infection lead to inflammatory response. The urinary tract infection the common microbial disease and the urinary tract infection the second most infection in women infection than men due to anatomy of women and type 2 diabetic is high risk to urinary tract infection than non diabetic.

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both and has been estimated that 347 million people worldwide have diabetes, and Nepal with the mortality rate . The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs especially the eyes, genitourinary system, nerves, heart, and blood vessels<sup>[3]</sup>.

Over time, patients with diabetes may develop cystopathy

High urine glucose content and defective host immune factors predispose to infection .the diabetes mellitus and obstruction of urinary tract is predominant risk factor for developing of urinary tract infection.

The most common organism causes the urinary tract infection are *Escherichia coli*, *klebsiella pneumonia* and *Candida*.

The diabetes type 2 is high risk of urinary tract than non diabetic patient .their antimicrobial drugs susceptibility and diabetes patient have higher severity urinary tract infection which can be cause of complication the women diabetic under premenopausal and postmenopausal period aside with sexual activity are considered increase risk for developing urinary tract infection. The diabetic patients have up to four times more urinary tract infection risk when they are in oral treatment or insulin injection. The high glucose level in the urine that make present of risk of urinary tract infection due to present of high bacteria in urine.

An antimicrobial is substance active against bacteria and used in treatment and prevention of such infection may either kill or inhibit the growth of bacteria . the antimicrobial susceptibility used to detective drug resistance in common

pathogen .the most widely method used are microdilution or rapid automated instrument and the common susiptability testing method are broth dilution method and antimicrobial gradient method, disk difufusion method ,automated instrument method .<sup>(1)</sup>

## **1.2 Rationale**

Urinary tract infection is one of most serious health problem in diabetes mellitus. In this study we want to detect and determine the causative agent of urinary tract infection and confirm in this study present of urinary tract infection in Type 2 is higher than type one .we attempt to urinary tract infection in the diabetes.urinary tract infection are not usually serious but they can be dangerous if the bacteria make their way into kidney, Specially little studies were conducted in this area.

## **1.3 Objectives**

### **1.3.1 General objective**

To determine the causative agents of urinary tract infection in diabetic patients

### **1.3.2 Specific objectives**

1. To determine the most common causative agents that causes UTI in diabetes.
2. To detect the most suitable antibiotic.
3. To evaluate which type of diabetes associated with urinary tract infection.
4. To Isolate and identify causative agent of UTI.
5. To study antibiogram of causative antibiotic against the bacteria isolates.



# Chapter Tow

## Literature review

## 2. Literature Review

### 2.1 Urinary tract infection overview:

Urinary tract infections (UTIs) are the most common infections seen in out patient practice <sup>(1)</sup>.

Normal urine is sterile; therefore infection could, theoretically, be diagnosed if a single bacterium was isolated from the urinary tract. In practice, voided urine becomes contaminated in the non-sterile distal urethra. Consequently, with logarithmic bacterial proliferation rates, most individuals diagnosed with urinary infection have a bacterial count of  $10^4$ – $10^5$ /ml. Quantitative urine culture is, therefore, a necessity for diagnosis <sup>(1)</sup>.

### 2.2 Pathogenesis:

#### 2.2.1 Bacterial factors:

Bacteria generally gain entry into the urinary system by ascending the urethra into the bladder and then, in some cases, ascending the ureters to the renal parenchyma. The organism that most commonly infects the urinary tract is *Escherichia coli*, and certain strains of *E. coli* are more likely to cause a UTI .

These strains possess advantageous virulence characteristics, including increased ability to adhere to the epithelial cells of the urethra and increased resistance to serumcidal activity and hemolysin production. *E.coli* adheres by their fimbriae or pili, distinct protein hair like structures on the bacterial surface. Pyelonephritis strains are the most adherent; cystitis strains tend to be intermediately adherent. Two types of fimbriae are important for determining whether *E. coli* causes lower or upper tract infection. Type I fimbriae specifically adhere to mannosylated proteins on the surface of bladder epithelial cells. Bacteria that adhere by type I fimbriae can be readily detached from epithelial cells by exposing them to mannose (“mannose-sensitive”). Some strains of *E. coli* have a second type of fimbriae called P fimbriae that adhere to glycopospholipids embedded in the outer surface of the plasma membrane of uroepithelial cells <sup>(2)</sup>.

### **2.2.2 Host factors:**

Urine contains high concentrations of urea and generally has a low pH. These conditions inhibit bacterial growth. The urine of pregnant women tends to be more suitable for bacterial growth, and patients with diabetes often have glucose in their urine, making that urine a better culture medium. These factors help to explain why pregnant women and diabetic patients have an increased incidence of UTI. Mechanical factors probably are the most important determinants for the development of UTI. Mechanical factors can be grouped into two risk categories<sup>(2)</sup>.

#### **2.2.2.1 Obstruction:**

The flushing mechanism of the bladder protects the host against infection of the urinary tract. When bacteria are introduced into the bladder, the organisms generally are cleared from the urine. Obstruction of urinary flow is one of the most important predisposing factors for the development of a UTI. Prostatic hypertrophy and urethral strictures can lead to bladder outlet obstruction<sup>(1)</sup>.

Defective bladder contraction associated with spinal cord injury also results in poor bladder emptying. These conditions result in a significant volume of urine remaining in the bladder after voiding (“increased post-void residual”), which markedly increases the likelihood of infection. Intrarenal obstruction caused by renal calculi, polycystic kidney disease, and sickle cell disease also increases the risk of renal infection<sup>(2)</sup>.

