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Uropathogens associated with urinary tract infections in Zimbabwe: A case study of Mashoko Christian Hospital, Bikita District in Masvingo Province, Zimbabwe.

BY

MCDONALD MBWIRIRE (B1852879)

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Biological Sciences Department

Faculty of Science and Engineering

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ABSTRACT

A urinary tract infection (UTI) is an infection caused by microorganisms, mostly bacteria, but sometimes fungi and, in rare circumstances, viruses. Urinary tract infections (UTIs) are among the most frequent community-acquired diseases globally, with *E. coli* being the most common pathogen, though underlying host characteristics such as patients' age and gender may have an impact on the incidence of causative agents. A urinary tract infection (UTI) is an infection that can occur anywhere in the urinary tract system. The urinary tract system consists of the kidneys, ureter, bladder, and urethra. The entire study was done with the aim of looking at factors like age and gender and how they affect the prevalence of most urinary tract infections. The research was carried out at Mashoko Christian Hospital in Masvingo. Urine samples were taken from patients suspected of having urinary tract infections and were examined microscopically and microbial cultured. For both age and gender, a random sampling procedure was used. The sampling period was at least two weeks. A total of twenty-five urine samples from patients, including controls, were collected and analysed. The samples were separated into three groups: the young (20-39 years old), the middle-aged (40-59 years old), and the elderly (60 and over). Urinalysis was performed to determine if the urine samples tested positive for bacteria. The samples were examined in two stages: first, using urine dipsticks to detect the presence of nitrites, proteins, and leucocytes, and then microscopically to determine the presence of bacteria in the urine. The positive samples were cultured. Total Bacterial Counts (TBC) and Total Coliform Counts (TCC) were performed in Blood Agar and MacConkey Agar for each urine sample. Using inoculum produced from urine samples, certain types of bacteria were then isolated and identified. According to the study's findings, women had the highest frequency of UTIs (67%), while men had 33%. There were no significant differences in age groups, and there was no variation in prevalence across all age groups. However, when gender was examined independently, the incidence increased with age, with the 20-39 and 40-59 age groups having the highest prevalence.

DECLARATION

I, **Mcdonald Mbwirire B1852879**, declare that everything that appears in this report is the result of my own personal work and effort from beginning to completion that I did not obtain or copy anything unlawfully from anyone, and any assistance, whether material or conceptual, that I have incorporated in my work has been openly and lawfully obtained and is approximately acknowledged in the report. I have not, in short, plagiarized anything from anyone.

Student Signature  Date 18/02/2025

Supervisor's signature: Dr. N. Mgocheki Date 18/02/2025

Chairperson's signature:  Date 18/02/2025

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DEDICATIONS

I dedicate this whole work to my family especially my father Mr T. Z. Mbwirire, my mother Mrs M. Zuweni Mbwirire and my sister Melinda M Mbwirire who have been always there to guide, support and encourage me. Through difficult times, they have been always there for me.

Contents

CHAPTER 1:

1.0 INTRODUCTION

1.1 Background information

A urinary tract infection (UTI) is an infection caused by pathogens, most commonly bacteria, fungi, and, in rare circumstances, viruses. UTI may occur anywhere throughout the urinary tract. The kidneys, ureters, bladder, and urethra comprise the urinary tract system. In the lower tract, most UTIs only affect the urethra and bladder. Urinary tract infections can also affect the upper tract, which includes the ureters and kidneys, albeit upper tract infections are less common than lower tract infections. UTIs are approximately 50 times more prevalent in women than in males among persons aged 20 to 50 years old (Jon, 1997). Because the female urethra is shorter and closer to the entrance, UTIs are significantly more likely than in males. As a result, pathogens from the intestines may enter the urine system considerably more easily. Bacteria can enter the bladder, ureters, and kidneys through the urethra and cause an infection. According to (Nicole *et al.*, 2005), *Escherichia coli*, which is commonly found in feces, causes the majority of UTIs. *Staphylococcus* and *Proteus* are the other two bacteria species genera that are prone to cause urinary tract infections. *Candida*, a yeast that can overgrow, can occasionally cause UTIs.

In addition to bacterial infections, UTIs can be caused by a sexually transmitted infection (STI). Most UTIs in normal urinary tracts are caused by *E. coli* strains, which account for 75 to 95% of all UTIs. The remaining gram-negative urine pathogens are primarily *Enterobacteria*, such as *Klebsiella* or *Proteus millabius*, and, on rare occasions, *Pseudomonas aeruginosa. Staphylococcus Saprophyticus* is identified from 5–10% of bacterial UTIs. *Enterococcus faecalis* (group D, *Streptococci*) and *Streptococcus agalactae* (group B *Streptococci*) are less frequent gram-positive bacterial isolates that might constitute contaminants. The prevalence of UTI rises in individuals over the age of 50, although the female-male ratio falls as the frequency of prostate enlargement in males rises. A urinary tract infection can cause a systemic illness, especially in the elderly. UTIs are classified as either complicated or

uncomplicated UTIs. Uncomplicated UTI develops in premenopausal adult women who have no anatomical or functional urinary tract abnormalities. Most UTIs in males occur in youngsters and the elderly, and they are caused mostly by anatomical anomalies. Complicated UTIs develop when there is a comorbidity present that raises the risk of infection (Griffin et al, 1979).

Women, particularly those aged 16 to 64 years, are more likely than males to have UTIs. Urinary infections affect both men and women of all ages, as well as specific groups of people such as pregnant women, the elderly, or patients with spinal cord injuries, catheters, or diabetes. Urinary tract infection produces short-term morbidity in the form of fever, dysuria, and lower abdominal pain (LAP), as well as irreversible kidney scarring. Nosocomial (healthcare-associated infections) or community-acquired urinary tract infections (CA-UTIs) are the two types of urinary tract infections. These are urinary system problems that occur in a person's life in the community or a hospital setting within 48 hours after admission. Nosocomial urinary tract infections are the second most prevalent kind of UTI in the community. These are urinary tract infections that emerge after 48 hours of hospitalization. The patient shouldn't continue incubating when admitted or within three days of discharge. Urinary tract infections (UTIs) can be chronic, acute, asymptomatic, complicated, or uncomplicated, and the clinical manifestations of UTIs are determined by the area of the urinary system that is affected, the seriousness of the infection, the etiologic microbes, and the patient's capacity to mount an immune defense against it. The signs and symptoms of UTIs vary depending on the age of the individual who is afflicted and the position of the infection in the urinary tract. A fever, a burning sensation while peeing, itching, blisters and ulcers in the vaginal region, genital and suprapubic discomfort, and pyuria, in general, are symptoms.

Gender, age, race, the act of circumcision HIV/AIDS status, diabetes, urinary catheter, genitourinary tract anomalies, pregnancy status, and hospitalization status are all associated risks for recurrent urinary tract infections (UTIs). However, for the present investigation of Mashoko Hospital patients, the emphasis will be only on the impact of gender and age.

1.2 Problem statement

Every year, over 150 million individuals worldwide experience UTIs, costing more than $6 billion in direct health care costs. In Algeria, the prevalence of urinary tract infections was estimated to be 4.5 percent amongst all patients hospitalized in acute care facilities for a period exceeding 48 hours. In Senegal, the frequency was reported to be 0.7% among patients hospitalized at Dakar University Hospital, while women had a greater prevalence compared to men. Research in Nigeria utilizing 12,458 urine samples found that the prevalence of community-acquired and nosocomial urinary tract infections was twelve percent and nine percent, respectively. Females had a frequency of fourteen percent, while men had a prevalence of seven percent. In Uganda, the frequency of urinary tract infections was 29/218 (13.3%), with a medication resistance rate of 20-60% among prenatal moms. UTIs were discovered to have a prevalence of 54/139 (38.8%) at Mulago Hospital, Uganda, and age, female gender, and marital status were shown to be linked with the condition among adults visiting the assessment center. The frequency of UTIs in Uganda's Bushenyi District was reported 67/300 (22.33%). Complications from UTIs include chronic kidney disorders (CDKs).

To the greatest extent of our understanding, relatively few extensive urinary tract infection studies were conducted in Zimbabwe. As a result, there was a requirement to research the incidence of urinary tract infections among admitted and non-admitted patients at Mashoko Christian Hospital which is located in rural areas of Zimbabwe, as well as whether or not there was a relationship between the age and gender of persons. Asymptomatic and symptomatic UTIs both represent a severe hazard to the public, lowering the standard of living and increasing job absenteeism.

1.3 Justification

Urinary tract infections have recently accounted for most admissions in hospitals, and they have many side effects to the infected. Being a community acquired infection;: some patients have acquired UTIs during the period of their admission. This study aims to uncover the relationship between certain bacterial species with age and sex of patients. After carrying out this study it shall be clear whether one strain of bacterial species is responsible for causing urinary tract infections in certain age range and sex group. This study will also contribute to knowledge by identifying the risk factors related to age and gender. Previously no studies have been conducted regarding UTIs in Bikita District therefore by carrying out this study at Mashoko Christian Hospital

will provide information about the prevalence of UTIs around the area. Since the hospital also provides health education to patients, after carrying out this research at the hospital it shall provide information which will add to health education concerning the issue of UTIs.

1.4 Aims and objectives

1.4.1 Main Aim

- To investigate the uropathogens associated with urinary tract infections in Zimbabwe, with a focus on age and gender, at Mashoko Christian Hospital in Bikita District, Masvingo Province.

1.4.2 Objectives

- To identify the bacterial species found in urine specimens from men and women of different ages.
- To quantify and compare bacterial counts in urine samples
- To determine risk factors associated with gender and age and the incidence of UTIs.

1.4.3 Research Questions

1. What are the common urobacterial pathogens associated with men and women of different ages?
2. Which are the more prevalent urogenital bacterial pathogens in urine samples?
3. What are the risk factors associated with gender and age and the incidence of UTIs?

