

Midlands State University



**PREVALENCE AND SUSCEPTIBILITY OF UROPATHOGENS CAUSING
URINARY TRACT INFECTIONS IN PATIENTS PRESENTING AT MASVINGO
GENERAL HOSPITAL**

BY

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ABSTRACT

Urinary Tract Infections (UTIs) are a serious problem globally and are some of the most common diseases encountered in medical practice today. Despite the widespread availability of antibiotics, UTIs remain a problem because of antimicrobial resistance. Antimicrobial susceptibility testing therefore provides information that allows physicians to select the most appropriate antibiotics for UTIs. This study was carried out to: (i) determine the prevalence of UTIs among patients presenting at Masvingo General Hospital, (ii) evaluate the sensitivity patterns of the identified isolates, and (iii) determine the efficacy of eight commonly used antibiotics (Ampicillin, Tetracycline, Nitrofurantoin, Nalidixic acid, Gentamicin, Ciprofloxacin, Kanamycin and Norfloxacin) against the isolates using Kirby Bauer disc diffusion technique. A total of 123 urine samples were collected from patients seeking treatment for UTIs Masvingo General Hospital. Sample processing and patient information collection were carried out at Genau Pathology Laboratories in Masvingo. Urine samples were analysed following standard microbiological techniques. The bacterial isolates recovered were *Escherichia coli*, *Klebsiella spp*, *Pseudomonas aureginosa* and *Staphylococcus aureus*. Of the 123 samples examined, 47 (38%) comprising of 36(77%) females and 11(23%) males were positive for UTIs. The most common uropathogen was *Escherichia coli* with a prevalence of 47%, followed by *Staphylococcus aureus* (21%), *Klebsiella spp* (17%) and *Pseudomonas aureginosa* (13%). Antimicrobial susceptibility tests showed that the most effective antibiotic was nitrofurantoin being effective against 79% of the isolates, followed by gentamycin (66%) and kanamycin (66%). The least effective antibiotic was ampicillin, which was effective against only 4 out of 47 (9%) isolates, followed by nalidixic acid, effective against 19 out of 47 (40%) of the isolates. *Escherichia coli* was highly resistant to ampicillin but highly sensitive to nitrofurantoin. *Klebsiella* species were highly resistant to Ampicillin but highly sensitive to Kanamycin. *Pseudomonas aureginosa* was totally resistant to ampicillin but four drugs were effective against it, namely, nitrofurantoin, tetracyclin, ciprofloxacin and norfloxacin. Lastly, *Staphylococcus aureus* was resistant to nalidixic acid and ampicillin but highly sensitive to nitrofurantoin. Overall, the results suggest that the most common uropathogen causing UTIs in patients presenting at Masvingo General Hospital was *Escherichia coli* and most isolates were resistant to ampicillin and nalidixic acid but highly sensitive to nitrofurantoin, gentamycin and kanamycin. Therefore nitrofurantoin, gentamycin and kanamycin are recommended for treatment against UTIs. The relatively high prevalence (38%) of UTIs at Masvingo General Hospital suggests that UTIs are a risk to people around Masvingo Province thus there is need to monitor the profile of etiological bacteria of UTIs and the antimicrobial resistance regularly. This would show emergence of resistance to newer therapeutic agents as well as keep track of effectiveness of serving therapeutic agents.

DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other university.

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ABBREVIATIONS AND ACRONYMS

ASB	Asymptomatic bacteriuria
CFU	Colony forming units
CLED	Cysteine lactose electrolyte deficient
EARSS	European Antimicrobial Resistance Surveillance System
ESBL	Extended Spectrum Beta-Lactamases
GI	Gastrointestinal tract
IMVIC	Indole test, Methyl red test, Voges-proskaur test and Citrate test
IVs	Intravascular devices
MDR	Multi Drug Resistance
MR	Methyl red
MRSA	Multi-resistant <i>Staphylococcus aureus</i>
NCCLS	National committee for clinical laboratory standards
NICE	The National Institute for Health and Clinical excellence
TMP-SMX	Trimethoprim-sulfamethoxazole
TSI	Triple Sugar Iron test
UTA	Urinary Tract Anomaly
UTI	Urinary Tract Infections
VP	Vogesproskaur
WHO	World health organisation

CHAPTER 1

INTRODUCTION

1.1 Background

Urinary Tract Infections (UTIs) are common microbial infections that affect different parts of the urinary tract and occur in both males and females (Ranganathan, 2014). Women are mostly vulnerable to UTIs due to their anatomy and reproductive physiology (Ranganathan, 2014). A shorter and wider urethra in women than in men makes it easier for bacteria to make their way to the urinary tract. Women also have more moisture around their urethra than men, causing microbes to be trapped (Andabati, 2010). Wiping from back to front enhances the spread of organisms to the urinary tract. In England and Wales, consulting rates in general practice for cystitis and other urinary infections were found to be approximately 3.5 % per 10,000 persons, whereas in Italy, in 2002, 2.4 % of a cohort of more than 450,000 people received a diagnosis of acute cystitis in preliminary care (Gallati *et al.*, 2006). In a study in Nigeria, a prevalence of 14.2 % was obtained by Aiyegoro *et al.*, (2007) among children in Ile-Ife.

Basically the urinary tract is made up of four main organs which are the kidneys, ureter, bladder and the urethra (Foxman, 2013). The upper urinary tract is composed of kidneys and ureters and the lower urinary tract has the bladder and urethra (Kass, 2002). Most infections occur in the lower urinary tract with very few cases of infection occurring in the upper urinary tract. Infection of the kidneys is called pyelonephritis, infection of the bladder is called cystitis and infection of the urethra is called urethritis. Migration of microbes from the bowel flora to the urethra is the most common route of infection in females (Foxman, 2013).

UTIs are usually caused by bacterial invasion of the lower and the upper urinary tract (Gallati *et al.*, 2006). *Escherichia coli*, *Klebsiella spp*, *Enterococcus spp*, *Proteus spp*, *Pseudomonas aeruginosa* and *Candida spp* are the most microbes associated with UTIs (Ronald, 2002). Ranganathan (2014) postulate that, *Escherichia coli* account for 80% to 85% of the infections followed by *Staphylococcus species* that constitutes 10% to 15% of the infection. In addition, bacterial species *Klebsiella*, *Pseudomonas*, *Proteus* and *Enterococcus* species play a minor role in conferring the infection (Ronald, 2002).

Microscopy, culture and urine chemistry are the most common laboratory diagnostic tools used to detect causative agents of UTIs and their antibiotic sensitivity patterns (Hooton *et al.*, 2010). Due to emerging resistance, antibiotic sensitivity patterns have become important in informing appropriate treatment (Zhanel *et al.*, 2006). Most health centres and hospitals in developing countries rely on the unreliable strip urinalysis method for screening urine sample in patients and this does not provide the true sign of the infection. This could in turn result in inappropriate use of antimicrobial agents as well as empirical practices (Gupta *et al.*, 2001).

The common symptoms of UTIs include dysuria, pyuria, nocturia, increased frequency of urination with little urine being passed out, urgency to urinate, cloudy or dark urine or hematuria, lower abdominal pain and back discomforts, nausea and spewing, loss of appetite, fever, fatigue and foul smelling urine (Tambyah and Maki, 2000). The symptoms associated with the bladder and kidney infections are different. Those of bladder (cystitis) infections includes painful and frequent urination whereas conditions like high fever and flank pain are commonly experienced in case of kidney contagion which is referred to as pyelonephritis (Tambyah and Maki, 2000).

Factors that enhance acquiring UTIs include diabetes, immune suppression, hypertension, allergies, increased sexual activity, catheterization, use of diaphragms, birth control pills and spermicidal agents, age and gender, delays in micturition and abuse of antibiotics (Foxman, 2014). In most developing countries, especially in Africa, malnutrition, poor hygiene and poverty are part of the risk factors of UTIs.