#### **2.2.2.2 Urethra Length:**

Women have a short urethra, which increases the risk of bacteria entering the bladder. The incidence of UTI in women (1% to 3% of women) is much higher than that in men (0.1% or less until later years). At least 10% to 20% of all women develop a symptomatic UTI at some point during their lifetime. Trauma to the urethra by sexual intercourse and the use of a diaphragm both increase the risk of UTI<sup>(2)</sup>.

### **2.2.2.3 Diagnostic of urinary tract infection**

Urine microscopy is the use of a microscope to look at urine. In patients with urinary tract infections, one can often find pyuria (elevated white blood cell[WBCs] in urine) and hematuria (red blood cells in urine), and sometimes bacteria can be seen. The presence of WBC casts indicates pyelonephritis rather than cystitis.

A urine sample that has abundant squamous epithelial cells suggests that it is contaminated and the results of the culture are not reliable. Urine dipsticks use different chemicals reagents on a strip that is dipped in urine.

To diagnose urinary tract disease positive leukocyte esterase, positive nitrite, and positive hemoglobin. Positive nitrite occurs from the conversion of nitrate, to nitrite by interobactercae..

Risk factors for the development of a UTI in women with type 1 diabetes developed a UTI. Risk factors for the development of a UTI in these women were sexual intercourse (in the week before study entry) and oral contraceptive use (Table 2). The presence of macrovascular complications at women against the development of a UTI.

Risk factors for the development of a UTI in women with type 2 diabetes developed a UTI. The only significant risk factor for the development of a UTI in these patients was the presence. In both patient groups, age, glycemic control (HbA1c levels), the presence of peripheral neuropathy, and macrovascular complications did not influence the risk of developing a UTI. In addition, the presence of postvoiding bladder residue did not increase the chance of developing a UTI.

### **2.3 Common Urinary Tract Pathogens:**

*Escherichia coli* (75%), *Klebsiella* (15%), *Proteus* (5%), *Enterococci* (2%), *Staphylococcus epidermidis* (2%), Group B streptococci (2%) and *Pseudomonas* (rare).<sup>(2)</sup>

## **2.4 Clinical Manifestations:**

Patients with cystitis usually experience acute-onset dysuria (pain, tingling, or burning in the perineal area during or just after urination). Dysuria results from inflammation of the urethra. In addition, patients need to urinate frequently, because inflammation of the bladder results in increasing suprapubic discomfort when the bladder is distended and may cause bladder spasms that interfere with bladder distension. Some patients note blood in the urine caused by inflammatory damage to the bladder wall <sup>(6)</sup>.

The clinical manifestations of upper-tract disease usually overlap with those of lower-tract disease. However, in addition to symptoms of cystitis, patients with pyelonephritis are more likely to experience fever and chills, costovertebral angle pain, nausea and vomiting, and hypotension. Certain risk factors increase the likelihood of upper-tract disease <sup>(6)</sup>.

Treatment is recommended in pregnant women because these patients are at increased risk of developing pyelonephritis. In preschool children, asymptomatic bacteriuria can result in renal scarring and interfere with normal growth of the kidneys <sup>(2)</sup>.

Urethritis (inflammation of urethra) can be confused with cystitis. The primary symptom is burning on urination. Colony counts resulting from urine culture are less than 10<sup>5</sup> organisms per milliliter, and the patient usually does not experience suprapubic pain or urinary frequency. Women with vaginitis can also experience burning on urination. Therefore, in a woman with symptoms suggestive of cystitis or urethritis accompanied by a vaginal discharge, (pelvic exam is warranted to exclude a pelvic infection). The physical findings associated with UTI are usually minimal. Patients with cystitis may have suprapubic tenderness <sup>(2)</sup>.

Patients with pyelonephritis often are febrile and may be hypertensive and have an elevated heart rate. They often are acutely ill, appearing toxic. Costovertebral angle or flank tenderness resulting from inflammation and swelling of the infected kidney may be noted. In elderly patients, pyelonephritis and gram-

negative sepsis may lead to confusion and somnolence <sup>(7)</sup>.

#### **2.4.1 Hyperglycemia:**

Hyperglycemia is an increase in plasma glucose level in healthy patient. During a hyperglycemia state insulin is secreted by the  $\beta$ -cells of the pancreatic islets of langerhans, insulin enhances membrane permeability to cells in the liver, muscle and adipose tissue. <sup>(1)</sup>

#### **Hyperglycemia may be due to:**

\*Diabetes mellitus

\*Intravenous infusion of glucose containing fluids.