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Age and Gender Association with Urinary Tract Infections

Urinary tract infection is a common infection that affects both men and women, albeit it affects women more frequently because of their anatomy. The microbial aetiology of UTIs has been extensively studied and established, with *Escherichia coli (E. coli)* regarded as the pathogen with a larger proportion than other pathogens in causing urinary tract infections in men and women of all ages. It is expected to be the pathogen in 50-80% of cases. Other *Enterobacteriaceae* species include *Klebsiella, Proteus,* and *Enterobacter* species, as well as *Enterococci, Streptococci, Staphylococci*, and *Pseudomonas* species, which account for the vast majority of the remaining positive urine cultures. If one of the aforementioned species causes an infection, empiric antibiotic therapy should be adopted. Due to localized variations in the frequency of urinary agents, the advent of new pathogens, and changes in antimicrobial resistance, it is advised that pathogen epidemiology be assessed regularly to adjust treatment recommendations. Because basic host characteristics might influence urinary etiology and antibiotic sensitivity, more in-depth research on specific patient groups is required. Among the risk indicators, patients' gender and age are widely available in microbiology laboratory surveys when patients' clinical characteristics are unknown (Magliano *et al.*, 2012). The current study was thus designed to look at the prevalence of urinary tract infections in different age groups of both genders.

A urinary tract infection is a condition that all women will experience at some point in their lives, and the frequency is greater among pregnant women. Urinary tract infections, as the name implies, affect portions of the urinary tract, including the upper and lower urinary tracts. Cystitis (bladder infection) and pyelonephritis (kidney infection) are two forms of diseases that are named after the organ that is afflicted. There are a variety of symptoms connected with bladder and kidney infections, and these symptoms differ. These include painful and frequent urination in cases of cystitis caused by bladder infections, and conditions such as high fever and flank pain in cases of kidney contagion, known as pyelonephritis. According to Magliano et al. (2012), the frequency of pyelonephritis in youngsters and the elderly is not well understood and is presently being researched. Bacteria are the primary agents responsible for human infection, but the roles of some fungi and viruses cannot be overlooked. The occurrence of UTI as a result of viral or fungal infection is a very unusual occurrence. While the infection appears to be innocuous in its early stages, the patient begins to exhibit specific symptoms as the stage proceeds, and if left untreated, it can lead to death in severe cases.

Urinary tract infections are a varied set of clinical syndromes and illnesses that vary in their beginning, epidemiology, location, and severity. In addition to the aforementioned characteristics, it conforms based on the expressed local symptoms, frequency of repetition, level of harm produced, the existence of complicating variables, and danger from their repeated occurrence. The increased prevalence of bladder infection has recently resulted in kidney infection, which has resulted in blood-borne infection, which can have disastrous implications, including death, if severe. As a result, UTI is a contagious disease that can claim lives in extreme cases, and correct treatment can result in a speedy recovery from the infection. The infection begins in pregnant women between the sixth and twenty-fourth weeks of their pregnancy. Although the incidence of bacteriuria during pregnancy is nearly identical to that of non-pregnant women, pregnancy increases the chance of infection in women (Anibijuwon, 2015).

The occurrence of urinary tract infections is at its peak during pregnancy, which can be influenced by a variety of different factors. However, the greater incidence of UTI during pregnancy cannot be regarded as a general truth because the notion is still in the early stages of study and various researchers are still attempting to find out the role of pregnancy in connection to urinary tract infection. Many researchers have previously attempted and are continuously attempting to find appropriate facts to correlate the occurrence of UTI during pregnancy. Pregnancy, on the other hand, is seen as a critical period that necessitates several

protective measures for both the mother and the unborn child (Magliano *et al.*, 2012).The notion of the occurrence of UTI among pregnant women is depicted as a problematic feature that has yet to be comprehended. Infection in the lower urinary system, which includes the bladder, results in cystitis, which leads to infection of the upper urinary tract, known as pyelonephritis. This might be the result of a blood-borne infection. Even though the bacteria *Escherichia coli* causes 80% of the infection, the involvement of other pathogens in generating urinary tract infections cannot be overlooked, and Gram Positive cocci is one of the agents responsible for UTI.. Gram-positive cocci have recently gained prominence throughout the world, with *Staphylococcus* species being one of the most important infections (Meynell & Malins, 1948). Pathogens responsible for UTI are known to display a feature known as biofilm production, which indicates the start of the infection.

2.2 Structure of the Urinary Tract

The urinary system, which includes the urinary tract, is very susceptible to infection since it can damage any section of the urinary tract. The urinary system is made up of numerous sections of the urinary tract, including the kidneys, bladder, renal artery and vein, ureter, urethra, and endowment for urine exit, as indicated in the Figure below. Kidneys, being the most important organs, are recognized to provide crucial regulatory functions (De Groat, 1993). Their function is to remove undesired water-soluble waste from the circulation while also allowing for the re-absorption of important elements such as water, glucose, and amino acids (Tanagho and McAninch, 2000).

Urine is produced by the kidneys and directed to the urinary bladder via thin tubular structures known as ureters (Abrams *et al.,* 1988). The urine bladder is yet another vital part of the urinary system. It is a muscular, flexible organ that collects urine from the kidneys before disposing of it (Finer and Landau, 2004). The urethra, which connects the urinary bladder and genitals, flushes away water-soluble waste in the form of urine from the genitals. The process of producing urine and disposing of it is referred to as systematic, and urinary tract infection significantly supports this process, which may result in the observation of a variety of symptoms that the patient meets throughout the contagion process (Abrams *et al.,* 1988).

The urethra is the most common route for an infectious pathogen to enter the urinary system and cause illness. The shorter urethra in women renders them more sensitive to such infections, which is thought to be one of the keys causes for the greater occurrence among women than males (Abrams *et al.,* 1988). Because the urethra is shorter in women than in males, women

are more susceptible to urinary tract infections (Finer and Landau, 2004). In women, the shortness of the urethra allows the pathogen to enter the bladder, resulting in bladder infection.

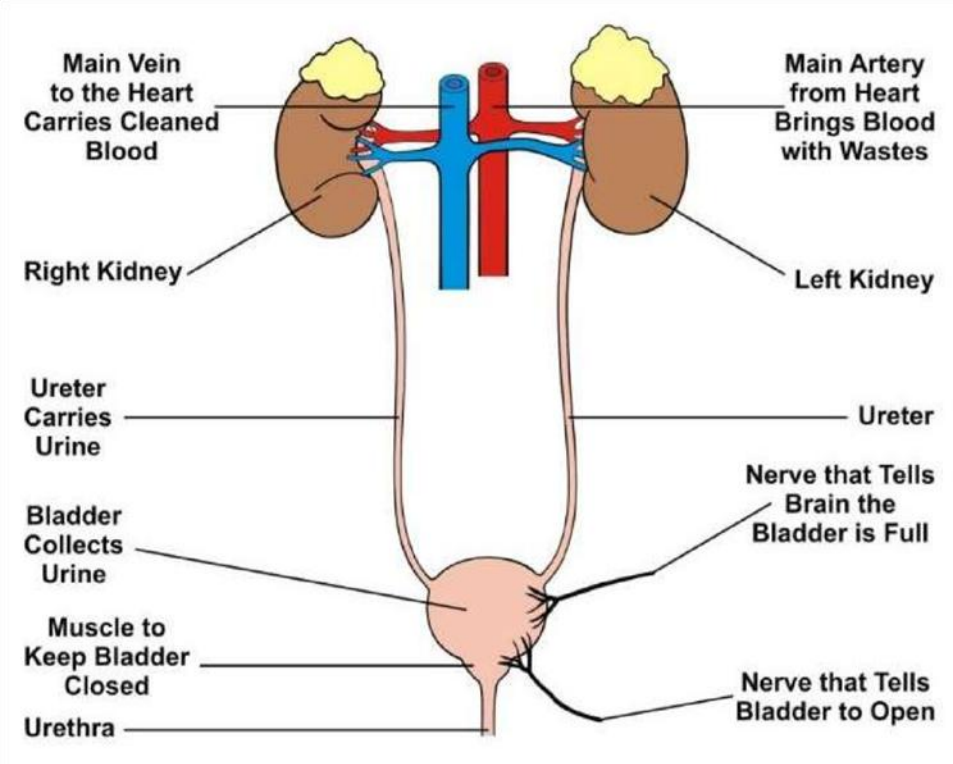

Figure 2.1: Structure of the urinary tract system. (www.livescience.com/27012-urinary-system.html)

2.3 Classification of urinary tract infections

Pyelonephritis /Complicated UTI

Pyelonephritis is an inflammation of the kidney parenchyma, calices (cup-shaped division of the renal pelvis), and pelvis (upper end of the ureter that is located inside the kidney) that is mainly caused by bacterial infection (Patricia, 2014). Complicated UTI can affect either sex at any age (Magliano *et al.*, 2012). It is frequently pyelonephritis or cystitis that does not meet the requirements for being uncomplicated. A UTI is considered complicated if the patient is a child, pregnant, or has any of the following: a structural or functional urinary tract irregularity and barrier of urine flow, comorbidity that increases the risk of infection or resistance to

treatment, such as poorly controlled diabetes, chronic kidney disease, or immune concession, and recent urinary tract instrumentation or surgery. Asymptomatic bacteriuria occurs when bacteria are identified in the urine without constricting the urinary tract or systemic indications of infection. It is a common condition that worsens with age and does not require treatment. UTI may be evaluated as a probable diagnosis in patients who have bacteriuria and systemic signs of infection without localizing genitourinary (GU) symptoms (Meynell & Malins, 1948). However, alternative reasons of the systemic symptoms should be considered, since the urine culture may only be showing asymptomatic bacteriuria. Pyelonephritis is an infection of the kidney parenchyma. Pyelonephritis symptoms include confined pain (costo-vertebral soreness, back or flank pain), and full indicators of infection include chills, fever, nausea, and vomiting. Complicated UTI, which implies that there is a cause for the UTI and also presents with localizing GU symptoms and systemic symptoms that may not be distinguishable from uncomplicated UTI based on these symptoms alone (Ezeadila *et al.*, 2015).

Pyelonephritis and complex UTIs are often caused by enteric bacteria migrating from the digestive tract into the urethra and then accumulating in the urinary system. The term "complicated" implies that there is a prejudicing reason for the stimulation of the infection, which could include the presence of abnormal rejecting factors such as neurogenic bladder, stricture, benign prostatic hypertrophy, or a foreign body such as a stone, stent, or catheter. There are other complex aspects such as known or suspected multi-drug resistance that might be risk factors. Complicated UTI can occur in either gender and at any age, but it is more common in men after the fifth decade due to the progression of benign prostatic hypertrophy (BPH) and in both men and women who have voiding abnormalities associated with other conditions (Price *et al.*, 2016). Most UTIs in young premenopausal women who are not pregnant, as well as in young adult men, are not complicated. If there is anomalous voiding as a result of the aforementioned factors, UTIs in diabetics or postmenopausal women should be considered complicated. Pregnancy, antibiotic resistance, and immunological suppression are additional risk factors for complicated UTIs, and these diseases complicate therapy. A history of urinary maintenance, recurrent UTI, or urinary events, such as stent or catheter placement, may indicate a higher risk of complex UTI in individuals with atypical voiding. Any illness or foreign body that causes a blockage of normal urine flow contributes to a complicated UTI. Diabetes is also a risk factor for perirenal edema and emphysematous pyelonephritis (Anibijuwon, 2015).