There are a number of drugs universally used against microbes causing UTIs such as tobramycin, kanamycin, gentamycin, ciprofloxacin, amikacin, and cotrimoxazole where some resistance has been detected (Williams and Craig, 2011). Studies have shown the resistance of *Escherichia coli* to a variety of antibiotic drugs such as sulfamethoxazole, ampicillin, cephalothin, ciproflaxin, amoxilin, augmentin, gentamycin and other medications (Zhanel *et al.*, 2006). There is an increase in resistance to commonly employed antibiotics caused by inappropriate use of the antimicrobial agents (Gupta *et al.*, 2001). Antimicrobial resistance of UTIs is requires an urgent attention in order to derive suitable remedy to overcome the problem. The extent of antimicrobial resistance shown by the pathogens towards the commonly employed drugs is an issue of global concern. Antimicrobial resistance patterns exhibited by the uropathogens vary with factors like the site of their isolation, environmental conditions as well as the stage of the infection (Gupta *et al.*, 2001).

1.2 Significance of the study

Urinary Tract Infections are some of the most common infections of patients visiting Masvingo General Hospital. The prevalence and diagnosis of UTIs in patients presenting at Masvingo General Hospital on the basis of sensitivity profiles and sex has not been done despite extensive published literature concerning UTIs coming from other medical institutions such as Parirenyatwa Hospital in Harare. This knowledge gap is giving rise to challenges for physicians as UTI treatment is dependent on the susceptibility profiles of uropathogens. An unpublished preliminary study suggests high prevalence of recurrent UTI cases among patients visiting Masvingo General Hospital. The high level of recurrence could be caused by the rising level of antibiotic resistance among the uropathogens causing UTIs. This implied that patients might have been prescribed ineffective treatments thus giving rise to the recurrence of UTIs.

The rising level of antibiotic resistance means there is a need to establish the prevalence of UTIs among the patients who present with symptoms in order to justify this practice of using antibiotics for UTI treatments and determine the involved uropathogens and their sensitivity patterns in this institution.

Moreover, the patterns of antibiotic resistance being exhibited by the isolated uropathogens will provide information on the antibiotics that are more effective and those that are not. This will hence improve effectiveness of treatments and reduce the levels of recurrence. The data generated will provide the health sector with information on the level of antibiotic resistance so that necessary interventions can be taken.

1.3 Objectives

The main objective of this study was to determine the prevalence and susceptibility of uropathogens causing UTIs in patients presenting at Masvingo General Hospital. The specific objectives of this study were:

- i. to isolate and identify bacteria causing urinary tract infections in patients presenting at Masvingo General Hospital using biochemical tests,
- ii. to determine the common microbes causing UTI among patients presenting at Masvingo General Hospital,

- iii. to evaluate the antibiotic susceptibility patterns of the isolates causing UTI among patients presenting at Masvingo General Hospital, and
- iv. to evaluate the drug effectiveness against isolated uropathogens.

CHAPTER 2

LITERATURE REVIEW

2.1 Urinary tract infections

Urinary Tract Infections (UTIs) are one of the most common bacterial infections encountered in many parts of the world. UTIs are often associated with significant morbidity and mortality. It is estimated that 150 million cases of UTIs occur globally per year resulting in more than 4 billion pounds (6 billion dollars) in direct health care expenditure (Zeyauallah and Kaul, 2015). According to Sewify *et al* (2016), urinary tract infections (UTIs) are a group of infections of the urinary tract. The most common UTI is cystitis, which is an infection of the bladder (where urine is stored). Other UTIs involve the urethra (urethritis) or kidneys (pyelonephritis). The symptoms of UTI are non-specific and may lead to vague diagnosis (Abejew *et al.*, 2014). Urinary tract infections occur as a result of the microbial colonization of urine and the invasion of any structure of the urinary tract by microbial organisms such as bacteria, viruses, yeasts and parasites (Ayoade *et al.*, 2013).

Microbial invasion being the basis of urinary tract infection could be seen in various clinical manifestations resulting in various disease conditions in both males and females of all ages. Young adults particularly females are, however, the most at risk of bacteriuria (Ayoade *et al.*, 2013). For example, in the United States, UTIs result in approximately 8 million physician visits and more than 100,000 hospital admissions per year of sexually active women treated annually for UTIs. Up to 95% of the UTI cases in the U.S are treated with antibiotics such as cotrimoxazole without bacteriological investigation since these infections are so routinely encountered in medical practice (Ayoade *et al.*, 2013). This kind of indiscriminate use of antibiotics is, however, fraught with the problem of pathogen resistance to antibiotics.

Urinary tract infection is a common health problem among pregnant women. Asymptomatic bacteriuria (ASB) is a common bacterial infection of the urinary tract requiring medical treatment in pregnancy (Gessese *et al.*, 2017). Diagnosis and treatment of ASB is important as approximately 20–40% of pregnant women, if untreated during pregnancy, symptomatic UTI will develop (Gessese *et al.*, 2017). Treatment of UTI is important in keeping with the goal of safe motherhood initiative; that women safely go

through pregnancy and childbirth and produce healthy babies. Untreated ASB is a risk factor for acute cystitis (40%) and pyelonephritis (25–30%) in pregnancy and could lead to adverse obstetric outcomes such as prematurity, low-birth weight, and higher fetal mortality rates (Gessese *et al.*, 2017). The relative frequency of uropathogens varies depending upon age, sex, catheterization, hospitalization and previous exposure of antimicrobials (Seifu and Gebissa, 2018).

In England and Wales, consulting rates in general practice for cystitis and other urinary infections were found to be approximately 3.5 % per 10,000 persons, whereas in Italy, in 2002, 2.4 % of a cohort of more than 450,000 people received a diagnosis of acute cystitis in preliminary care (Gallati *et al.*, 2006). In a study in Nigeria, a prevalence of 14.2 % was obtained by Aiyegoro *et al.*, (2007) among children in Ile-Ife. In studies that have been done regionally the prevalence of UTI was found to be 13.3 % in Uganda (Andabati and Byomugisa, 2010).

2.2 Structure of the Urinary tract

The whole urinary system comprising of the parts of the urinary tract is at risk as the infection can affect any part of the urinary tract. Figure 2.1 depicts the urinary system comprising of the various parts of the urinary tract including the renal artery and vein, kidneys, bladder, ureter, urethra and provision for urine exit. Kidneys are the organs of utmost significance and are known to perform crucial regulatory functions (De Groat, 1993). These acts as innate filters and play a vital role in removing the unwanted water soluble waste from the blood and also enables the re-absorption of essential ingredients like water, glucose and amino acids (Tanagho and McAninch, 2000).

Kidneys are known for the production of urine which is diverted to the urinary bladder by means of thin tubular structure known as ureter (Abrams *et al.*, 1988). The urinary bladder is a muscular flexible organ which accumulates the urine collected from the kidneys before they are disposed (Finer and Landau, 2004). The collected water soluble waste in the form of urine is then flushed out from the genitals by means of urethra which connects the urinary bladder and genitals. This process of production of urine and its disposal is systematic and urinary tract infection greatly influences this process and may result in a variety of symptoms which the patient experiences during the process of contagion (Abrams *et al.*, 1988).

Entry of an infectious pathogen in the urinary tract causes the infection which usually occurs through the urethra. This is one of the prime reasons for higher incidence among women than men due to the shorter length of urethra in women which makes them vulnerable to such infections (Abrams *et al.*, 1988). Since the urethra is shorter in women when compared to men, they are more prone to infections associated with the urinary tract (Finer and Landau, 2004). The shorter length of the urethra in women enhances the scope for the pathogen to invade the bladder resulting in bladder infection.