\*Severe stress. <sup>(3)</sup>

#### **2.5 Diabetes mellitus:**

Diabetes mellitus is group of metabolic disorder of carbohydrate metabolism caused by an absolute or relative insulin deficiency. It has been defined by the world health organization (Who) as fasting venous plasma glucose concentration greater than 140 mg/dl or greater than 200 mg/dl. <sup>(3)</sup>

##### **2.5.1 Classification of diabetes mellitus:**

Diabetes is diagnosed by criteria proposed in 1979 by a work of group of the national diabetes data group and adopted by the WHO committee. Insulin dependent diabetes mellitus (IDDM, type 1) (formerly juvenile – onset diabetes) .the patient is require to insulin therapy because they are prone to develop ketoacidosis usually present during childhood they destroyed the  $\beta$ -cells of the pancreatic islets. The patient suffering from polyuria, polydipsia and rapid weight loss and insulinpenia .the disease initiated by an environmental factor or infection .non insulin dependent diabetes mellitus (NIDDM, type II) (formerly adult –onset diabetes)the patients are much less likely to develop ketoacidosis than those with IDDM and insulin may sometimes be needed but not essential for survival onset is most usual during adult life and no genetic markers. There is familial tendency or due to variety of inherited disorders may be responsible for the syndrome such as reducing insulin secretion and also due to factor made deposit high plasma insulin include obesity, stress, and patient take oral

hypoglycemic agent. diabetes mellitus associated with certain conditions and syndromes. absolute insulin deficiency due to pancreatic disease, relative insulin deficiency due to excessive growth hormones, glucocorticoid secretion, drugs such as thiazide diuretics .this formerly termed secondary.<sup>(4)</sup>

### **2.5.2 Pathophysiology of diabetes mellitus:**

The type 1 and type2 diabetes, the individual will be hyperglycemic which can be severe glycosuria the individual with type 1 diabetes has higher tendency to produce ketones, in type1 there is an absence of insulin an excess of glucagon this permits gluconeogenesis and lipolysis.type1 insulin is present as is hyperinsulinemia , glucagon is attenuated fatty acid oxidation is inhibited causes convert the fatty acid into triglycerides for release as very low density lipoproteins.cholesterol synthesis is also increased and increase plasma low density lipoproteins and increased break down of protein may causes muscle wasting .the laboratory finding of patient with diabetes with keto acidosis made dehydration, electrolyte disturbances, acidosis ,aceto acetate, Beta -hydroxy butyrate, acetone due to oxidation of fatty acid .Bicarbonate and carbon dioxide decrease, sodium concentration tend to be lower, increase triglyceride, hyperkalemia.The patient with type 1 diabetes is non ketotic hyperosmolar state there appears to be an imbalance production and elimination in urine, this state is precipitated by heart disease, stroke. The lab finding of non ketotic hyperosmolar coma include increase plasma glucose to 1000mg/dl ,normal or elevated plasma sodium and potassium, slightly decreased bicarbonate, elevated blood urea nitrogen and creatinine an elevated osmolality.<sup>(5)</sup>

The incidence of urinary tract infections (UTIs) is common in both diabetic and non diabetic patients. Since the microorganisms causing UTI vary in their susceptibility to antimicrobials from place to place and time to time, hence constant screening of trends and susceptibility pattern of predominant organisms against antimicrobials is essential.<sup>(5)</sup>

### **2.5.3 Complication of Diabetes Mellitus:**

The vascular disease is a common complication of diabetes mellitus, macrovascular disease due to abnormalities of large vessel may present as coronary artery , cerebro vascular made alteration in lipid metabolism, Micro vascular disease due to abnormalities of small blood vessels particularly affects the retina (diabetic retinopathy), abnormalities in central nervous system, kidney disease is associated with several abnormalities including proteinuria and progressive renal failure, diffuse nodular glomerulosclerosis may cause the nephrotic syndrome and present of small amounts of albumin in urine. <sup>(3)</sup>

Diabetic nephropathy is the most common cause of chronic renal failure patients with type 1 diabetes have a 30%--40% chance of developing nephropathy after 20 years, type 11 15%--20% patient develop neuropathy, the kidney may be damaged by diabetes in three main ways, glomerulosclerosis, ischemic lesion, infective lesion, the risk factors for developing diabetic nephropathy poor control of blood glucose, long duration of diabetes, presence of other microvascular complication, pre existing hypertension, family history of diabetic nephropathy, initially there is micro albuminuria, proteinuria and renal failure with raised urea and creatinine <sup>(5)</sup>.

The renal complication may partly due to the increased glycation of structural protein in the arterial walls supplying the glomerular basement membrane, azotemia is increase urea due to catabolism of tissue protein and increase glyconeogenesis and increase the plasma creatinine level, increased the phosphate in the urine, Acute metabolic complication of diabetes mellitus is diabetic keto acidosis is more severe form of metabolic may be precipitated by infection or by vomiting, insulin may be mistakenly, in absence of insulin then increased lipid and protein break down enhanced hepatic glyconeogenesis and impair glucose entry into cells, the clinical feature of diabetic keto acidosis hyperglycemia causing plasma hypersmolality, metabolic acidosis, glucosuria



## **2.6 Urinary tract infection in diabetes:**

The prevalence of urinary tract infections has also increased. Hyperglycemia and hypertension are the major risk factors for initiation of chronic kidney disease but other factors, such as repeated episodes of acute kidney injury (infections, drugs, or nephrotoxins) can also contribute to its progression. In diabetic patients, it is generally accepted that infections are frequent causes of morbidity and mortality. Immunologic defects contribute to the increased risk for infection: impaired neutrophil function, low levels of prostaglandin E, thromboxane B<sub>2</sub>, leukotriene B<sub>4</sub>, decreased T cell-mediated immune response. Other conditions such as incomplete bladder emptying due to autonomic neuropathy and high glucose concentration in the urine allow urinary colonization by microorganisms. The presence of bacteria in urine is bacteriuria. There is evidence that patients with diabetes have an increased risk of asymptomatic bacteria and urinary tract infections (UTIs) with UTIs being the most common bacterial infections in diabetic patients. <sup>(9)</sup>.