If the catheter is in place, a urine culture with more than 104CFU/mL of an uropathogenic supports the diagnosis. This approach only applies to patients who have been diagnosed with UTI symptoms. Positive urine culture in the absence of symptoms is classified as asymptomatic bacteriuria and should not be treated except in extremely specific circumstances such as pregnancy. In patients with a total urinary tract barrier, a culture of the first urine from a freshly implanted nephrostomy tube can aid in the identification of the uropathogen. Blood cultures may be positive in patients with pyelonephritis or systemic indications of infection, and they should be able to dispute the urine pathogen (Ezeadila *et al.*, 2015). If the blood and urine isolates do not match, a search for the source of the blood isolate should be undertaken, and a diagnosis other than complicated UTI should be considered (the urine isolate might indicate asymptomatic bacteriuria).

Pyelonephritis is a bacterial infection of the parenchyma of the kidney. Unless the infection has been diagnosed, pyelonephritis should not be used to characterize tubulointerstitial nephropathy. Pyelonephritis is responsible for around 20% of community-acquired bacteraemia in women. Pyelonephritis is uncommon in males with normal urinary tracts. Bacterial growth in the urinary system causes around 95% of instances of pyelonephritis. Although obstructions such as strictures, calculi, tumors, neurogenic bladder, and VUR can lead to pyelonephritis, past research has shown that the majority of women with pyelonephritis have no obvious functional or anatomic abnormality. Pyelonephritis in males is usually caused by a functional or anatomic deficit. Reflux can be caused by Cystitis or by anatomical abnormalities. When ureteral peristalsis is restricted, for example during pregnancy, by obstruction, or by gram-negative bacteria endotoxins, the danger of bacterial surmounting is considerably reduced (Meynell & Malins, 1948).

Pyelonephritis is a typical side effect of bladder catheterization in young girls and pregnant women. Pyelonephritis, which is not caused by bacterial ascent but rather by haematogenous dissemination, is more common in infectious organisms such as *S. aureus, P. aeruginosa, Salmonella* species, and *Candida* species (Magliano *et al.*, 2012). The infection is urgent and intermittent, starting in the pelvis and medulla and spreading into the cortex as an increasing wedge. Cells that promote chronic inflammation emerge within a few days, perhaps leading to medullary and subcortical expansion. Normal parenchymal tissue between foci of infection is prevalent in this situation. Papillary necrosis may be seen in acute pyelonephritis owing to hyperglycemia, obstruction, sickle cell disease, pyelonephritis in renal transplants, pyelonephritis related to candidiasis, or analgesic nephropathy. In children, acute

pyelonephritis is usually accompanied by renal scarring, but in adults, the same damage is not visible in the absence of reflux or obstruction (Price *et al.,* 2016).

Cystitis

Cystitis is a mutual bladder infection in women, with episodes of uncomplicated cystitis typically preceded by sexual intercourse (honeymoon cystitis). Bacterial infection of the bladder in males is often complicated, coming from the urethra or prostate or after urethral instrumentation (Magliano *et al.,* 2012). Chronic bacterial prostatitis has been identified as the most prevalent cause of recurrent cystitis in males. Cystitis is a medical word that describes inflammation of the bladder. This bacteria-caused ailment is known as a urinary tract infection (UTI). If the infection spreads to the kidneys, it can be dangerous. A bladder infection may be both painful and inconvenient. Cystitis can sometimes develop as a side effect of another medical condition. Antibiotics can be used to treat bacterial cystitis, however, therapy for other kinds of cystitis is dependent on the underlying reason. A strong, persistent urge to urinate, a burning sensation when urinating, passing frequent, small amounts of urine, blood in the urine (haematuria), passing cloudy or strong-smelling urine, pelvic discomfort, a feeling of pressure in the lower abdomen, and finally low-grade fever are common signs and symptoms of cystitis. New instances of inadvertent daytime wetness in young children may indicate a urinary tract infection (Ezeadila *et al.,* 2015).

Urethritis

Bacteria infect the many periurethral glands in the bulbous and pendulous sections of the male urethra, as well as the whole female urethra, producing urethral infection. Sexually transmitted pathogens such as *Chlamydia trichomonas, Neisseria gonorrhoea,* and *Trichomonas vaginalis*, as well as herpes simplex virus, are prevalent causes in both sexes. Urethritis is a disorder in which the urethra, or the tube that transports urine from the bladder to the outside world, becomes inflamed and irritating. The male urethra also transports sperm. Urethritis often causes urination discomfort and an increased need to urinate. The most common cause of urethritis is a bacterial infection (Meynell & Malins, 1948).

Acute urethral syndrome

The acute urethral syndrome affects women and is characterized by dysuria, frequency, and pyuria (dysuria-pyuria syndrome), which is comparable to cystitis. In contrast to Cystis, acute

urethral syndrome, and regular urine cultures are either negative or indicate colony numbers lower than the usual criteria for bacterial cystitis diagnosis. *Chlamydia trachomatis* and *Urea plasma urealyticum* are the causal agents of urethritis, a probable cause of Acute Urethral Syndrome, and they are not identified on standard urine culture (Price *et al.*, 2016). Non-infectious reasons have also been hypothesized, although the associated evidence is inconclusive, most non-infectious causes cause little or no pyuria. Anatomic flaws such as urethral stenosis, physiologic abnormalities such as pelvic floor muscle dysfunction, hormonal imbalances such as atrophic urethritis, restricted trauma, GI system symptoms, and inflammation are all possible non-infectious causes (Anibijuwon, 2015).

Asymptomatic bacteriuria

Asymptomatic bacteriuria is defined as the absence of signs and symptoms in a patient whose urine culture meets the criteria for UTI. Pyuria may or may not be present. Because it is asymptomatic, this kind of bacteriuria develops mostly when high-risk individuals are checked or urine cultures are performed for other reasons. Asymptomatic bacteriuria is specified in patients such as pregnant women at 12 to 16 weeks gestation or the first prenatal visit if the bacteriuria is untreated, if later than the stated period, because of the risk of symptomatic UTI, which includes pyelonephritis, during pregnancy; and adverse pregnancy outcomes, such as low-birth-weight neonate and premature delivery (Magliano *et al.*, 2012).Patients who have undergone a kidney transplant within the last 6 months, young children with a gross VUR, and before some invasive GU operations that might induce mucosal bleeding (for example, transurethral resection of the prostate) are among the other groups of patients. Postmenopausal women, people with managed diabetes, and patients who utilize urinary tract foreign objects such as stents, nephrostomy tubes, and indwelling catheters) may have chronic asymptomatic bacteriuria and, in certain cases, pyuria (Meynell & Malins, 1948). Asymptomatic patients should not be frequently examined since they are at low risk. Treatment of asymptomatic bacteriuria in patients with indwelling catheters frequently fails to eliminate the bacteriuria and merely contributes to the growth of antibiotic-resistant organisms.

2.4 Diagnosis of urinary tract infection

Detection of bacteriuria by urine microscopy.

Using gram staining Bacteriuria can be identified microscopically in urine specimens that have not been centrifuged, by Gram staining of centrifuged specimens, or by direct examination of bacteria in urine specimens. Gram staining of uncentrifuged urine specimens is a straightforward procedure. A sample of urine is placed on a glass microscope slide, allowed to dry, stained with Gram stain, and then microscopically examined. Because of the various criteria used to establish a positive test result, the routine aspects of the test are not well defined. The test was reported to be effective for detecting 105 colony-forming units per millilitre of urine but insensitive for detecting smaller amounts of bacteria in one investigation (Carroll *et al.,* 1994).

Detection of bacteriuria by Nitrite test.

Bacteriuria is also chemically detectable when bacteria create nitrite from nitrate. The biochemical response observed by the nitrite test is associated with members of the *Enterobacteriaceae* family, which are the organisms most usually responsible for UTIs. Because nitrite generation is not related to urinary tract infections such as *Staphylococcus saprophyticus, Pseudomonas* spp, or *Enterococci*, the efficacy of this test is limited (Pappas, 1991). The test also necessitates examining a specimen of the first urine produced in the morning, as bacteria take 4 hours to convert nitrate to nitrite at detectable levels.

Detection of pyuria by urine microscopy.

Pyuria can be identified and measured microscopically by examining the urine leukocyte excretion rate. Leukocytes can be counted using a haemocytometer, in urine specimens using Gram staining, or in a centrifuged specimen. Leukocytes, leukocyte casts, and other cellular features are directly detected using urine microscopy. One downside of urine microscopy is that leukocytes degrade fast in urine that is not fresh or has not been well stored. Furthermore, each of these approaches has drawbacks that restrict its use as a regular test (Carroll *et al.,* 1994). As a result of these drawbacks, urine microscopy should be reserved for individuals suspected of having pyelonephritis or other more severe illnesses.

2.5 Bacterial pathogens associated with Urinary Tract Infections

Staphylococcus saprophyticus species

One of the coagulase-negative *staphylococci* is *Staphylococcus saprophyticus*. This bacterium is mostly responsible for urinary tract infections in young sexually active women. Its involvement in inducing UTI in males is yet unknown (Price et al., 2016). *Staphylococcus saprophyticus* is a major bacterium that causes cystitis in young women. *S. saprophyticus* has clinical characteristics with *Escherichia coli*-caused urinary tract infections, but it differs in etiology, seasonal fluctuation, and geographic distribution. This pathogen is only seen in humans and is associated with uncomplicated urinary tract infections. *Staphylococcus saprophyticus* has unique urotropic and ecological characteristics that distinguish it from other *staphylococci* and *Escherichia coli.* Coagulase-negative staphylococci were thought to be urinary contaminants until Torres Pereira reported the isolation of coagulase-negative staphylococci with antigen 51 from the urine of women with acute UTI in the 1960s and 1962. This hypothesis has been validated by several research in future years (Price *et al.,* 2016). The investigations revealed that the bacterium belongs to micrococcus subgroup 3 and was later renamed *S. saprophyticus*. Urease development and renal ureteral infection are linked to *S. saprophyticus* infection. *S. saprophyticus* can also cause urinary tract infections in men of all ages; the organism has previously been identified in young boys, male homosexuals, and elderly men with urinary catheters (Magliano *et al.,* 2012).