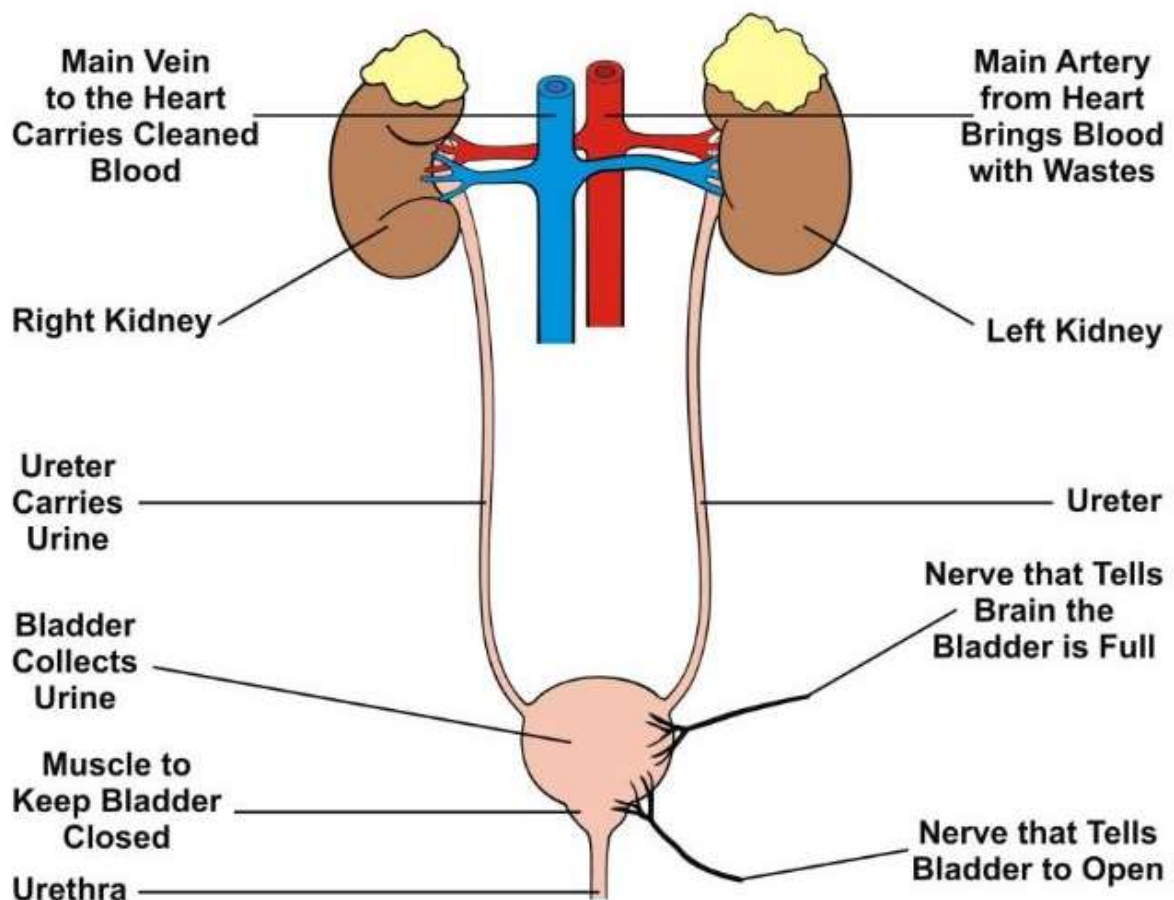


Fig 2.1: Structure of the urinary tract system

2.3 Causative uropathogens

2.3.1 Bacterial UTI

Many different microorganisms can cause UTIs though the most common pathogens causing the simple ones in the community are *Escherichia coli* and other Enterobacteriaceae, which accounts approximately 75% of the isolates. In complicated urinary tract infections and hospitalized patients, organisms such as *Enterococcus faecalis* and highly resistant Gram-negative rods including *Pseudomonas spp.* are comparatively more common (Beyene and Tsegaye, 2011). The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization (Beyene and Tsegaye, 2011). Urinary tract infection is mostly caused by Gram-negative aerobic bacilli found in gastrointestinal tract. The most common are: *Escherichia coli*, *Klebsilla pneumoniae*, *Enterobacter*, *Citrobacter*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. Other common pathogens include *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Enterococcus spp* and *Serratia spp* which presumably result in UTI following colonization of the genito urinary tract (Gessese *et al.*, 2017). *Escherichia coli* (60–70%), *Klebsiella spp* (10%), *Proteus spp* (5–10%) and *Pseudomonas spp* (2–5%) are the dominant Gram-negative bacteria causing UTI (Gessese *et al.*, 2017). Among Gram-positive bacteria pathogens *Streptococcus spp* and *Staphylococcus spp* are frequently isolated from cases of UTIs.

2.3.2 Fungal and viral UTIs

Urinary tract infection may be caused by viruses and fungi. Fungi, such as *Candida*, is the second most cause of nosocomial UTI in children, it can be spread systemically and can be life threatening (Yildiz *et al.*, 2007). Fungi infections are seen in infants and children who are on long term antibiotics, patients who are immunocompromised, or patients using invasive devices like IVs, and catheters (Watson, 2004). *Candida* and fungal infections are more prevalent in children with Urinary tract Anomaly (UTA); it is associated with infections after instrumentation of the urinary tract (Yildiz *et al.*, 2007). The prevalence of UTI due to *Candida* increases gradually with the duration of hospitalization. Treatment of Candiduria includes stopping antibiotics, removing or changing indwelling catheters, and starting antifungal therapy with antifungal agents like oral fluconazole, parental or

intravesical amphotericin B. Viral UTI can be caused by adenoviruses types 11 and 21, polyomavirus BK, and herpes simplex viruses (Watson, 2004).

2.4 Signs and Symptoms of urinary tract infection

The symptoms of a urinary tract infection can be classified according to the type of infection one has. However, each individual may experience symptoms differently. The symptoms of a urinary tract infection may resemble other conditions or medical problems and it is advisable to consult physicians for proper diagnosis.

2.4.1 Acute pyelonephritis (affects the kidneys)

The symptoms of acute pyelonephritis usually show within 48 hours of infection. Its common symptoms include high fever (more than 38.9°C), chills, nausea and vomiting, abdominal pain, pain in the back, groin, or side, fatigue, painful or burning sensation when urinating, cloudy urine, pyuria (pus in urine), hematuria (blood in urine), urinary urgency and frequency and lastly distinct fishy smell in urine (Foxman, 2010).

2.4.2 Cystitis (affects the bladder)

This is the most common type of urinary tract infection and its symptoms are low-grade fever, persistent urge to pass urine, frequent urination but only in small amounts, painful or burning sensation when urinating, cloudy urine, hematuria (blood in urine), pelvic pain or discomfort, lower abdominal (pelvic) pressure, abnormal urine odor (strong-smelling) (Foxman, 2010).

2.4.3 Urethritis (affects the urethra)

The symptoms of urethritis involve difficult or painful urination (dysuria) and urinary urgency and frequency.

2.5 Diagnosis of urinary tract infection

2.5.1 Detection of bacteriuria by urine microscopy.

Bacteriuria can be detected microscopically using Gram staining of un-centrifuged urine specimens, Gram staining of centrifuged specimens, or direct observation of bacteria in urine specimens. Gram stain of un-centrifuged urine specimens is a simple method. A volume of urine is applied to a glass microscope slide, allowed to air dry, stained with Gram stain, and examined microscopically. The performance characteristics of the test are not well-defined, because different criteria have been used to define a positive test result. In one study, the test was found to be sensitive for the detection of $\geq 10^5$ colony forming units per millilitre of urine but insensitive for the detection of lower numbers of bacteria (Carroll *et al.*, 1994).

2.5.2 Detection of bacteriuria by nitrite test.

Bacteriuria can be detected chemically when bacteria produce nitrite from nitrate. The biochemical reaction that is detected by the nitrite test is associated with members of the family *Enterobacteriaceae* (the pathogens most commonly responsible for UTIs), but the usefulness of the test is limited because nitrite production is not associated with urinary-tract pathogens such as *Staphylococcus saprophyticus*, *Pseudomonas spp.*, or *Enterococci* (Pappas, 1991). Another limitation to the test is that it requires testing a specimen of the first urine produced in the morning, as ≥ 4 h are required for bacteria to convert nitrate to nitrite at levels that are reliably detectable.

2.5.3 Detection of pyuria by urine microscopy.

Pyuria can be detected and quantified microscopically by measuring the urinary leukocyte excretion rate, counting leukocytes with a hemocytometer, counting leukocytes in urine specimens using Gram staining, or counting leukocytes in a centrifuged specimen. The advantages to urine microscopy are that leukocytes, leukocyte casts, and other cellular elements are observed directly. One disadvantage to urine microscopy is that leukocytes deteriorate quickly in urine that is not fresh or that has not been adequately preserved. In addition, each of these methods has disadvantages that limit its usefulness as a routine test

(Carroll *et al.*, 1994). Because of these disadvantages, urine microscopy should be limited to patients in whom pyelonephritis or other more serious infections are suspected.