The increased prevalence of asymptomatic bacteria (ASB) and symptomatic UTI in diabetic patient may be the result of difference in host responses between diabetic and non-diabetic patients or to a difference in infecting bacterium itself. Patients with diabetes have a 10- fold increased risk of UTI when compared to non-diabetics and diabetics have a longer hospitalization than non-diabetics. Diabetics are more prone to UTIs and to upper UTI. The reason for this predisposition is not completely understood, but the most important is likely to be bladder dysfunction caused by diabetic neuropathy. In diabetic women, there is higher incidence of bacteriuria and of asymptomatic kidney infection. UTIs are more common in women than men. Females are more commonly affected with UTI than males and are about thirty times more common among females than males. UTIs occur in females throughout life and tend to increase with age. Silent infections occur about 1% for each ten years of life. They can suddenly become symptomatic and produce considerable discomfort particularly among

women prone to repeated infections and during the last three months of pregnancy<sup>(9)</sup>.

## 2.7 Previous Study

**Acharya D et.al** In Kathmandu University Hospital found 55 (30.5%) samples showed significant growth. No significant difference among culture positivity rate was noted between diabetic and non diabetic patients (34.5% Vs 26.7%). *Escherichia coli* was the most frequent organism (64.5% in diabetic and 66.7% non diabetic) followed by *Klebsiella* spp (22.6% in diabetic and 12.5% in non diabetic). Gentamycin and nitrofurantoin were highly sensitive to *E. coli* isolated in diabetic patients among the tested antimicrobials followed by cotrimoxazole, norfloxacin and ciprofloxacin. Least sensitivity rate was observed with ampicillin and cephalixin.<sup>(10)</sup>

**Hamdan Z et.al** in sudan – Khartoum found 121 (60.5%) men and 79 (39.5%) women; 193 (96.5%) had type II DM. The overall prevalence of UTI was 39 (19.5%). Among the total population, 17.1% and 20.9% had symptomatic and asymptomatic bacteriuria, respectively. According to multivariate logistic regression, none of the investigated factors (age, sex, type of DM and duration) were associated with UTI. The predominant isolates were *Escherichia coli* (22, [56.4%]), and *Klebsiella pneumoniae*, [9, (23%)]. Eight of 22 *E. coli*, four of nine *K. pneumoniae* and one of five *Enterococcus faecalis* isolates originated from symptomatic patients. Six, four, three, and two of 22 *E. coli* isolates showed resistance to ampicillin, co-trimoxazole, nitrofurantoin, and amoxicillin-clavulanic acid, respectively. Two, two, one and one of nine *K. pneumoniae* isolates were resistant to ampicillin, co-trimoxazole, cephalixin, and amoxicillin-clavulanic acid. All 22 *E. coli* isolates were sensitive (100%) to gentamicin and cephalixin. All nine *K. pneumoniae* were sensitive to gentamicin (100%) and 88.8% were sensitive to cephalixin.<sup>(11)</sup>

Urine may be static or even bactericidal against uropathogens under certain situations. In diabetes mellitus modification of chemical composition of urine occurs which alters this ability of urine and support the growth of pathogens. It

has been experimentally shown that osmotic diuresis secondary to glycosuria predispose to ascending *Escherichia coli* infection in laboratory animals.

The successful management of UTI in diabetics depends on the proper identification of the bacteria responsible and the selection of effective antibiotics against them. The emergence of resistant bacterial strains in hospitals poses a continued challenge to treat and control the spread of infections. Furthermore, the indiscriminate use of antibiotics often results in the increased resistance of urinary pathogens to most commonly used antimicrobials. Updated knowledge of the susceptibility pattern of bacteria is therefore important for the proper selection and use of antimicrobial drugs and for the development of an appropriate prescribing policy.

Owing to the fact that the incidence of UTI is increasing worldwide, this study was undertaken to identify the responsible microbial culprits for the urinary tract infection in diabetic patients including their susceptibility patterns to various antibiotics in a tertiary care teaching hospital of Eastern Nepal that covers the patients of both rural as well as urban areas and provides teaching hospital of Eastern Nepal that covers the all emergency management facilities.

The clinical features of UTI in patients diabetes.

Occasionally fever may be less apparent, particularly in individuals with metabolic disturbance secondary to diabetes.

Reduced levels of consciousness may occur from metabolic changes or sepsis. Hematuria or flank pain secondary to sloughing papillae may be noted in patients with papillary necrosis. Occasionally patients may experience pneumaturia if gas has been produced within the urinary tract, or rarely, gas in tissues may be palpated in the flank or groin. The investigation of possible UTI in patients with diabetes requires urinalysis and urine culture by catheterization if necessary, prior to initiation of antibacterial treatment together with assessment of renal function and glycemic status. Although there are no studies, most physicians have an increased concern to make specific diagnoses because

of the complication that may ensue. A blood culture should be routine inpatients with presumed acute pyelonephritis.