The pathogen can also cause urethritis, epididymitis, prostatitis, and nephrolithiasis in males, but it is uncommon in hospitalized men. Other coagulase-negative staphylococci are typically isolated from elderly individuals hospitalized with urinary indwelling catheters or other urinary tract interventions. The ability of *S. saprophyticus* to cling to uroepithelial cells is the primary cause for this distinction, although other coagulase-negative staphylococci can colonize indwelling catheters. *S. saprophyticus* virulence factors include adhesion to urothelial cells via a surface-associated protein, lipoteichoic acid; a hemagglutinin that binds to fibronectin, a haemolysin; and extracellular slime formation (Anibijuwon, 2015). According to Hedman et al, he described the epidemiological and clinical aspects of 270 randomly selected episodes of UTI caused by *S. saprophyticus* and matched them with 276 episodes of UTI caused by other organisms, and they matched according to each subject's sex and age, as well as the temporal occurrence of each episode.

Escherichia Coli

Escherichia coli causes between 65% and 90% of urinary tract infections (UTIs) in children. *E. coli* is a gram-negative bacillus and a facultative anaerobe bacteria that colonizes the gastrointestinal tracts of warm-blooded mammals. When the immune system cooperates or sickness results from environmental exposure, several strains of *E. coli* are capable of producing disease (Rane, 2011; Ayulo *et al.,* 1994). In addition to urinary tract infections, *E. coli* can cause infections in the wound, biliary tract, and stomach cavity. Septicaemia, neonatal meningitis, infantile gastroenteritis, and haemorrhagic diarrhoea can all be caused by *E. coli* (Ayulo *et al.,* 1994). Renal failure, pancreatitis, and diabetes mellitus are all recognized *E. coli* infections. Drowsiness, seizures, and coma are also possible neurological signs (CDC, 2015). This bacterium can be transmitted by unsanitary activities such as not washing hands and a lack of personal hygiene. There are about 700 recognized *E. coli* serotypes, which are characterized by their "O" somatic and "H" flagella antigens, both of which come from the bacteria's cell surface (Todar, 2014; Hamilton *et al.,* 2006). More than 50 *E. coli* serogroups have been identified. EHEC sickness is more frequent in the summer and is caused by the intake of raw meat, water, unpasteurized milk, or fruit juice (Shanson, 1999). The bacteria continually develop and mutate, gaining new pathogenic characteristics, making treating *E. coli* O157: H7 infections challenging (Todar, 2014).

Enterobacter species

Enterobacter (genus *Enterobacter*) is a rod-shaped bacterium belonging to the *Enterobacteriaceae* family. They are classified as facultative anaerobes, but they are also gram-negative bacteria, which implies they can thrive in both aerobic and anaerobic settings. Many *Enterobacter* species contain flagella, indicating that they are mobile. Motility, as well as specific biochemical features, such as the capacity to produce an enzyme known as ornithine decarboxylase, are used to distinguish *Enterobacter* from the extremely similar and closely related *Klebsiella* bacteria (Meynell & Malins, 1948). *Enterobacter* is the collective name for the organism's primary natural environment, animal intestines (from Greek enteron, meaning "intestine"). *Enterobacter* is prevalent, and its existence in animal digestive tracts contributes to its widespread dispersion in soil, water, and sewage (Ezeadila *et al.,* 2015).

Several *Enterobacter* species, including *E. cloacae, E. aerogenes, E. gergoviae,* and *E. agglomerans,* have been identified as adaptive pathogens (disease-causing organisms) in humans. Urinary tract infections are caused by pathogenic *Enterobacter*. Infections caused by

Enterobacter cloacae or *E. aerogenes* are frequently related to exposure to the organisms in nosocomial settings such as hospitals or nursing homes (Anibijuwon, 2015). Non-beta-lactam antibiotics including fluoroquinolones, such as ciprofloxacin, can cause resistance in *Enterobacter* due to unique cellular and genetic processes. Ciprofloxacin-resistant *E. aerogenes* and multidrug-resistant *E. aerogenes*, which are resistant to ciprofloxacin and imipenem, are two examples of bacteria that exploit similar processes. Resistance to aminoglycosides in *Enterobacter* species has been linked to a bacterial genetic component known as an integron (Price *et al.,* 2016). Integrons are made up of genes that confer antibiotic resistance and are incorporated into bacterial genomes by genetic recombination.

Klebsiella species

Klebsiella pneumonia is a gram-negative bacteria that arises in normal oral, cutaneous, and gastrointestinal flora. It is also the most important human pathogen under the genus *Klebsiella*, causing a wide range of illnesses in hospitals, including urinary tract infections. They are also seen in long-term care institutions and communities across the world, and they can be found in various places such as the lung, abdominal cavity, surgical sites, and soft tissue infections, bacteremia. This Gram-negative bacterium is also the third most often isolated pathogen in sepsis patients' blood cultures (Magliano *et al.,* 2012). A novel hyper virulent (hypermucoviscous) variety of *K. pneumoniae* has been identified and is causing serious and life-threatening infections such as pyogenic liver abscesses, endophthalmitis, and meningitis. *Klebsiella pneumoniae* is a prevalent pathogen in nosocomial infections, and it is multidrug-resistant (Meynell & Malins, 1948).

The major targets of this pathogenic bacterium are hospitalized, immune-impaired individuals with significant underlying disorders, which implies that a patient can have a urinary tract infection while also having another infection. The molecular pathways involved in the pathogenesis of illnesses caused by these bacteria are not fully understood. Caveolin-1, a protein that regulates endocytosis, is linked to inflammatory responses in *K. pneumoniae* infection (Anibijuwon, 2015). Lyn, a Src tyrosine kinase, is implicated in monocyte-related phagocytosis via FcR via tyrosine phosphorylation in immunoreceptor tyrosine-based activation patterns. This may result in lipid raft coordination and affect the cellular function of caveolin-1 (Ezeadila *et al.,* 2015). Lyn is found near lipid rafts and can thus be translocated into stimulated membrane areas to transmit cellular signals, which may lead to host cell defense against *K. pneumonia* through the instruction of phagocytosis processes and the regulation of

inflammatory responses. Lyn can work with lipid rafts to regulate inflammatory responses in *K. pneumoniae* infection via the p38/NF-B pathway (Meynell & Malins, 1948).

Proteus millabillus species

Proteus mirabilis, a common pathogen, has been identified as the primary cause of severe urinary tract infections and is also responsible for bacteremia. Previous research suggests that most occurrences of *P. mirabilis* bacteremia are caused by a UTI (Price *et al.*, 2016); however, the risk factors for bacteremia and fatality rates from *P. mirabilis* UTI have yet to be discovered. Because of the increased mortality associated with bacteremia *P. mirabilis* UTIs, clinicians should manage cases with the risk factors for bacteremia, which include community-acquired infection, hyperthermia or hypothermia, hydronephrosis, band neutrophils accounting for >10% of the white blood cell count, and a high level of C-reactive protein (Ezeadila *et al.*, 2015). *Proteus mirabilis* is well recognized in clinical laboratories and microbiology survey courses as the species that swarm over agar surfaces, outnumbering any other organisms present. Urease production and robust swarming motility are two of this organism's key recognized guarantees. This species is a Gram-negative, motile, urease-positive, lactose-negative, indole-negative rod that generates hydrogen sulfide. It is a member of the same bacterial family as *E. coli (Enterobacteriaceae). P. mirabilis* can cause symptomatic infections of the urinary system, such as cystitis and pyelonephritis. *P. mirabillis* was also discovered in instances of asymptomatic bacteriuria, primarily in elderly type 2 diabetic patients (Magliano *et al.*, 2012). *P. mirabillis* infections can also cause bacteremia, which can progress to potentially fatal urosepsis. Infections with *P. mirabilis* can also result in the production of urinary stones (urolithiasis). *P. mirabilis* is mostly found in the gastrointestinal system. The majority of *P. mirabilis* urinary tract infections are assumed to be the consequence of bacteria surmounting from the gastrointestinal system, while some are likely to be the result of person-to-person transmission, particularly in healthcare settings such as hospitals (Anibijuwon, 2015). *P. mirabilis* causes 1-10% of all urinary tract infections, with the percentage varying depending on the geographic area of the research, the types of samples obtained, and the characteristics of the individuals studied.

2.6 The implications associated with urinary tract infections: Chronic Kidney Diseases (CDK)

Chronic kidney disease (CKD) is increasingly being recognized as a global public health issue, and it has recently become a major concern. People with CKD and renal failure are more likely to have infection complications (UTIs). The modulation of renal metabolism of immunologically active proteins and specific therapeutic effects can be caused by uremic intoxication, which is a secondary immune modification in uraemia (Magliano et al, 2012). End-stage renal disease (ESRD) is associated with significantly increased susceptibility and mortality due to cardiovascular disease (CVD) and infections, including urinary tract infections that can spread from the urinary system to the cardiovascular system, which account for approximately 50% and 20% of total mortality in ESRD patients, respectively (Meynell & Malins, 1948).All of these issues are thought to be related to changes in immune system function in ESRD. Chronic renal failure increases the chance of developing urinary tract infections (UTIs) due to metabolic abnormalities that cause secondary immunological changes that disrupt numerous components of the immune system. Uraemia is associated with immune dysfunctions, including a reduced immune response component that contributes to the high prevalence of infections in these patients, as well as a persistent immune stimulation component that results in inflammation, which may contribute to CVD (Price *et al.,* 2016). Enhanced atherosclerosis in ESRD may involve interconnected processes such as oxidative stress, endothelial dysfunction, and vascular calcification in a milieu of constant low-grade inflammation with reduced function of T cells and neutrophils, as well as a deregulated cytokine network, with the proinflammatory cytokines IL-6 and TNF-alpha playing key roles in the improvement of the imbalance and CVD (Ezeadila *et al.,* 2015). Furthermore, UTIs are common following kidney transplantation in individuals with chronic renal insufficiency.