2.5.4 Bacterial urine cultures.

Urine culture may not be necessary as part of the evaluation of outpatients with uncomplicated UTIs (Stamm and Hooton, 1993). However, urine cultures are necessary for outpatients who have recurrent UTIs, experience treatment failures, or have complicated UTIs (Wing *et al.*, 2000). Urine cultures are also necessary for inpatients that develop UTIs. The bacterial culture remains an important test in the diagnosis of UTI, not only because it helps to document infection, but also because it is necessary for determination of the identity of the infecting microorganism(s) and for antimicrobial susceptibility testing. This is particularly true because of the increased incidence of antimicrobial resistance. The most commonly used criterion for defining significant bacteriuria is the presence of $\geq 10^5$ colony forming units per millilitre of urine (Stamm *et al.*, 1982).

2.6 Treatment of urinary tract infections

The infection is confirmed after analysis of the patient's urine. This analysis identifies the bacteria and antibiotic sensitivity tests provides with information to which antibiotic the bacteria are sensitive to. The patients suffering from UTI are prescribed antibiotics. They are supposed to take plenty of fluid to wash off the bacteria from the urinary tract. After the therapy is finished the patient is supposed to give another sample of urine. This will show whether the infection is cured or the antibiotics has failed to eradicate the bacteria. Simple UTI infections are usually treated with Sulfamethoxazole - trimetoprim, Amoxicillin, Ciprofloxacin and Levofloxacin. Patients may be also prescribed analgesics which numb the bladder and relieve certain symptoms of the infection. Frequent urinary infections may require a longer course of antibiotics. In menopausal women, doctors can prescribe vaginal estrogen therapy which reduces the recurrence of the infection. In severe form of infection patients may need to be hospitalization and given intravenous antibiotics.

Ampicillin and sulfonamides generally should not be used for empiric therapy because more than one third of isolates demonstrate in vitro resistance (Hooton and Stamm, 1997). More than 15% to 20% of *Escherichia coli* strains causing uncomplicated cystitis are now resistant to these agents in several areas of the United States and other countries (Gupta

et al., 1999). The prevalence of resistance to nitrofurantoin among *Escherichia coli* is < 5%, although non-*Escherichia coli* uropathogens are often resistant. Resistance to the fluoroquinolones remains < 5% in most studies of uropathogenic strains. Three-day regimens are recommended because they are associated with better compliance, lower cost, and lower frequency of adverse reactions than 7 to 10 day regimens (Warren *et al.*, 1999).

Several studies and clinical experience have confirmed the effectiveness of 3 day regimens of trimethoprim, trimethoprim-sulfamethoxazole, or a fluoroquinolone for treatment of acute uncomplicated cystitis, and these agents are generally recommended for empiric therapy (Warren *et al.*, 1999). In comparison, 3 day regimens with beta-lactams are less effective than 5 days of therapy (Warren *et al.*, 1999). Nitrofurantoin is a safe and generally effective agent, but it should be administered for a minimum of 7 days. Single-dose regimens are somewhat less effective than 3 to 7 day regimens, even with fluoroquinolones (Hooton and Stamm, 1997). First-line treatment suggested by the Infectious Disease Society of America in 1999 was trimethoprim-sulfamethoxazole (TMP-SMX) in a 3-day regimen (Warren *et al.*, 1999). Given the increasing prevalence of TMP-SMX resistance among uropathogens, it is important to examine risk factors predicting *in vitro* resistance. These are diabetes, recent hospitalization, antibiotic use in the past 3 to 6 months (for any reason), and recent TMP-SMX use (Wright *et al.*, 1999).

2.7 Drug Resistance

Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population (Ayoade *et al.*, 2013). Antimicrobial resistance is a growing problem and a cause of major concern in many countries. Amongst the numerous recommendations to combat resistant bacteria is the necessity for national and international surveillance programmes to monitor the level of antimicrobial resistance. Several programmes have been investigated which have looked at pathogens from a variety of common infections (Kahlmeter, 2003). These include the WHO Antimicrobial Resistance Monitoring Programme, the European Antimicrobial Resistance Surveillance System (EARSS), the Hospitals in Europe Link for Infection Control through Surveillance (HELICS), the European Study Group on Nosocomial Infections and the Alexander Project on pathogens in lower respiratory tract infections. In the field of urinary tract infections (UTIs), there has been a steady increase in the level of resistance to commonly used

antibiotics, including ampicillin and trimethoprim.^{3,4} There have also been reports of resistance emerging to fluoroquinolones in some countries (Kahlmeter, 2003).

Drug resistance among bacteria causing UTI has increased since introduction to UTI chemotherapy. The etiological agents and their susceptibility patterns of UTI vary in regions and geographical location (Kibret and Abera, 2014). Besides, the etiology and drug resistance change through time. Knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred in time so that updated recommendation for optimal empirical therapy of UTI can be made (Kibret and Abera, 2014). The initial treatment for UTIs is generally empirical and is based on the known antimicrobial resistance patterns of urinary pathogens. However, the prevalence of antimicrobial resistance among urinary pathogens has been increasing, due to the increased and inappropriate prescription of antibiotics (Li *et al.*, 2017). Antibiotic resistance is a growing concern in China, where clinical isolates of *E. coli* have exhibited high rates of resistance to amoxicillin with clavulanic acid (20.6%–27.9%), ciprofloxacin (64.7%–74%), and piperacillin (71.1%–80.1%) (Li *et al.*, 2017).

2.8 Risk factors

Most infections begin in the urethra, the tube that drains the bladder. It is theorized that because in women the opening of the urethra is in close proximity to the anus and vagina in women, organisms can more readily move from these openings to the urethra. This is said to account for the higher infection rate in women. In women the risk of UTI increases with sexual activity and age. Post-menopausal women may experience bladder or uterine prolapse or a shifting of these structures from their normal position (Kahlmeter, 2003). The shift can lead to incomplete emptying of the bladder and create conditions conducive to bacterial colonization. Postmenopausal women also experience changes in hormone production, particularly estrogen, which can alter vaginal flora, the good organisms that populate the vagina and fight bacteria (Kahlmeter, 2003).

Other risk factors for UTIs are obstructions in the urinary tract such as kidney stones. Poor bladder emptying and bladder control in the elderly put them at risk. In men an enlarged prostate may impede the flow of urine and increase risk. People who have catheters placed for diseases or surgical procedures are at risk despite extraordinary sanitization procedures employed during catheter placement and maintenance (Koshariya *et al.*, 2015). Disorders such as diabetes that alter or weaken the immune system raise the risk of UTI by lowering

natural resistance. Several studies have suggested that women who use a diaphragm have a higher incidence of UTIs than those who use other means of birth control. In some women, intercourse may trigger the onset of a UTI although the reason for this has yet to be determined (Koshariya *et al.*, 2015).

Hospital-acquired urinary tract infection (UTI) is the most common infection acquired in hospitals. Up to 25% of hospitalised patients undergo urinary catheterisation, a similar proportion of patients cared for in residential homes will have long term indwelling catheters. (Koshariya *et al.*, 2015). Although often necessary intervention, indwelling urinary catheters are a leading cause of nosocomial infections and have been associated with both morbidity and mortality (Koshariya *et al.*, 2015). In developing countries, UTIs are among the most common health problems affecting women in their reproductive ages (Onyango *et al.*, 2018). Pregnant women are more susceptible to UTIs due to a combination of hormonal and physiologic changes that predispose them to bacteriuria. The incidence of acute pyelonephritis in pregnant women is also significantly increased (Onyango *et al.*, 2018). Factors such as history of recurrent urinary tract infection, diabetes, poverty, increasing maternal age and anatomical abnormalities of the urinary tract have also been associated with a two fold increase in bacteriuria during pregnancy, but the risk factors associated with UTIs in Africa remains poorly investigated (Onyango *et al.*, 2018).

2.9 Recurrence of urinary tract infections

Recurrent UTI is defined as 2 uncomplicated UTIs in 6 months or, more traditionally, as 3 positive cultures within the preceding 12 months (Gopal *et al.*, 2007). This is estimated to affect 25% of women with a history of UTI. When there is recurrent infection with the same organism despite adequate therapy, it is considered a relapse. Reinfection is defined as recurrent UTI caused by a different bacterial isolate or by the previously isolated bacteria after a negative intervening culture or an adequate time period (2 weeks) between infections (Gopal *et al.*, 2007). Reinfection is more common than relapse (Hooton, 2001).