Pregnant women with diabetes presumably have an increased prevalence of bacteriuria. The concomitant illnesses of diabetes and UTI could be expected to increase the complications of pregnancy, but data are sparse. No population-based studies have identified the risk factors for acute cystitis in sexually active women, comparing women with diabetes to controls. This remarkably common illness has an incidence in otherwise healthy women, which exceeds 0.4 episodes per person per year. Studies are needed in sexually active women with diabetes. Acute pyelonephritis is a common presentation of UTI in patients with diabetes. During the pre-antibiotic era, 7.6% of 307 autopsied patient with diabetes died of pyelonephritis compared to 1.6% of 2000 non-diabetic patients, and pyelonephritis ranked sixth in 'cause of death. The increased risk of acute pyelonephritis with diabetes had not been quantitated until recently. Diabetes was overwhelmingly a risk factor for 'admission with acute pyelonephritis'. Among individuals over the age of 40 years, 36% of 797 hospitalized women and 21% of 402 hospitalized men had diabetes. Diabetes was estimated to increase the probability of acute pyelonephritis requiring hospital admission by 20–30-fold under the age of 44 and by 3–5-fold among both men and women of 45 years and over. <sup>(7)</sup>

# Chapter Three

## **Materials and Methods**

### **3. Methodology**

#### **3.1. Study design:**

This is Hospital based Descriptive cross section study.

#### **3.2 Study area:**

The area is Shendi locality which is located 172.01 Kilometers north to the capital, Khartoum southern part of river Nile and covering area about 30 Km<sup>2</sup>.

There are several general centers for different services and purposes, also there is Shendi University with various faculties as faculty of medical laboratory sciences, Education, Economics, Law, Community and science technology.

Shendi has 3 hospitals the biggest is Elmek Nimer university hospital which have different departments and provide a good health for this town population.

#### **3.3 Study population:**

Diabetic patients in Shendi hospital.

##### **3.3.1 Inclusion criteria:**

Diabetic patient in different ages with urinary tract infection.

##### **3.3.2 Exclusion criteria:**

Specimens of diabetic patients negative for urinary tract infection were excluded.

#### **3.4 Ethical Considerations:**

The study proposal was reviewed and ethically approved by the scientific and the ethical committee of Shendi University and ethical committee of hospital.

#### **3.5 Methodology:**

##### **3.5.1 Sample collection:**

Hundered sample of Midstream urine (MSU) is collected as follows:

1. Patient was given a sterile, dry, wide-necked, leak proof container and requested a 10–20 ml specimen. Container was labeled with the date, the name and number of the patient, and the time of collection. When immediate delivery to the laboratory is not possible, refrigerate the urine at 4°C. <sup>(8)</sup>

### **3.5.2 Interpretation of culture growth:**

The plates were examined for any significant bacterial growth. The isolated bacteria were then identified by colonial morphology, Gram stain and biochemical test. To isolate the bacteria causing UTI in a diabetic patient, the following steps were followed: culture the samples, Gram stain, Biochemical test, and Antimicrobial susceptibility test.

### **3.5.3 Characterization and identification of isolated microorganism**

Clean voided midstream urine samples were collected in a sterile container after giving proper instructions, and samples were processed in the laboratory within 2 hours of collection. Urine cultures were performed using a semi-quantitative technique where urine samples were inoculated on cysteine-Lactose electrolyte deficient (CLED) medium plates with a calibrated loop (0.001 ml) and incubated at 37°C for 18-24 hours. Urine culture reports that exhibited colonies. After 24 hours of incubation, the culture plates were examined and the appearance, size, color, and morphology were observed. Gram stain reaction, Catalase, Coagulase, Indole, Oxidase, Citrate utilization tests were carried out as Gram stain. The test detects the type of microorganism isolated based on its staining reaction.

### **GRAM STAIN: Principle of Gram staining:**

When the bacteria is stained with primary stain crystal violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by alcohol. The cell walls of protein-sugar complexes called peptidoglycan and lipid content is low. Decolorizing the cell causes this thick cell wall to dehydrate and shrink, which closes the pores in the cell wall and prevents the stain from exiting the cell. So the ethanol cannot remove the crystal violet-iodine complex that is bound to the thick layer of peptidoglycan of gram positive bacteria and appears blue or purple in color.

In case of gram negative bacteria, cell wall also takes up the CV-iodine complex but due to the thin layer of peptidoglycan and thick outer layer which is formed of lipid, CV-iodine complex gets washed off. When they are exposed to alcohol,

decolorizer dissolves the lipids in the cell walls, which allows the crystal violet-iodine complex to leach out of the cells. Then when a gram stained with safranin they take the stain and appears red in color. <sup>(6)</sup>

**Procedure:**

1) A dried smear was made and fixed 2) The fixed smear was covered with crystal violet for 30 seconds The stain was rapidly washed off with water The water was tipped off and the smear iodine for 60 seconds. The iodine was washed off with clean water The smear was decolorized rapidly for few seconds with acetone water. It was washed immediately with clean water The smear was covered with safranin for few minutes. The stain was washed off with clean water The back of the slide was wiped clean and placed in a draining rack for the smear to air-dry.

Gram positive bacteria appeared purple while Gram negative appeared pale to dark red.

**3.5.4 BIOCHEMICAL TESTS**

Biochemical tests Including Catalase test, Coagulase test, Oxidase test, Indole test, Citrate were carried out on the colonies to ascertain organisms isolated.

**Catalase test**

This test detects the presence of Catalase an enzyme that catalyses the release of oxygen from hydrogen peroxide.

**-Procedure**

- 1) 2ml of hydrogen peroxide solution was poured into test tubes for each isolate.
- 2) Several colonies of the test organisms were removed using a sterile wooden stick and immersed into the hydrogen peroxide solution in the test tube.
- 3) Immediate bubbling was looked for. Active bubbling indicates positive catalase test

No bubbles indicates negative catalase test.

**-Coagulase test**

This test detects the presence of coagulase enzyme.