2.7 Risk factors of urinary tract infections in both gender of different ages

The use of spermicide-coated condoms is one of the leading causes of UTI in women (Meynell & Malins, 1948). The increased incidence of UTI in women who use antibiotics or spermicides is very definitely related to alterations in vaginal flora that allow Escherichia coli proliferation. In older women, soiling of the perineum owing to faecal incontinence raises the risk of UTI.UTI risk factors include anatomic, structural, and functional abnormalities. Vesicoureteral reflux (VUR) is a frequent anatomic abnormality that affects 30 to 45% of young infants with symptomatic or complicated UTIs (Anibijuwon, 2015). Urethral valves (a congenital obstructive malformation), delayed bladder neck development, bladder diverticulum, and

urethral duplicates are some of the anatomic anomalies that contribute to UTI. Urine flow obstruction and inadequate bladder discharge are also anatomical and functional urinary tract abnormalities that predispose to UTI. Calculi and tumors can also significantly reduce urine flow. Neurogenic dysfunction, pregnancy, uterine prolapse, cystocele, and prostatic enlargement can all impede bladder emptying (Ezeadila *et al.,* 2015). UTIs caused by congenital causes often occur throughout childhood. Instrumentation, such as bladder catheterization, stent appointments, cystoscopy, and recent surgery, are additional risk factors for UTI. The elderly have a higher prevalence of most other risk factors.

2.8 Treatment of urinary tract infections

The infection is verified after a urine examination of the patient. The bacteria are identified by analysis, and antibiotic sensitivity tests are done to determine which antibiotics the bacteria are sensitive to. Patients who are discovered to have a UTI infection are given antibiotics. Patients are advised to drink plenty of fluids to flush bacteria from their urinary tract. When the therapy is completed, the patient is expected to provide another urine sample for confirmation. This test will determine if the illness has been treated or whether the antibiotics were ineffective in eliminating the germs. Sulfamethoxazole trimetoprim, Amoxicillin, Ciprofloxacin, and Levofloxacin are routinely used to treat simple UTI infections. Analgesics, which numb the bladder and reduce certain symptoms of infection, may also be recommended to patients. Recurrent urinary tract infections may necessitate a lengthier course of antibiotics. Doctors may recommend vaginal oestrogen treatment to menopausal women to minimize the recurrence of the illness. Patients with serious infections may need to be hospitalized and given intravenous antibiotics. In certain locations of the United States and other countries, more than 15% to 20% of *Escherichia coli* strains causing uncomplicated cystitis are now resistant to ampicillin and sulphonamides (Gupta *et al.,* 1999). Because more than one-third of isolates demonstrate in vitro resistance, ampicillin and sulphonamides should not be utilized as empiric treatment (Hooton and Stamm, 1997). Even though non-*Escherichia coli* uropathogens are frequently resistant to nitrofurantoin, the incidence of resistance among *Escherichia coli* is 5%. In most investigations of uropathogenic strains, resistance to fluoroquinolones remained at 5%. Three-day treatments are recommended because they are associated with higher compliance, cheaper costs, and fewer adverse responses than 7 to 10-day regimens (Warren *et al.,* 1999). Several trials and clinical experience have demonstrated the efficacy of 3-day regimens of trimethoprim, trimethoprim-sulfamethoxazole, or a fluoroquinolone for the treatment of acute uncomplicated cystitis, and these drugs are typically suggested for empiric therapy (Warren *et*

al., 1999). In contrast, 3-day beta-lactam regimens are less successful than 5 days of treatment (Warren *et al.,* 1999). Nitrofurantoin is a safe and typically efficient antibiotic, however, it should not be used for longer than 7 days. Even with fluoroquinolones, single-dose treatments are marginally less efficacious than three to seven-day regimens (Hooton and Stamm, 1997). Trimethoprim-sulfamethoxazole (TMP-SMX) in a 3-day regimen was recommended as first-line therapy by the Infectious Disease Society of America in 1999 (Warren *et al.,* 1999). Given the rising prevalence of TMP-SMX resistance among uropathogens, it is critical to investigate risk variables that predict in vitro resistance. Diabetes, recent hospitalization, antibiotic usage in the last 3 to 6 months (for whatever cause), and recent TMP-SMX use are all examples (Wright *et al.,* 1999).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study site

This research was conducted at Mashoko Christian Hospital, which is located in Bikita District, Masvingo Province. Its coordinates are -20.4833 and 31.7667. All urine samples were obtained from the hospital's Out Patients Department (OPD) under complete Quality care. The microbiological analysis and identification of bacteria from urine samples were separated into two sections: biochemical and microscopical analysis and urine culture. Mashoko Hospital is located 135 kilometres from Masvingo town in Zimbabwe's natural region four. Mashoko's typical temperature ranges from 25 to 38 degrees Celsius.

3.2 Sampling

A total of 24 urine samples were chosen at random from the samples of suspected UTI patients. During collection, some precautionary measures were taken, such as collecting mid-stream urine and labelling each sample with the patient's name, age, and gender, even though the name was irrelevant in the study; this helped to maintain the accuracy of the results, and avoid any inconveniences. Across all age groups, at least 50ml of urine was collected, however, only 10ml of each sample was saved for culture. The age groups to be investigated were separated into three categories: young, middle-aged, and old. The young were aged 20-39, the middle-aged were aged 40-59, and the elderly were aged 60 and over. A total of 8 samples were taken from each age group, with an equal number from both genders, implying that each gender in each age group received four samples. A control sample was also taken. To avoid contamination of the samples, procedures such as urinalysis, microscopy, urine culture, and microbe identification were performed at Mashoko laboratory under strict quality control.

3.3 Laboratory Analysis

Urinalysis and Microscopy

Urinalysis is a test done on a urine sample to determine the biochemistry and presence of pathogens such as bacteria. Urinalysis was done in this study to check for biochemistry components such as leucocytes and Nitrites, which are usually indicated on the Urine dipstick. These components are linked to the presence of bacteria in urine samples. If the urine sample is leucocyte and Nitrite positive, it indicates a bacterial

infection. To perform this test, at least 30ml of each sample was spun in test tubes at 3000 revolutions per minute (rpm) for 10 minutes. The supernatant was utilized for urinalysis (Nitrite, Protein, and leukocyte test), while the sediment was saved for bacterial examination under the microscope. A urine dipstick was dipped into each sample and the findings were examined after at least 1 minute. The outcomes of each sample were documented. The microscopical examination was also done on all samples to determine if the urine included bacteria or not. If the urine contained bacteria, the sample was saved for culture, and all positive samples were preserved in low temperatures.

Figure 3.1: An illustration of the process of Urinalysis using a dipstick. (www.jeanhailes.org.au/news/dipstick-tests-miss-up-to-half-of-utis).

Preparation of culture media

The first step was to weigh 50g of Mackonkey media on a balance before suspending it in 1L of distilled water. After withdrawing the approximate amount, the tops of the ingredients bottles should be sealed as soon as feasible. It was necessary to utilize clean glassware and equipment. The Scott bottles used for media preparation had a capacity

of at least double the volume of the medium to be prepared. The water was distilled using a glass still. The Scott bottles were labelled with the type of medium that was to be produced. Water containing chlorine, lead, copper, or detergents was avoided since it kills the target organism. The components, which were powdered, were added to the water and mixed to dissolve. The medium was dissolved in the mixture by stirring while heating and managing the heat to prevent boiling and foam formation, which harm the medium. When the ingredients were entirely dissolved, they were autoclaved at 121 degrees Celsius. After then, the medium was allowed to cool to 45 degrees Celsius. The medium was well mixed before being put into plates.

Sterilization of glass, Petri dishes, tubes and other glassware

A hot air oven was used to sterilize the items. The glassware was sterilized by dry heat for 60 minutes at a temperature of 160°C, and the time was recorded using a clock from the moment the items in the oven reached the temperature. As a result, a one-hour heating period was permitted. There was also time allotted for the goods in the oven to cool. The oven lid was kept closed until the temperature inside dropped below 50°C.

Checking for pH of a culture medium

Most culture mediums should have a pH close to neutral. The pH of the medium was tested using a pH meter.

Sterilization of the culture media

The majority of the media was autoclaved for sterilization.

Dispensing sterile media into Petri dishes

On a flat surface, sterile Petri dishes were placed. The flax was gently rotated to mix the media. This procedure was carried out to prevent the production of bubbles. For sterilization, the flask's neck was flamed. Each 100mm diameter Petri plate received 20 ml of media. To achieve a uniform layer of agar, the Petri dishes were rotated on the bench surface. The medium was left to gel. After the medium had gelled and cooled, the plates were stacked and wrapped in plastic bags to prevent moisture loss and contamination. It also guarantees that the plates are not exposed to strong light, particularly sunshine. The plates were kept at 8 degrees Celsius.

Inoculation

Immediately before starting inoculating a culture medium, the medium was inspected for visual contamination or any changes in appearance that may indicate medium degradation. Aseptic procedures were employed throughout this practice to prevent contamination of cultures and specimens, as well as infection of the surrounding environment. Aseptic procedures used include the use of sterile wire loops before and after utilizing a Bunsen burner with a protective tube. The specimen bottles and tubes' necks were flamed after and before removal, as well as before restoring caps and bungs. It was also ensured that the lids and tops of tubes and bottles did not come into contact with unsterile surfaces. Racks were employed for holding specimens and culture medium tubes. After inoculating the culture media, specimens were prepared for slide preparation, and the work benches were decontaminated with ethanol before and after the day's work. Inoculation is a technique for inoculating medium into Petri dishes that should result in single colonies. Inoculation may also tell if a culture is pure or mixed. This ensures that a pathogen is isolated in pure culture before being tested for antimicrobial sensitivity. Playing out or looping out refers to the process of the inoculating medium in Petri dishes. The surface of the medium was dried to allow for the growth of single colonies, and the cover of the plate was removed and put facing up on an incubator shelf. The medium-containing base was inverted and placed at an angle on the lid. The medium was incubated in an electric incubator at 37°C for 35 minutes. The inoculum was applied to a tiny portion of the plate using a sterile loop, and the loop was flame sterilized. Inoculate all of the specimens on the plates with a sterile loop.