Most recurrences occur within the first 3 months after the primary infection, and there can often be clustering of infections (Kraft and Stamey, 1977). When the initial infection is caused by *E. coli*, there is a higher risk of reinfection within the first 6 months (Foxman *et al.*, 2000). There are as many options for prevention and management of recurrent UTI as there are studies on the issue (Nicolle, 2002). A Cochrane review of 19 trials including 1120 patients, showed that antibiotics are better than placebo in reducing the number of clinical

and microbiological recurrences in pre- and postmenopausal women with recurrent UTI (Albert *et al.*, 2004).

2.10 Prevention of urinary tract infections

2.10.1 Cranberries

Cranberries (particularly in the form of cranberry juice) have been touted as an effective home remedy for the prevention and treatment of UTIs for several decades. So far, no definite mechanism of action has been established. The main suggestion is that cranberries prevent bacteria (particularly *Escherichia coli*) from adhering to uroepithelial cells (Schmidt and Sobota, 1988). Without adhesion, the bacteria cannot infect the mucosal surface of the urinary tract. A review of 10 studies with a total of 1049 subjects showed some evidence that cranberry juice and derivatives may decrease the number of symptomatic UTIs over a period of 12 months, particularly for women with recurrent UTIs (Jepson and Craig, 2008).

2.10.2 Acupuncture

Two small RCTs evaluated the role of acupuncture compared with sham acupuncture or no treatment in the prophylaxis of recurrent UTIs (Aune *et al.*, 1998). During a 6-month period, both studies demonstrated that acupuncture could play a significant role in preventing recurrent UTIs. Authors concluded that it seems a worthwhile alternative to antibiotic strategy.

2.10.3 Probiotics

The instillation of *Lactobacillus* into the vagina is believed to stop the ascension of uropathogens into the bladder. Available studies suggest that probiotics can be beneficial, and most authors consider this approach promising, but further research is needed before probiotics can be recommended for prevention of UTI (Falagas *et al.*, 2006).

2.10.4 Vaccines

An injectable vaccine developed in Switzerland was found to be effective, with no adverse effects observed in pregnant women or their offspring (Grischke and Rüttgers, 1987). In order to obviate some adverse reactions of the parenteral vaccine, four mucosal vaccines were developed as a vaginal suppository or an oral tablet, but the vaccine's benefits seemed to decline after the last dose (Uehling *et al.*, 2001). The only parenteral vaccine currently under development, FimCH, has proven to be safe in a phase I clinical trial (Hopkins *et al.*, 2002). A phase II clinical trial has been completed, but data are not yet available (Hopkins *et al.*, 2002).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study area

This research was carried out at Masvingo General Hospital in Masvingo Province, Zimbabwe. The hospital is located in the south-eastern parts of Zimbabwe (20°03'36.6"S 30°49'41.1"E). Masvingo General Hospital is a referral hospital catering for patients from areas the province.

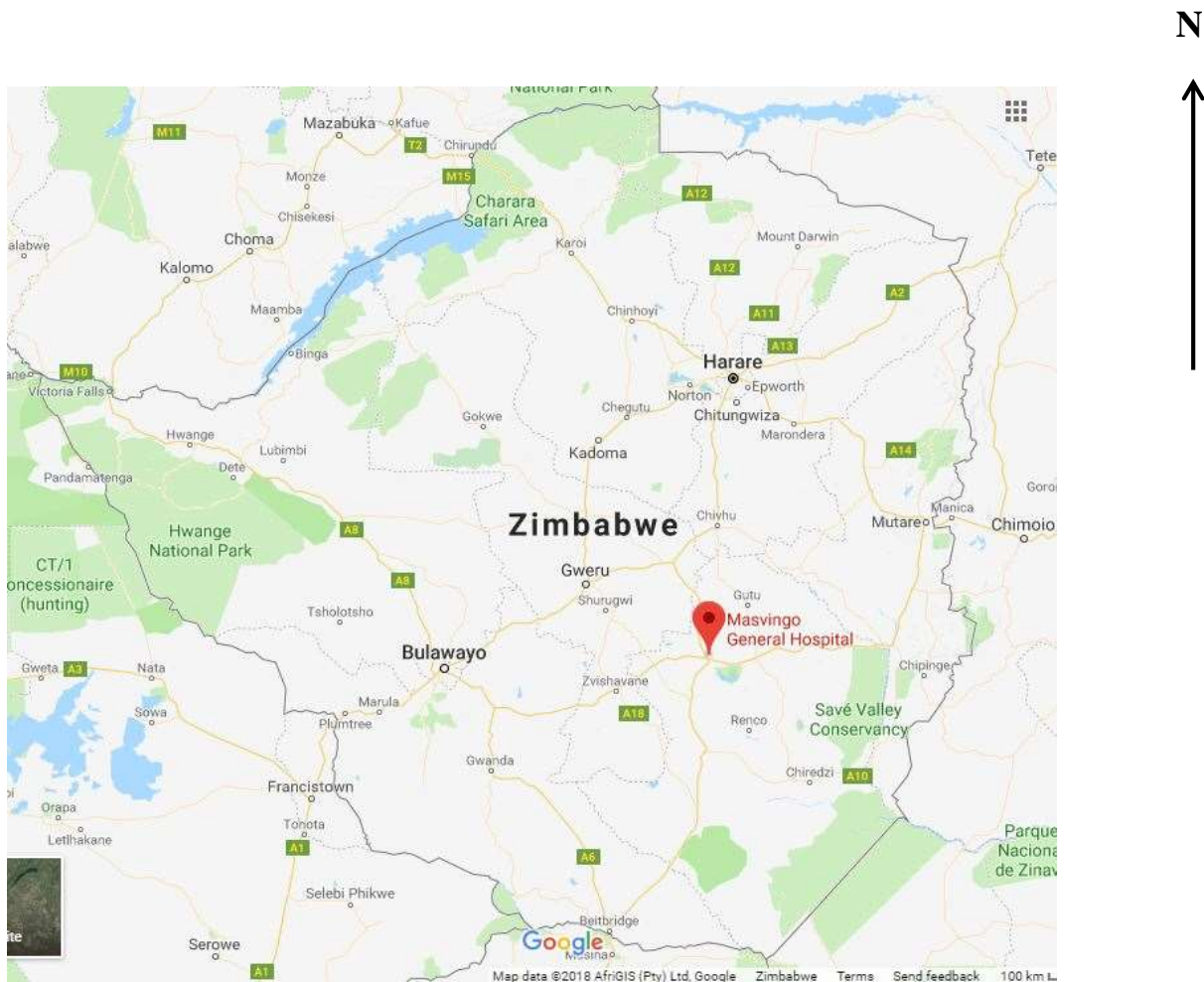


Figure 3.1: Map of Zimbabwe showing study area

3.2 Sampling method

Purposive sampling was used to select patients presenting with urinary tract infection symptoms. Simple random sampling was then carried out to select the patients that would take part in the study. During the sampling the inclusion and exclusion criteria were strictly followed with all the participants given a consent form to volunteer to participate in the research. For children the parents were approached to give the consent. This was done until the required sample size was achieved.

3.3 Study population and sample size

The study was targeting all the patients with urinary tract infection symptoms and those visiting Masvingo General Hospital seeking treatment for the urinary tract infections. Symptoms looked at included foul smelling urine, lower abdominal pain, cloudy urine, loss of bladder control and lastly burning sensation during urination. The patients were divided into six age groups with respect to age which are (0-10 years), (11-20years), (21-30years), (31-40years), (41-50years) and lastly 51+ years. Also two groups were created according to gender thus females and males.

3.3.1 Sample size

The sample size was determined using the formula below:

$$N = Z^2 \times PQ / D^2$$

where: N = Desired minimal sample size

Z = Standard normal deviation (1.96 from tailed normal table)

P = Prevalence of condition under study (9%) according to Mbanga (2010)

Q = 1 - P

D = Precision required for the study at 95 % confidence level (0.05)

$$N = (1.96)^2 \times 0.14(1-0.14) / (0.05)^2$$

= 125 samples were supposed to be tested

3.3.2 Inclusion criteria

All the patients presenting with symptoms at Masvingo General Hospital seeking treatment were included in the study. Only the patients who consented to be part of the research and also not under any sort of antibiotic treatment for the past two weeks before study were included.