Procedure for Coagulase test



1) A drop of water was placed on the end of two separate grease-free slides for each isolate.

2) A colony of the test organism was emulsified in each of the drops to make suspensions.

3) A loopful of plasma was added to one of the suspensions. It was mixed gently and clumping of the organism was looked for within 10 seconds.

Clumping within 10 seconds indicates that the organism is *Staphylococcus aureus* growth.

No clumping within 10 seconds indicates that there is no bound Coagulase.

### **-Oxidase test**

This test detects the production of oxidase enzyme by some microorganisms

#### **Procedure**

1) A piece of filter paper was placed in a clean petri dish and 3 drops of freshly prepared oxidase reagent was added.

2) Using a piece of stick, a colony of the test organism was removed and smeared on the filter paper.

Development of blue-purple color within a few seconds indicates a positive oxidase test.

No blue-purple color indicates a negative oxidase test.

### **-Indole test**

The test detects the production of indole in tryptophan containing medium by some bacteria when Kovac's reagent is added to it.

#### **Procedure**

1) The test organism was inoculated in tube for up to 48 hours.

3) 0.5ml of Kovac's reagent was added to the bijou bottle. It was shaken gently. A red color in the surface layer within 10 minutes indicates a positive indole test.

No red surface layer indicates a negative indole test.

### **Citrate test**

This test detects the presence of *Klebsiella* spp

## **Procedure**

- The test organism was inoculated into sterile peptone water broth and incubated for few hours.
- A sterile straight wire was then used to inoculate Simmons citrate agar with the broth culture.
- It was incubated at 37°C for 48 hours. Development of a blue color growth indicates a positive citrate test.

### **3.5.5 Antimicrobial susceptibility test**

Antimicrobial agents include naturally occurring anti-biotics, synthetic derivatives of naturally occurring antibiotics (semi-synthetic antibiotics) and chemical antimicrobial compounds (chemotherapeutic agents). Generally, however, the term ‘antibiotic’ is used to describe antimicrobial agents (usually anti-bacterial) that can be used to treat infection. Compared with antibacterial agents, fewer antiviral and antifungal agents have been developed. Many antiviral agents have serious side-effects.

Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method employing Mueller-Hinton Agar plate as Laboratory Standards Institute .The antibiotic discs . These groups of antibiotics have been in use for management of UTI in our hospital. The inoculated plates were incubated at 37°C for 18-24 hours. Diameter of the zone of inhibition around the disc was measured.

#### **3.5.5.1 Antimicrobial discs:**

Commercially available antimicrobial discs were used to determine the drug sensitivity and resistance pattern of the bacteria isolates

Antimicrobial sensitivity testing of bacteria isolates was performed on Mueller-Hinton agar plate by the Kirby-Bauer disk diffusion method .Isolates were tested for their susceptibility against antimicrobial agents .Standardized inoculum conforming to 0.5 McFarland standard turbidity of each isolate was inoculated on Mueller–Hinton agar plates using a sterile cotton swab by streaking the swab over the entire sterile agar surface three time. Then into each plate antimicrobial

disk was placed .All plates were aerobically incubated at 37°c for 18 hours before the zone sizes were recorded.

# Chapter Four

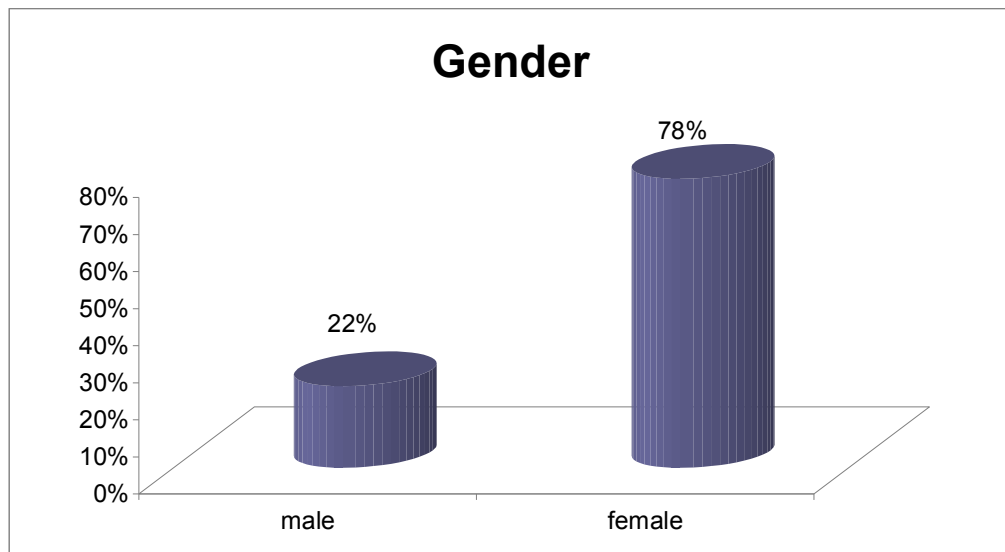
## Results

## 4. Results

Total number of 100 urine sample conducted in Shendi town during period from May-August2018 aimed to determined the urinary tract infection in diabetes mellitus and susciptability testing . The data obtained explained in tables (1,2,3,4,5,6,7,8,9).growth in 76 sample and No growth in24 sample.