Labelling of inoculated media

Using a marker pen, each of the inoculations was labelled with the patient's age and gender. Dates were also labelled, and all labelling was done at the base of the culture plates. To avoid concealing the culture, a slope label was placed on the underside of the media. Because the medium was incubated aerobically, it was labelled O2.

3.4 Microbial Isolation and identification

3.4.1 Identification of Escherichia coli

Gram staining

Mackonkey agar plates with growth were chosen, and distinct colonies were chosen for gram staining in each plate. Microscope slides were labelled according to their particular sample labelling, and the ringer's solution was placed into each slide using a dropper. Colonies of interest were selected using a flamed inoculating loop and suspended on glass slides. The slides got fixed by running them through a flame. The suspensions were rinsed with distilled water after being saturated with crystal violet dye for 2 minutes. The slides were then soaked with iodine for about 1 minute and cleaned with distilled water. Acetone was applied to each slide for 10 seconds to decolorize bacteria within cell walls before being rinsed away with distilled water. The slides were saturated with safranin counter stain for 2 minutes before being rinsed with distilled water. The slides were dried in a 27°C drying oven. On a compound light microscope, a drop of immersion oil was applied to each slide, which was then examined under a high-power lens x100. All findings were documented and tabulated.

Sub culturing

Mackonkey agar plates with mixed growth were sub cultured. Selecting single colonies of interest using a sterilized inoculating loop was used for sub culturing. Inoculation was carried out in marked Petri dishes using fresh Mackonkey agar plates. The plates were incubated for 24 hours at 37°C.

Indole test

Gram-negative colonies were collected from Mackonkey agar plates with a sterilized inoculating loop, and the suspension was produced in tryptone water with Kovac's reagent. In a bijou bottle containing 3 ml of sterile tryptone water, the test organism was inoculated. The inoculum was incubated for 24 hours at 37°C. 0.5ml of Kovac's reagent was used to perform the test. Examinations were conducted, and the results were recorded and tallied.

3.4.2 Identification of *Klebsiella* species

Citrate test

Simenon's citrate agar was used in the experiment. The medium slopes were made in Bijou bottles and kept at 8°C. Using a sterile wire loop, the slope was streaked with a saline suspension of the inoculum, and the butt was stubbed. After incubating the

medium at 35°C for 24 hours, samples were tested for a bright blue colour to indicate a positive test.

3.4.3 Isolation and identification of *Staphylococcus saprophyticus* species

Catalase test

This test is used to distinguish between staphylococci and streptococci. This test necessitates the use of hydrogen peroxide. Using a sterile wooden stick, 2ml of Hydrogen peroxide was placed into a test tube, and numerous colonies of the inoculum were extracted and submerged in Hydrogen peroxide. Because the inoculum was cultivated on blood agar, extreme caution was used because catalase is present in red cells. The reactions were observed and documented.

Coagulase test

Glass slides were labelled with the names of the specimens that were cultured. A pipette was used to place sterile Ringer's solution on slides. Gram-positive colonies were isolated from blood agar and suspended in saline. On each slide, a drop of blood plasma was mixed with a bacterial solution. The slides were gently shaken and agglutination was detected.

Manitol Salt test

Gram-positive colonies were chosen from sub cultured blood agar and inoculated into labelled Petri plates using a sterile loop. The Petri plates were incubated in an incubator at 37°C for 24 hours. The plates were checked after incubation for any yellow colouring on the infected regions.

3.4.4 Isolation and Identification of *Proteus millabilis* species

Urease test

Testing for glucose enzyme activity is essential for distinguishing *Enterobacteria*. *Proteus* strains produce a much of urease. Christensen's urea broth was used in the experiment. In a Scott bottle holding 3ml of sterile Christen's modified urea broth, the

inoculum was vigorously inoculated. The medium was incubated in a water bath for 12 hours at 37°C. Colour changes were seen and documented.

CHAPTER 4

4.0 RESULTS

Table 4.1: Summary of urinalysis and microscopy according to age groups

Age Group	**20-39**		**40-59**		**60+**	
Biochemical component	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Nitrite	4	4	5	1	6	1
Protein	3	5	4	1	4	3
Leucocytes	3	5	5	2	6	1
Microscopy						
White blood cells	5	3	5	1	6	1
Bacteria	5	3	4	2	6	1

The Nitrite test yielded the most positive results in the 40-59 and 60+ age ranges, with 5 and 6 instances, respectively. With one case each, these age groups got the lowest negatives for the same test. The 20-39 age group had the lowest number of positive cases in the Nitrite test, as well as an equal number of positives and negatives. In the protein test, the age groups 40-59 and 60+ had an equal number of positive cases, and they were listed as the age categories with the most positive instances, with four positive cases in each category. There were just 2 and 3 negative cases in these two age categories, respectively. Positive cases were lowest in the 20-39 age group, whereas negative cases were greatest, with 5 cases. For the leucocytes test, the 60+ age group had the most positive cases with 6 cases out of 7 samples obtained, followed by the 40-

59 age group with 5 cases, and finally the 20-39 age group with three cases out of eight. With five cases, the 20-39 age group had the highest negatives.

Table 4.2.: Summary of urinalysis and microscopy according to gender

Gender	**Males**		**Females**	
Biochemical component	**Positive**	**Negative**	**Positive**	**Negative**
Protein	5	5	8	3
Leucocytes	4	6	9	2
Nitrites	5	5	10	1
Microscopy				
Bacteria	5	5	11	0
White blood cells	7	3	10	1

The highest positives on the Protein test were observed in females, with 8 out of 11 samples collected. Males had an equal number of positive and negative protein tests, which totalled 5. Males had 4 positives on the leucocyte test, whereas females had 9 out of 11. On the nitrite test, females had the highest number of positives (ten out of eleven), whereas males had an equal number of positives and negatives.

Table 4.3: Identification and isolation of coliforms

Mackonkey Agar(MAC)	Shape	Gram status	Indole	Citrate	Suspected bacteria
Pink	Mucoid	Negative rods	-	+	*Klébsiella spp*
Pink	Bulgy	Negative rods	+	-	*E. coli*

KEY

MAC (Mackonkey Agar)

Positive (+)

Negative (−)

4.1 Isolation and Identification of *Staphylococcus saprophyticus*

Only one of the 15 urine samples that tested positive for *S. saprophyticus* was infected. When Hydrogen peroxide was applied to glass slides containing the colonies during a catalase test, the isolated coliforms created an effervescent. A salt Manitol test was also done, and the isolates fermented MSA, resulting in a colour change from phenol red to yellow. The isolates were also able to coagulate blood plasma, indicating a positive Coagulase test.

Table 4.4: Identification of Staphylococcus saprophyticus.

Blood Agar-shape	Coagulase	MSA	Catalase	Suspected bacteria
Small whitish Cocci shaped	+	+	+	*Staphylococci saprophyticus*

KEY

Positive (+)

4.2 Bacterial Isolation

Four bacterial species were isolated from the 15 positive urine samples. *Escherichia coli, Staphylococcus saprophyticus, Klebsiella species,* and *Proteus mirabillis* were among the species isolated and identified. *Klebsiella species* were identified as gram-positive rods, and biochemical assays including Indole yielded positive results, and the rods were observed as pink colonies on Mackonkey agar. Colonies of *E. coli* were also isolated and characterized as gram negative rods. The Indole test was positive, the citrate test was negative, and the colonies were pink in colour. The gram-positive cocci *S. saprophyticus* was also identified. The catalase, coagulase, and Manitol salt agar tests on *S. saprophyticus* were all positive. *P. millabilis* bacteria were also isolated and identified as gram positive.

Table 4.5: UTI Bacterial prevalence in males and females

Type of bacteria	Prevalence in females (%)	Prevalence in males (%)
Escherichia coli	55	40
Klebsiella species	27	0
P. mirabillis	9	40
S. saprophyticus	0	20

The prevalence of different bacteria isolated from males and females presenting UTI at Mashoko Christian Hospital is shown in Table 4.3, and four different species were isolated from both males and females. *E. coli, Klebsiella* species, and *P. mirabillis* were isolated from females. *E. coli* was isolated with a 55% prevalence, *Klebsiella* species with a 27% prevalence, and *S. saprophyticus* with a 9% prevalence. Three distinct bacterial species were identified from males, with *E coli* accounting for 40%, *P. mirabillis* accounting for 40%, and *S. saprophyticus* accounting for 20%. There were no *Klebsiella* species isolated from males.

4.3 A summary of bacteria isolated from urine samples of different age groups

Bacterial prevalence was also examined in relation to age groups. Only one type of bacteria, *E. coli*, was isolated and identified among people aged 20 to 39. From the four positive samples, *E. coli* was found to be the one that was most prevalent. Five samples from people aged 40 to 59 tested positive, and three different species of bacteria were isolated: *P mirabillis, Klebsiella*, and *E. coli*. Only one sample was infected with *E. coli*, whereas three samples were infected with *Klebsiella*, and *P mirabillis* was isolated in one sample. Six samples were cultured and found to be positive in the old age group of persons 60 years and older. *E coli* was identified in three samples, *P mirabillis* in two samples, and *S. saprophyticus* in one sample.

Table 4.6: UTI bacterial prevalence in different age groups

Age group(years)	**20-39**	**40-59**	**60+**
Bacterial species	**%**	**%**	**%**
Escherichia coli	80	20	50
P. mirabillis	0	20	33
Klebsiella species	0	60	0
S. saprophyticus	0	0	17

Bacterial prevalence was also examined in relation to age groups. The prevalence of *E. coli* has been found to be 80% in the 20-39 age range. *Klebsiella* species had the highest incidence of 60% in the 40-59 age range, while *E. coli* and *P. mirabillis* both had a 20% prevalence. *E. coli* showed the highest prevalence of 50% in people aged 60 and older, followed by *P. mirabillis* (33%), and *S. saprophyticus* (17%).

Table 4.7: Distribution of UTIs amongst patients who were suspected to have UTIs at Mashoko Christian Hospital.

Type of UTI	Number of males	Percentage (%)	Number of females	Percentage (%)
UTI	5	50	10	91
non UTI	5	50	1	9
Total	10	100	11	100
Total in %	33		67	

Eleven urine samples were obtained from females, ten from males, and three were control samples from a total of 24 urine samples collected. Ten of the fifteen samples were found to be UTI positive; ten samples were from females and five samples were from males.