3.3.3 Exclusion criteria

Patients who refused to take part in the study and parents who did not give consent for their children to participate were excluded. Patients already on treatment for the past two weeks and those not presenting with any symptoms were also left out.

3.4 Sample Collection

Urine specimens were collected by means of sterile urine jars. The urine jars were labelled with the patient study number, date of collection, date of birth, sex and age. Patients were instructed to collect only midstream urine up to half full capacity of the urine jar. For very young children unable to pass urine voluntarily some urine bags were used. Collection was done with specialised personnel to ensure the reliability of the samples.

3.5 Laboratory procedures

3.5.1 Sample reception

When samples arrived at the medical laboratory, a sample ID number and other patient information were entered into a log book before examination was commenced.

3.5.2 Macroscopic and microscopic examination of samples

After entering the patient information, physical properties of the urine were noted down. These included urine colour, smell and appearance. This was the first step of sample diagnosis and was used as a mere indicator of the kind of results to expect from the urine culture. Under microscopy the main components that were looked for are the white blood cells as their presence in urine is a good indication of bacterial infection of the urinary tract.

First five millilitres of urine were transferred into a centrifuge tube. The tube was then centrifuged at 3000rpm for one minute. The supernatant was then discarded to remain with the debris at the bottom. The debris was placed on a glass slide and then the slide was covered by a cover slip. The slide was then viewed under the microscope using the X10 for focusing and the X40 for cell counting. The number of white blood cells and red blood cells in the urine were recorded as well as other components that might be in the urine e.g Yeasts.

3.5.3 Urine culturing

For urine cultures, cysteine lactose electrolyte deficient agar (CLED) was used as selective media to isolate uropathogens from the urine samples. The media were prepared according to the manufacturer's instructions and poured into sterile petri dishes. The prepared media in petri dishes was stored under sterile conditions in the fridge. To culture a sample one petri dish was used for each urine sample. The petri dish was first labelled with the patient ID number and then the date of culture. The culturing process was done under sterile conditions inside a laminar flow cabinet. A Bunsen burner was used to sterilise the inoculating loop before transferring the urine to the media. An amount of 0.001 ml of urine was then inoculated onto media using the inoculating loop. Cultured plates were then incubated for 24 hrs at 37°C. After the 24 hrs of incubation the number of pure colony forming units was then counted to determine the number of micro-organisms per millilitre in the original specimen of urine. The information was recorded under the result section in a log book. Those with $\geq 10^5$ colony forming units per millilitre of urine were selected for further tests. Plates with no growth or tiny colonies were returned to the incubator for another 24 hrs before discarding the plates since antimicrobial treatment or other factors may inhibit initial growth.

3.5.4 Bacteria identification

The bacteria were identified using colony properties and also biochemical tests. Colony properties used included colour, shape, arrangement and size of the bacterial colonies. The biochemical tests were used as confirmatory tests for the suspected bacterial isolate. The biochemical tests that were used are Indole test, Citrate test, oxidase test, Hydrogen sulphide production, lactose fermentation, gas production, catalase and coagulase tests. Also the gram staining method was used for bacterial classification as gram negative and gram positive bacteria.

3.5.4.1 Gram staining

After obtaining isolates from the CLED agar, gram staining was done to determine whether the bacteria were gram positive or gram negative. First a pure colony from the CLED agar plate was smeared evenly on a glass slide. Heat fixing was then done using a Bunsen burner flame by passing the slide three times across the flame. The slide was then placed on a slide rack and left to cool down. Crystal violet was then poured on to the smear and left to stand for one minute. The crystal violet was then removed and the slide was washed with running tap water. Iodine was then added to the slide and left to stand for 30 seconds. The iodine was then washed from the slide using running tap water. The slide was then decolourised with the addition of acetone for 5 seconds and washed with running water. Safranin was then added for 30 seconds and also washed using running water. The slide was then left to air dry and then viewed under the light microscope using the oil immersion lens. The results of the gram staining were then recorded in the result log book.

3.5.4.2 Triple sugar iron test

This test was used in Gram negative colonies. TSI agar has glucose with a 0.1 % concentration and lactose and sucrose with a concentration of 1 %. Sterile TSI slants with agar were taken from the refrigerator and wiped using a dry cotton towel. The cap was removed and then the neck was flamed. An inoculating straight loop was sterilized in the blue flame of the Bunsen burner and then allowed to cool. A colony of the suspected organism from CLED agar was picked, stabbed into the medium up to the butt of the TSI tube and then it was streaked back and forth along the surface of the slant. Again the neck of the TSI was flamed, capped and placed in the incubator for 18 hours at a temperature of 37°C. Triple sugar iron agar tube was used to test for the fermentation of only glucose (yellow butt), fermentation of lactose and sucrose (all over yellow), CO² formation (crack in agar), or ferrous ammonium sulphate produced (black precipitate).

3.5.4.3 Catalase test

This test was used to differentiate suspected *Staphylococci* spp. colonies which appeared with a uniform yellow colour. Two drops of 3 % hydrogen peroxide were put onto a

clean glass slide using a dropper; a pure colony of the organism was picked from CLED agar using a wooden applicator stick. Placing the colony on the hydrogen peroxide on the glass slide; emulsification was done. Observation for bubble formation was done within 30 seconds.

3.5.4.4 Free coagulase test

This test was used to differentiate suspected *Staphylococcus aureus* (pathogenic) from *Staphylococcus albus* which is non-pathogenic. Dilute plasma from human blood was used with peptone water. A loopful of the test organism was put into the diluted plasma which made a complete suspension. Incubation of the suspension was done at a temperature of 37°C then examination for clot formation was made.

3.5.4.5 Indole test

This test was used in organisms suspected to be *Escherichia coli* and *Klebsiella*. Indole test determines the presence or absence of the tryptophanase, an enzyme which breaks down tryptophan. A 1% Tryptone broth was used during the test. Kovac's reagent was added to the Tryptone broth and if indole was present a red coloration formed at the top.

3.5.4.6 Citrate test

Citrate test was used to test for the presence of citrate which is the sole source of carbon for bacteria. An agar slant with synthetic medium containing small amounts of mineral salts (citrate and ammonium) was used to perform the test. Bromothymol blue (pH indicator) was added to the agar slant and if there is growth (presence of citrate) the agar is blue and if there is no growth the agar is green.

3.5.4.7 Oxidase test

One colony of the suspect organism was transferred to a filter paper soaked with oxidase reagent (tetramethyl-p-phenylenediaminedihydrochloride). Appearance of a blue colour within 10 seconds indicates a positive result.

3.5.5 Bacterial antibiotic susceptibility assays

After identification of bacterial isolates, antimicrobial susceptibility assays were done using the disk diffusion technique with commercially available antibiotic drugs namely ampicillin, nalidixic acid, tetracycline, norfloxacin, ciprofloxacin, kanamycin, gentamycin, and nitrofurantoin. All the eight antibiotic drugs were combined on a single ring. Mueller Hinton agar plates were used for the susceptibility assays. Antibiotic disk rings viability was quality controlled using *E. coli* ATCC 25922 after every week according to WHO standards. The agar was prepared according to manufacturer standards and guidelines.

A pure colony from the CLED agar plate was picked using a sterile inoculating loop. The loop was sterilized using a Bunsen burner. The colony was then streaked over the surface of the muller hinton agar plate rotating the plate after each application to ensure an even distribution of the bacteria on the agar.

Antimicrobial disk rings containing specified concentrations in micrograms of the eight antibiotics were placed on the agar plates using a pair of sterile forceps and then gently pressed down on the agar to ensure contact. The plates were inverted, and then incubated at a temperature of 37°C for 24 hrs. After incubation the zone diameters with complete inhibition, including the diameter of the disk were measured using a ruler and recorded in millimetre on the under surface of the plate without opening the lid. The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate and sensitive.