**Table (1) Show distribution the study population according to gender**

Gender	Number	%
Male	22	22
Female	78	78
Total	100	100



**Table (2): Shows distribution the study population according to age.**

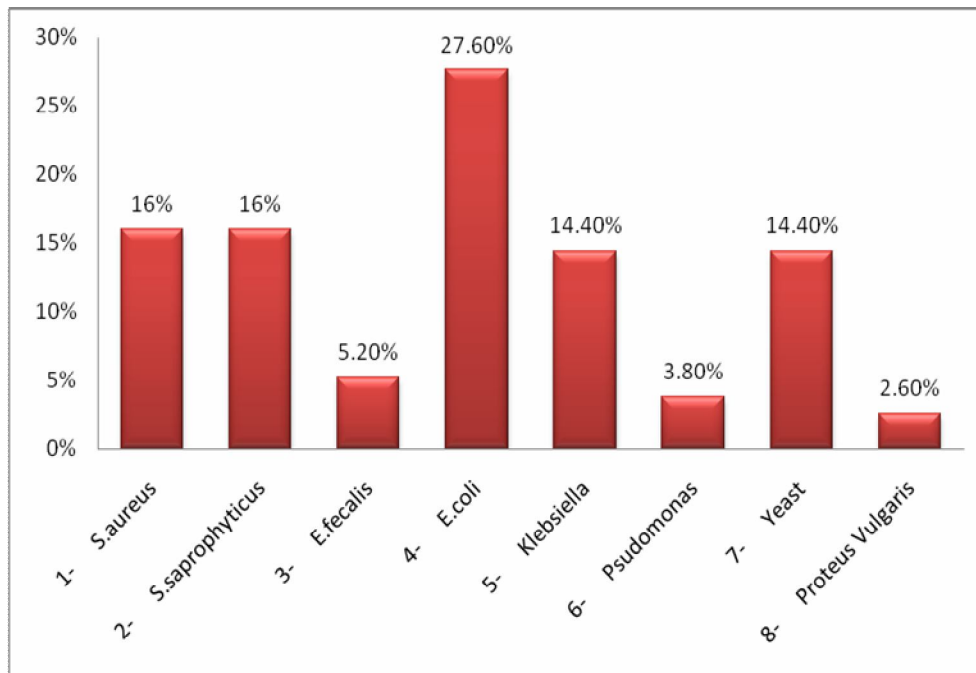
<b>Age group</b>	<b>Number</b>	<b>%</b>
Less than 24	8	8
25 – 34	13	13
35 – 44	19	19
More than 44	60	60
<b>Total</b>	<b>100</b>	<b>100</b>

**Table (3): Shows distribution the study population according to resident.**

<b>Trimester</b>	<b>Number</b>	<b>%</b>
Rural	70	70
Urban	30	30
<b>Total</b>	<b>100</b>	<b>100</b>

**Table (4): Shows percentage of microorganism isolated from urine among study population**

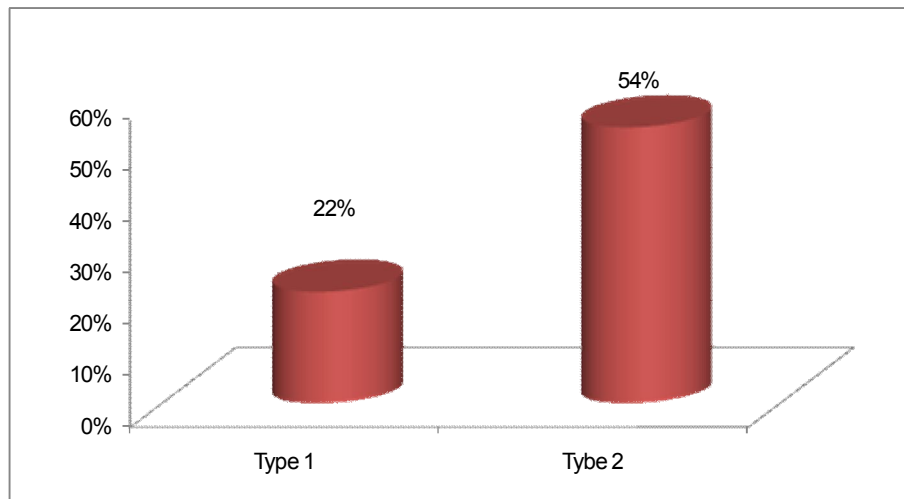
Bacteria name	Number	%
<i>S.aureus</i>	12	16
<i>S.saprophyticus</i>	12	16
<i>E.fecalis</i>	4	5.2
<i>E.coli</i>	21	27.6
<i>Klebsiella</i>	11	14.4
<i>Pesudomonas</i>	3	3.8
<i>Yeast</i>	11	14.4
<i>Proteus Vulgaris</i>	2	2.6
<b>Total</b>	<b>76</b>	<b>100</b>



**Figure 1: Shows percentage of microorganism isolated from urine among study population**

**Table (5) Shows distribution the study population according to type diabetes**

Type of diabetes	Number	Percentage
Type (1)	22	22
Type(2)	54	54
Total	76	76





**Table (6): Show percentage of antibiotic (gentamicin )sensitivity and resistance.**

<b>Bacteria</b>	<b>Antimicrobial agent</b>	<b>Sensitive</b>	<b>Resistance</b>
E.coli	Gentamicin	20	01
E.feacilus	Gentamicin	02	02
S.saprothiticus	Gentamicin	09	03
S.aureus	Gentamicin	10	02
Pseudomonas	Gentamicin	02	01
Yeast	Gentamicin	10	01
Protus vulgaris	Gentamicin	02	00
Klebsiella	Gentamicin	8	03