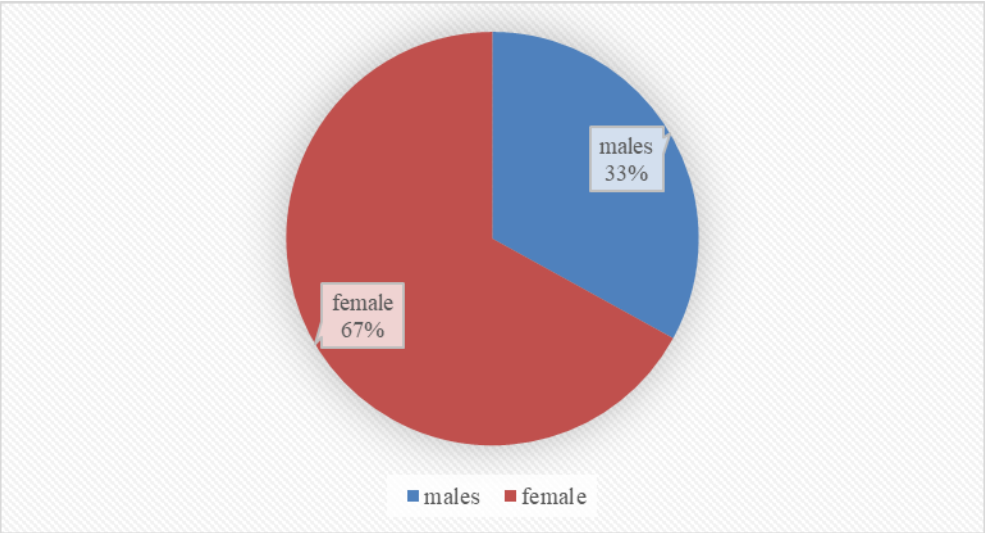

Figure 4.1: Patients presenting UTI at Mashoko Hospital

According to gender, 33% of UTI isolates were from males and 67% were from females, indicating that females had a higher prevalence of UTIs than males.

Table 4.8: prevalence of pathogens isolated from 15 UTI patients

Micro-organism	Number of isolates	Percentage (%)
Gram negative		
Escherichia coli	8	53
Proteus mirabillis	4	27
Klebsiella species	2	13
Gram positive		
Staphylococcus saprophyticus	1	7

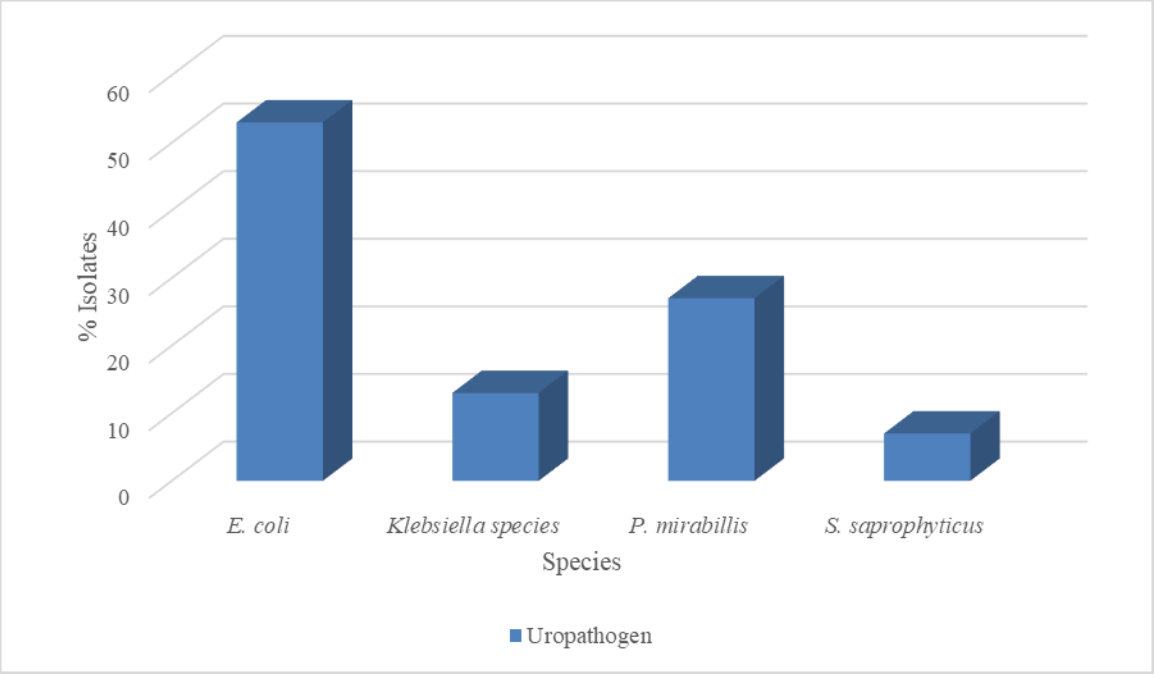

Figure 4.2: Prevalence of uropathogens isolated from positive samples

Among the six uropathogens isolated, *E. coli* had the highest prevalence in causing UTIs with a prevalence rate of 53.3%, followed by *P. mirabillis* with a prevalence of 27%, *Klebsiella* spp. with a prevalence of 13.3%, and *S. saprophyticus* with a prevalence rate of 7%.

4.4 **Isolation of Coliforms**

E. coli was found in eight of the 15 positive urine samples (Table 4.5). Both the Indole and citrate tests were positive for the isolates. The isolates were negative for the Indole test but positive for the Citrate test, indicating that two samples were contaminated with *Klebsiella* species. Only one sample contained *S. saprophyticus*. Four samples were used to isolate *P. mirabillis.*

CHAPTER 5

5.1 DISCUSSION

5.1.1 Microscopy and urinalysis

As part of the testing processes, microscopy and urinalysis were performed. 21 urine samples were collected from patients, and urinalysis and microscopy were done on the samples to assess their biochemical status as well as their microscopy. The first batch of urines was tested by age. Nitrites, proteins, and leucocytes were among the biochemical components to be tested in the samples. Microscopy was used to investigate the presence of white blood cells and bacteria, which indicate a person has a UTI. Urinalysis was performed on all 21 urine samples based on their age. The presence of white blood cells in urine indicates that the body has a defense system in place to combat the illness.

The Nitrite test yielded the most positive results in the 40-59 and 60+ age groups, with five and six instances, respectively. Such results could be attributed to high bacterial counts. These age categories likewise had the lowest negatives for the same test, with one case apiece. The 20-39 age group had the lowest number of positive cases in the Nitrite test, as well as an equal number

of positives and negatives. In the protein test, the age groups 40-59 and 60+ had an equal number of positive cases, and they were recorded as the age groups with the most positive cases, with four positive cases in each group. There were just two and three negative cases in these two age categories, respectively. Positive instances were lowest in the 20-39 age group, whereas negative cases were highest, with five and six cases respectively. For leucocytes tests, the 60+ age group had the most positive instances, with six cases out of seven samples obtained, followed by the 40-59 age group with five cases, and finally the 20-39 age group with three cases out of eight. With five cases, the 20-39 age group had the highest negatives. Six samples were collected from people aged 40 to 59 and examined for Nitrite, proteins, and leucocytes. Five of the samples run through the tests were positive for Nitrites, leukocytes, and Proteins. Microscopy was also performed on the samples, and different numbers of bacteria and white blood cells were seen. Few bacteria were visible under the microscope in the negative sample, which could be attributed to contamination during sample collection from the patient because there were no proteins, leucocytes, or nitrites discovered in the sample. Seven samples were obtained and examined for Urinalysis from people aged sixty and up. Six samples were found to be positive, with bacteria and white blood cells visible under the microscope. In addition to the 21 samples, three control samples were collected from healthy patients who did not exhibit any signs or symptoms of UTI. The controls were also subjected to the same biochemical assays for confirmation of negatives, and when inspected under a light microscope, no bacteria or white blood cells were visible.

Microscopy and urinalysis was also performed on the samples according to gender. On the Protein test the highest positives were found in females with a recording of eight out of eleven samples collected. Males had an equal number of those which were positive and negative for protein tests which is five. On the leucocyte test females had the highest record of nine out of eleven and males had four positives. On the nitrite test females had a highest record of positives which is ten out of eleven and males had a same record of positives and negatives. And to complete the test, microscopy was done on the samples and white blood cells and bacteria were seen in different quantities. Few bacteria were seen in some negative samples when viewed under the microscope and this could be due to contamination when the samples were being collected. When some negative samples were examined under a microscope, few bacteria were found, which could be due to contamination during the collection process.

Microscopy was also performed on the samples, and white blood cells and bacteria were visible in varying numbers when seen under the microscope. When some positive samples were

examined under a microscope, they were discovered to have fewer bacteria, such as those indicated by a (+) sign. The patient's having very few bacteria while having UTI could be due to the patient being in an early stage of UTI, which is known as symptomatic bacteriuria (uncomplicated UTI). Furthermore, some samples had (+++) bacteria, which is considered to be the highest quantity of bacteria, and the reason for a patient having bacteria in such quantity could be that the patient was suffering from Asymptomatic bacteuria or complicated UTI.

Female patients were shown to have a higher prevalence of UTIs (67%) than male patients, who had a frequency of 33%. The findings of this study are similarly consistent with those of Gynsa-Lulterodt et al (2014), who found a prevalence of 67% in females and 33% in males. The results are also consistent with a study conducted by Magliano et al (2012), who discovered that 80% of all UTI isolates were from females, while males had a frequency of 20% and the male to female ratio was 8: 2. The investigation was carried out at the Centro Diagonostico Italiano (CDl) bacteriological laboratory in Italy, where urine samples were collected from outpatient clinics in a densely populated urban region. The reason for a higher proportion of females than males is that women have a shorter urethra than men, which reduces the distance germs must travel to reach the bladder (Kahlmeter, 2003). Women are also more likely than men to develop UTIs because they use diaphragms and spermicidal drugs for birth control, both of which cause UTIs. It is also believed that changes in circulating estrogen after menopause in women cause a change in the urinary tract that makes women more vulnerable to UTIs, which is why we saw in this study that UTIs in females increase with age (Stanm and Norrby, 2001).