3.6 Data analysis

The overall prevalence of UTIs was calculated using the formula:

$$\text{Prevalence} = (\text{number of positive cases} \div \text{total number of samples}) \times 100\%$$

Prevalence was also calculated according to different characteristics which are gender, age group, type of uropathogen, and hospital status (outpatients or inpatients). Collected data for antibiotic sensitivity test was analysed using the Chi-Square test for homogeneity of proportions in SPSS to check for differences in the proportions of sensitive isolates across the eight antibiotics used in this study. The chi-square was also used to check for any differences of UTI prevalence according to individual characteristics i.e age group, gender, type of uropathogen and hospital status. Evaluations were carried out at 95 % confidence level and $P < 0.05$ was considered statistically significant.

3.7 Quality control

Quality control of antimicrobials was done weekly using *E.coli* ATCC 25922 (Figure 3.2). The results of the quality control showed that the drugs that were used throughout the study were properly working. The diameters recorded for all the antibiotics were in the same range.

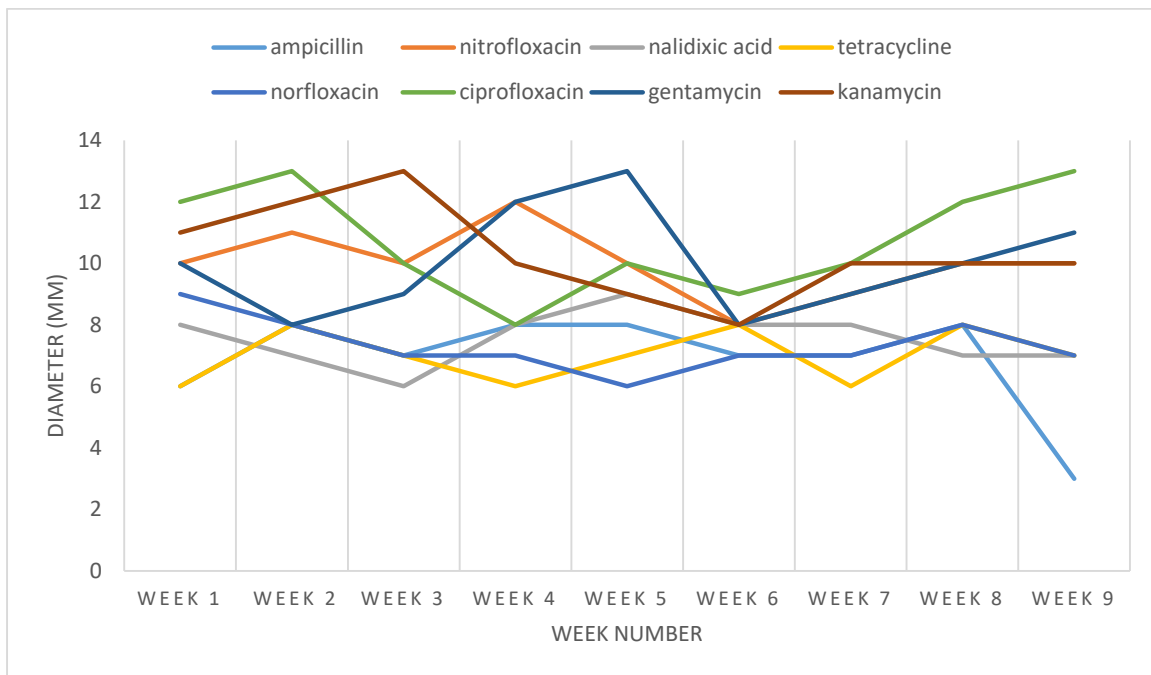


Fig 4.3: Quality control of antimicrobials using *E. coli* ATCC 25922

3.7.3.8 Ethical considerations

The research was granted permission by the medical officer in charge at Masvingo General Hospital. Ethical approvals were applied for and granted by the Research Ethics Board at Midlands State University, Gweru, Zimbabwe and Medical Research Council of Zimbabwe (MRCZ) after the project proposal was approved by the supervisor. The study was carried out with the help of qualified and registered medical laboratory scientists. For the sake of patient information confidentiality study identification numbers were assigned to each sample and the data was stored using the study ID numbers instead of patient names. Patients were approached to first give consent to participate in the research and for young children consent was sought from their parents/guardians.

CHAPTER 4

RESULTS

4.1 Demographic characteristics of the sampled population at Masvingo General Hospital

A total of 123 fresh urine samples two short of the original sample size were collected from patients by the standard mid-stream catch method in sterile universal bottles. The sample comprised 33 males and 90 women. Forty seven of the patients, comprising 36 females and 11 males, had UTIs (**Table 4.1**)

Table 4.1 Distribution of UTIs among patients presenting at Masvingo General Hospital

Status	No of patients		Percentage %
	Male	Female	
Positive	11	36	38%
Negative	22	54	62%
Total	33	90	

Collection of urine samples was done in accordance of six age group categories which are 0-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years and lastly 51+ years. Most of the urine samples were collected from the 31-40 years age group with the least amount being in the 0-10 years age group. In the age group 0-10 years, only one female was tested for UTI and the result was negative, 11-20 years two females presented with UTI, 21-30 years 11 females had UTI, 31-40 years 2 males and 13 females had UTI, 41-50 years only 4 males and 4 females had UTI, 51+ years 5 males and 6 females had UTI (**Table 4.2**). The age group that presented with most UTI cases was between 31-40 years (15 patients) and the least age group with UTI cases was between 0-10 years (0 patients). Most female patients with UTI cases were between the age group 31-40 years (13 female) whereas most male were in the 51+ years (5 male) (**Table 4.2**). There were no statistically significant differences of prevalence among all the age groups ($X^2 = 3.783$, P Value > 0.05, Appendix A, Table A1).

Table 4.2: Prevalence of Urinary tract infections across different age groups that presented at Masvingo General Hospital.

Age group	UTI status	Sex		Total
		Male	Female	
0-10	Negative	0	1	1
	Positive	0	0	
11-20	Negative	1	1	4
	Positive	0	2	
21-30	Negative	5	15	31
	Positive	0	11	
31-40	Negative	5	23	43
	Positive	2	13	
41-50	Negative	8	8	24
	Positive	4	4	
51+	Negative	3	6	20
	Positive	5	6	
TOTAL		33	90	123

4.2 Distribution of UTIs according to gender

The highest number of positive cases was found in women than in men. The total percentage of females with UTIs was 77% while that of the males was 23% (**Fig 4.1**).

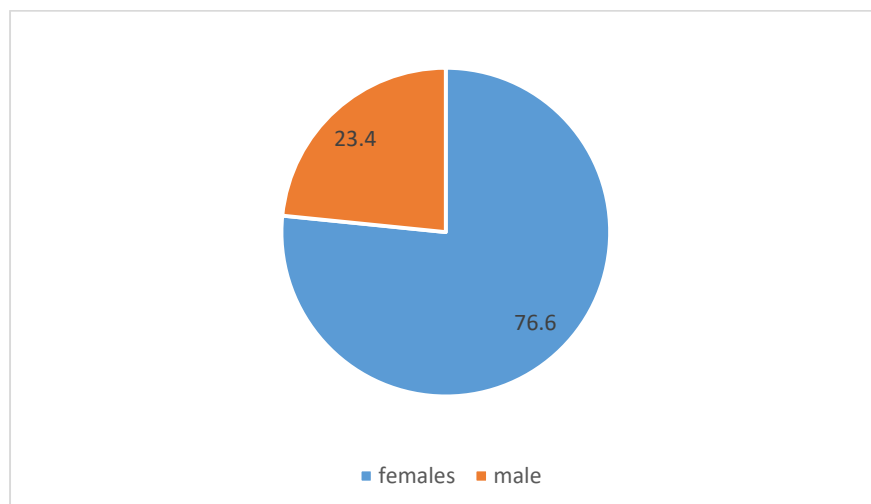


Fig 4.1 The distribution of UTIs among the male and female patients

4.3 Hospital status of the patients presenting with UTI symptoms at Masvingo General Hospital

The hospital had both inpatients and out patients attending for their UTI services offered. Among the population sampled, majority, 78% were out patients while only 22% were in patients. Patients who had UTI were mainly outpatients and only a few inpatients (**Table 4.3**). There were no significant difference in UTI prevalence between the outpatients and inpatients ($X^2= 1.447$, p value > 0.05, Appendix A, Table A3).