**Table (7): Show percentage of antibiotic (ceftazidime) sensitive and resistance.**

<b>Bacteria</b>	<b>Antimicrobial agent</b>	<b>Sensitive</b>	<b>Resistance</b>
E.coli	Ceftazidime	00	21
E.feacilus	Ceftazidime	00	04
S.saprothiticus	Ceftazidime	02	09
S.aureus	Ceftazidime	01	10
Pseudomonas	Ceftazidime	01	02
Yeast	Ceftazidime	00	11
Protus vulgaris	Ceftazidime	01	01
Klebsiella	Ceftazidime	03	08

**Table(8): Show precentage of ant biotic(ceftriaxone) sensitivity and resistance.**

<b>Bacteria</b>	<b>Antimicrobial agent</b>	<b>Sensitive</b>	<b>Resistance</b>
E.coli	Ceftriaxone	20	01
E.feacilus	Ceftriaxone	03	01
S.saprothiticus	Ceftriaxone	10	01
S.aureus	Ceftriaxone	09	02
Pseudomonas	Ceftriaxone	03	00
Yeast	Ceftriaxone	01	10
Protus vulgaris	Ceftriaxone	02	00
Klebsiella	Ceftriaxone	08	03

**Table (9): Show precentage of antibiotic (cloxacillin)sensitivity and resistance.**

<b>Bacteria</b>	<b>Antimicrobial agent</b>	<b>Sensitive</b>	<b>Resistance</b>
E.coli	Cloxacillin	19	02
E.fecaulis	Cloxacillin	04	00
S.saprothiticus	Cloxacillin	08	03
S.aureus	Cloxacillin	04	00
Pseudomonas	Cloxacillin	02	01
Yeast	Cloxacillin	01	10
Protus vulgaris	Cloxacillin	01	01
Klebsiella	Cloxacillin	10	01

# **Chapter Five**

**Discussion**

**Conclusion**

**Recommendations**

## 5.1 Discussion

Urinary tract infection is very often encountered in patient they may present themselves as asymptomatic bacteruria with diabetic patient. The urinary tract infection lead to inflammatory response. The urinary tract infection the common microbial disease and the urinary tract infection the second most infection in women infection than men due to anatomy of women and type 2 diabetic is high risk to urinary tract infection than non diabetic.

One hundred urine samples were collected and examined by using bacteriological techniques to identify causative agents of urinary tract infection. The study found that *E.coli* is the most prevalent bacteria about (27.6%) that cause urinary tract infection among diabetes followed by *S.aureus* and *S.saprophyticus* in percentage about (16%), and *E.feacilus* about 5.2%, *klebsiella* and yeast about 14.4% and follow by *Pseudomonas* about 3.8% and *Protus vulgaris* about 2.5%.

Gentamicin has high percentage of activity about (63%) against isolated bacteria and Ceftazidime has high percentage of resistance about (35%), and ceftriaxone and cloxacillin are low sensitive and high resistance.

Type 2 is high risk of UTI about 54% than type 1 about 22%. Female is high risk of UTI about 78% than male about 22%. The age of adult of more than 40 year old people is high frequent exposure to UTI about 60% than young people about 40%.

Acharya, Liferiora are determine as high prevalence organism in diabetic patients are *E.coli*, *Klebsiella*, *E. faecalis* that similar to this study and susceptible to antimicrobial agent gentamicin but the May Sewify Shinu Nairin, Pramod Kumar Jha are differ in this study in antimicrobial agent are Ciprofloxacin, Ampicillin in this study are resistant to ceftazidime but share in micro organism caused UTI in diabetic patient. <sup>(10)</sup>

## 5.2 Conclusion

The study found that:

- The common isolate bacteria are *E.coli*, *s .saprophyticus* ,*E.fecalis*, *klebsiella*, *pseudomonas*,*protus vulgaris* ,*yeast* .
- Gentamicin is the most active antibiotic against isolated bacteria.
- Ceftazidime is the most resistant antibiotic against isolated bacteria.
- Type 2 is high risk of urinary tract infection than type 1.
- The female is high risk to urinary tract infection than male.
- The age of type of people of young is low frequent of exposure to urinary tract infection than adult of more than 40 years old.

### **5.3 Recommendations**

The study recommends that:

The best antibiotic can be used for multidrug resistant bacteria is Gentamicin.

The patients must complete antibiotic courses to avoid development of multi drug resistant strains.

The Ceftazidime must not be used to treat urinary tract infection due to high percentage of resistance.

-culturing and education of diabetic patient about the hazard of diabetes mellitus excess of sugar whether by insituation of colloquium or through informational system or by friends of diabetes patient.

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# Appendices

## Appendix 1

### Questionnaire:

بسم الله الرحمن الرحيم

Shendi University

#### **The determine of causative agents of urinary tract infection in diabetic patient and susceptability testing**

1. Age.....
2. Type of diabetic .type1.....type 2.....
3. Patient suffering fro urinary tract infection .....  
If yes...how is treated..... name of drug
4. Recurrent suffering from urinary tract infection .....  
if ye s ..... how is treated ..... name of drug
5. Resident of patient .....

## Appendix 2: Plates



**Plate 1: antimicrobial susceptibility for Genatmicin, Ceftazidime, Cefrixone and Amikacin .**