Bacterial prevalence was also examined in relation to age groups. *E. coli* was found to be most prevalent in the 20-39 age bracket, with a prevalence of 80%. *Klebsiella* species had the highest incidence of 60% in the 40-59 age range, while *E. coli* and *P. mirabillis* both had a 20% prevalence. *E. coli* showed the highest prevalence of 50% in people aged 60 and older, followed by *P. mirabillis* (33%), and *S. saprophyticus* (17%).

At Mashoko Christian Hospital, the prevalence of UTls was 71% across all age groups and both genders. A similar situation was discovered in a research done on female students on the campus of the University of Ado Ekiti in Nigeria (Abijuwon, 2010). Although a much larger sample was used, the prevalence of UTIs among these students was 65%. Nigeria, like Zimbabwe, is a developing country, which explains why the prevalence rates are nearly identical. As a result, the greater occurrence of UTIs at Mashoko Christian Hospital could be

attributable to limited access to health care, given the hospital is located in a rural location where clinics are scarce.

There were no significant differences in age groupings, and there was no prevalence across all age groups. The findings revealed that the prevalence was the same across all age groups. However, when gender was examined separately, the prevalence increased with age. The prevalence was found to be high in the age group of 20-59. This age group has a higher prevalence because it is more involved in sexual activities, which increases the risk of acquiring UTIs, particularly in females. For example, Staphylococcus species is a member of the skin flora, and the uropathogen may remain on the skin and be transmitted during sexual intercourse. According to Magliano et al (2012), he suggested that age factors may influence the etiology of UTIs; however, no significant variations were seen in the age groups until the age groups were divided into males and females.

5.1.2 Bacterial isolation

After urinalysis and microscopy, all urine samples from Mashoko Christian Hospital patients were cultured. As part of the aims, uropathogens were isolated and identified. *E. coli, Klebsiella* spp, *P. mirabillis*, and *S. saprophyticus* were the uropathogens isolated. These uropathogens were also isolated in a study that was undertaken in a retrospective investigation (Rai *et al.,* 2007). The goal of this study was to identify the causative agent of urinary tract infections, and it was conducted in Kathmandu Hospital in Nepal. The five uropathogens isolated are the most common causative agents of UTI globally, but other pathogens that cause UTIs include *S. faecalis*, *Pvulgaris, citrobacter* species, *Paureginosa, S. aureus, Streptococcus agalactae,* and many others.

E. coli was discovered to be the most common uropathogen causing UTI among the five uropathogens, with a prevalence rate of 53%. This was also similar to previous research (Magliano *et al.,* 2012) who conducted a study on the age and gender etiology of community acquired urinary tract infections at a bacteriological in Italy between March 2008 and December 2009. Following the study, they discovered that 67.7% of the isolates were *E. coli* pathogens. *P. mirabillis* was the second most common uropathogen isolated, with a prevalence of 27%, followed by *Klebsiella* species with a prevalence of 13%, and *S saprophyticus* with a prevalence of 7%. The findings are also consistent with a 2009 study in lran, which indicated *E. coli* to be the most common pathogen, followed by *P. mirabillis*, *Klebsiella*, and finally *S. saprophyticus* (Farajnia, 2008). Among the uropathogens isolated, *E. coli, P. mirabillis,* and

Klebsiella species were all gram negative, with *S. saprophyticus* being the only gram positive uropathogen. UTIs caused by *Staphylococci* may be caused by increased usage of equipment such as bladder catheters.

Although patients were taught how to collect specimens aseptically, maximum efficiency cannot be guaranteed because the majority of patients are not sterility conscious. The highest *E. coli* prevalence (53%) may be associated with faecal contamination due to the availability of organisms from toilets and the urethra's length (Nicole, 2001). The prevalence of *P. mirabillis* (27%) and its status as the second most common uropathogen, as well as its strong relationship with UTI, may be related to its active motility and swimming capacity as compared to other microorganisms isolated, as they may effortlessly transverse the urethra. Furthermore, there may be a link between the prevalence of UTI and personal hygiene among Mashoko Hospital patients. Because the hospital is located in a rural area with limited facilities such as water supply, some of the patients may have acquired UTIs from the hospital toilets while admitted, as there is insufficient water to clean and flash the toilets on a regular basis. When there is dirt, urine sediments accumulate, resulting in the production of thick scum, and in this scenario, someone can become infected while urinating.

CHAPTER 6

6.1 RECOMMENDATIONS AND CONCLUSIONS

6.1.1 RECOMMENDATIONS

Because the prevalence of UTIs in this study was higher in females than in males, women are encouraged to get screened for UTIs if they have asymptomatic bacteria. Women must be taught how to prevent UTIs through awareness campaigns because their physiology makes them more susceptible to infections than males.

To avoid the growth of bacteria in toilets and the emergence of UTIs within the hospital, the hospital should make an effort to provide a constant flow of water into the hospital grounds. This study can also be conducted on a wider scale to meet the frequency of UTIs throughout Masvingo province and to allow for a much higher sample size. Continuous study should be

conducted to monitor the prevalence as well as the effectiveness of the antibiotics that will be in use at the moment.

There is also a requirement to regularly check the profile of etiological bacteria of UTIs and antibiotic resistance. This would demonstrate the establishment of resistance to newer therapeutic agents while also tracking the effectiveness of serving therapeutic treatments. Because several studies have shown that nitrofurantoin is the most effective antibiotic against all uropathogens, it should be used as a treatment option for the bacterial uropathogens isolated in this study.

6.1.2 CONCLUSION

UTIs were found in 71% of the samples collected from patients who presented to Mashoko Christian Hospital with UTI symptoms. The UTIs were caused by five uropathogens: *E. coli, Klebsiella, S. saprophyticus, Enterobacter,* and *P. mirabillis*. The most prevalent bacteria recovered were *E. coli* (53%), while the uropathogens with the lowest prevalence were *S saprophyticus* and *Enterobacter* spp (7%, respectively). Females were more prone to UTI than males, with a frequency of 67% against 33%. There were no significant differences in age groupings, and there was no prevalence across all age groups. The findings revealed that the prevalence was the same across all age groups. However, when gender was examined separately, the prevalence increased with age, with the 20-59 age group having the highest prevalence because this age group is more sexually active.

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APPENDICES

Case Processing Summary

Unweighted Cases[a]		N	Percent
	Included in Analysis	21	95.5

Selected Cases	Missing Cases	1	4.5
	Total	22	100.0
Unselected Cases		0	0
Total		22	100.0

Depending Variable Encoding

Original Value	Internal Value
Negative	0
Positive	1

Categorical Variables Codings

		Frequency	Parameter coding
			(1)
gender	Female	11	1.000
	Male	10	.000

Uropathogen

uti_status			Frequency	Percent	Valid percent	Cumulative Percent
negative	valid	No growth	5	100.0	100.0	100.0
		E. coli	8	50.0	50.0	50.0
		Klebsiella species	3	18.8	18.8	68.8
positive	valid	No growth	1	6.3	6.3	75.0
		P. mirabillis	3	18.8	18.8	93.8
		S. saprophyticus	1	6.3	6.3	100
		Total	16	100.0	100.0	

Table 6.1 Results for urinalysis and microscopy for different age groups

Age	Nitrites	leucocytes	Protein	White blood cells	Bacteria
20-39					
38	positive	Positive	Positive	++	++
36	negative	Negative	negative	rare	+
20	positive	Positive	negative	+	++

23	positive	negative	Positive	+	++
33	negative	Negative	negative	+	–
21	positive	Positive	Positive	+++	+
20	negative	Negative	negative	few	–
30	negative	Negative	negative	rare	–
40-59 41	positive	Positive	positive	+	+++
49	positive	Positive	positive	+	–
50	negative	Negative	negative	Few	–
54	positive	Positive	Negative	+	++
59	positive	Positive	Positive	++	++
55	positive	Positive	Positive	++	++
60+ 88	positive	Positive	Positive	+	++
65	positive	Positive	Positive	+	+
68	positive	Negative	Positive	+	++
72	positive	Positive	Positive	++	++
60	positive	Positive	Positive	+	+++
66	positive	Negative	Positive	+	+++
77	negative	Negative	negative	Few	–

Controls

20	negative	Negative	Negative	Nil	Nil

45	negative	Negative	Negative	Few	Nil
78	negative	Negative	Negative	Rare	Nil

KEY

Positive (+)

No bacteria identified (nil)

Table 6.2 Results for urinalysis and microscopy for males

Gender	Nitrites	leucocyte	Protein	WBC	Bacteria
38	Positive	positive	Positive	+	+++
20	Negative	negative	negative	+	_
33	Negative	negative	negative	+	_

50	Negative	negative	negative	Few	_
88	Positive	positive	Positive	++	++
68	Positive	positive	Positive	+	++
72	Positive	positive	Positive	+	+++
59	Positive	positive	positive	++	++
77	negative	negative	negative	Few	_
30	negative	negative	negative	Rare	_

Control

45	negative	negative	negative	Nil	Nil

Key

Positive (+)

No bacteria identified (nil)

WBC (white blood cell count)

Table 6.3 Results for urinalysis and microscopy for females

Gender	Nitrites	leucocyte	Protein	WBC	Bacteria
36	negative	negative	negative	Rare	+
23	Positive	positive	Positive	+	++
21	Positive	positive	Positive	++	+

41	Positive	positive	Positive	+	++
54	Positive	positive	Positive	+	++
49	Positive	positive	Positive	++	++
65	Positive	positive	Positive	+	+
60	Positive	positive	Positive	+	+++
66	Positive	positive	Positive	+	++
20	Positive	positive	positive	+	+++
55	Positive	positive	positive	++	++

Control

20	Negative	negative	negative	nil	Nil
78	Negative	negative	negative	rare	Nil

Key

Positive (+)

No bacteria identified (nil)

WBC (white blood cell count)

Table 6:4 A summary of bacteria isolated from urine samples of both males and females

Age	Bacteria isolated in females	Age	Bacteria isolated in males
20	*Escherichia coli*	38	*Escherichia coli*
23	*Escherichia coli, Enterobacter species*	59	*Escherichia coli*

21	*Escherichia coli*	88	*P. mirrabillis*
41	*P mirrabillis*	68	*S saprophyticus*
54	*Klebsiella species*	72	*P mirrabillis*
49	*Klebsiella species*		
65	*Escherichia coli*		
60	*Escherichia coli*		
66	*Escherichia coli*		
55	*Klebsiella species*		