Table 4.3: The proportions of inpatients and outpatients

Type	No of patients	Percentage	No of positive patients	No of negative patients
In patients	27	22 %	13 (27.7%)	14
Out patients	96	78 %	34 (72.3%)	62

4.4 Recurrence of UTIs among patients presenting at Masvingo General Hospital

Most of the UTI patients (62%) who attended Masvingo General Hospital did not experience any recurrence. Only 38% had experienced recurrence of a UTI (**Table 4.4**). There were significant differences noticed in UTI prevalence between the recurrent and non-recurrent cases ($X^2= 34.096$, P value < 0.05, Appendix A, Table A4).

Table 4.4: The proportions of recurrent and non-recurrent cases

	Recurrent	Percentage %	Non-recurrent	Percentage %
Males	5	10%	6	13%
Females	13	28%	23	49%
Total	18	38%	29	62%

4.5 Identification of uropathogens isolated from patients presenting at Masvingo General Hospital

Various biochemical tests were performed to identify the uropathogens. They were identified as follows (**Table 4.5**).

Table 4.5: Biochemical tests used to identify the organisms isolated from patients presenting at Masvingo General Hospital

Indole	MR	VP	citrate	oxidase	catalase	coagulase	motility	Gram status	Conclusion
P	P	N	N	N	N	N	P	N	<i>E Coli</i>
N	N	P	P	N	N	N	N	N	<i>Klebsiella</i>
N	N	N	N	N	P	P	N	P	<i>S aureus</i>
N	N	N	N	P	N	N	N	N	<i>P aureginosa</i>

Key: **P** - POSITIVE **N** - NEGATIVE **MR** – METHYL RED **VP** – VOGES PROSKAUR

Four genera of bacterial agents were isolated. Three Gram negative rods including *E. coli* and one Gram positive cocci (**Table 4.6**). There were significant differences in the prevalence of UTIs across all the uropathogen proportions ($X^2=1.230$, P value > 0.05, Appendix A, Table A5).

Table 4.6: Etiological bacterial agents isolated from patients presenting with UTI at Masvingo General Hospital

MICROORGANISM	NUMBER OF ISOLATES	PERCENTAGE %
GRAM POSITIVE COCCI		
		7 females
<i>Staphylococcus aureus</i>	10	3 males 21%
GRAM NEGATIVE RODS		
		17 females
<i>E. Coli</i>	22	5 males 47%
	8	5 females 17%
<i>Klebsiella</i>		1 males
	7	5 females 13%
<i>P. aureginosa</i>		2 males
TOTAL	47	100%

4.6 Prevalence of uropathogens among patients presenting with UTI at Masvingo General Hospital

With reference to the culture results obtained, the overall prevalence of UTI was 47 out of 123 samples (38%). UTI prevalence among the male patients was 11 out of 33 (33%) while among the female patients was 36 out of 90, (40%). *Escherichia coli* was isolated in five males out of the 11 who tested positive for the UTIs and in females it was isolated from 17 out of 90 female patients who participated. Prevalence of *Staphylococcus aureus* among male patients was 3 out of 33. Among the females, prevalence of *Staphylococcus aureus* was 7 out of 90. *Klebsiella spp* was isolated in only 1 male out of 33 and 5 females out of 90. *Psuedomonas aeruginosa* was isolated in 5 females out of 90 and 2 male patients out of 33 male participants.

4.7 Antibiotic susceptibility patterns of isolates to antibiotics used in the treatment of UTI in patients presenting at Masvingo General Hospital

Antimicrobial susceptibility test showed that *Escherichia coli* was highly resistant to ampicillin with 21 out of 22 isolates being resistant to it. *Escherichia coli* was most sensitive to nitrofurantoin with 21 isolates being sensitive out of the 22 isolates (**Table 4.7**). For the bacterial isolate *E. coli*, there were significant differences of the sensitive proportions across all the eight antibiotics used in this study ($X^2= 54.199$, P value < 0.05, Appendix A, Table A6). There were no significant difference in susceptibility noticed for *E. coli* for the five best antibiotics (kanamycin, gentamycin, ciprofloxacin, norfloxacin and nitrofurantoin) ($X^2= 8.100$, P value > 0.05, Appendix A, Table A7).

Klebsiella spp were highly resistant to Ampicillin with seven resistant isolates out of a total of 8 isolates. The drug with the highest potency against *Klebsiella* species was Kanamycin with 7 sensitive isolates out of a total of 8 isolates. For *Klebsiella spp*, the study showed significant differences of sensitive proportions of isolates across all the eight antibiotics used ($X^2 = 25.144$, P value < 0.05, Appendix A, Table A9). For the four best antibiotics (nitrofurantoin, kanamycin, gentamycin and nalidixic acid) no significant differences were observed for susceptibility ($X^2 = 8.242$, P value > 0.05, Appendix A, Table A8).

Pseudomonas aureginosa was totally resistant to ampicillin with all the 7 isolates being resistant. There were four drugs that were effective for *Pseudomonas aureginosa* which are nitrofurantoin, tetracyclin, ciprofloxacin and norfloxacin. All these drugs had a potency against 5 isolates out of the total 7 *Pseudomonas aureginosa* isolates (**Table 4.7**). The proportion of sensitive *P aureginosa* isolates was significantly different across all the eight antibiotics ($X^2= 25.173$, P value < 0.05, Appendix A, Table A11). For the four best drugs they were no statistically significant differences of sensitive proportion of *P aureginosa* ($X^2= 4.317$, P value > 0.05, Appendix A, Table A12).

Lastly *Staphylococcus aureus* species were highly sensitive to nitrofurantoin with all the 10 isolates being sensitive and the most resisted drugs were Nalidixic acid and Ampicillin both with 8 resistant isolates out of the 10 *Staphylococcus aureus* isolates. However all the four uropathogens were generally more resistant to Ampicillin (**Table 4.7**). *S. aureus spp* had significantly different proportion of sensitive isolates across all the antibiotics used in this study ($X^2 = 24.554$, P value < 0.05, Appendix A, Table A14). The four best antibiotics were

gentamycin, ciprofloxacin, norfloxacin and nitrofurantoin. No statistically significant differences were noticed for the sensitive proportions of these drugs ($X^2 = 6.276$, P value > 0.05, Appendix A, Table A15).

Table 4.7: Susceptibility profiles of bacterial isolates from patients presenting At Masvingo General Hospital to antibiotics

Antibiotics	Bacteria isolated											
	<i>E Coli</i> N = 22			<i>Klebsiella</i> N = 8			<i>P aureginosa</i> N= 7			<i>S aureus</i> N=10		
	S	I	R	S	I	R	S	I	R	S	I	R
AMPICILLIN	1	4	17	1	0	7	0	0	7	2	0	8
NITROFURAN TOIN	19	2	1	4	1	3	5	0	2	9	1	0
NALIDIXIC ACID	12	0	10	4	0	4	1	0	6	2	4	4
TETRACYCLI NE	12	5	5	3	2	3	4	1	2	3	2	5
NORFLOXACI N	15	1	6	1	3	4	5	0	2	5	1	4
CIPROFLOXA XIN	15	1	6	3	2	3	3	2	2	6	2	2
GENTAMICIN	17	0	5	6	0	2	3	1	3	5	2	3
KANAMYCIN	16	0	6	7	1	0	4	2	1	4	1	5

Key: **N** – NO OF ISOLATES **R** – RESISTANT **S** – SENSITIVE **I** – INTERMEDIATE

Susceptibility of the four bacteria strains showed that, *Escherichia coli* was more susceptible to Nitrofurantoin with a percentage of 96%; *Pseudomonas aeruginosa* was more susceptible to Nitrofurantoin, Tetracycline, Norfloxacin and Ciprofloxacin all having a percentage of 71%; *Klebsiella spp* was more susceptible to Kanamycin with a percentage susceptibility of 88%; *S. aureus* was more susceptible to Nitrofurantoin with a percentage of 90 % (**Fig 4.2**).

Pseudomonas aeruginosa was susceptible to only 7 out of the 8 tested antibiotics; *Staphylococcus aureus*, *E. coli* and *Klebsiella* were susceptible to all the 8 tested antibiotics (Fig 4.2). Generally all the bacterial strains highly resisted the antibiotic Ampicillin.

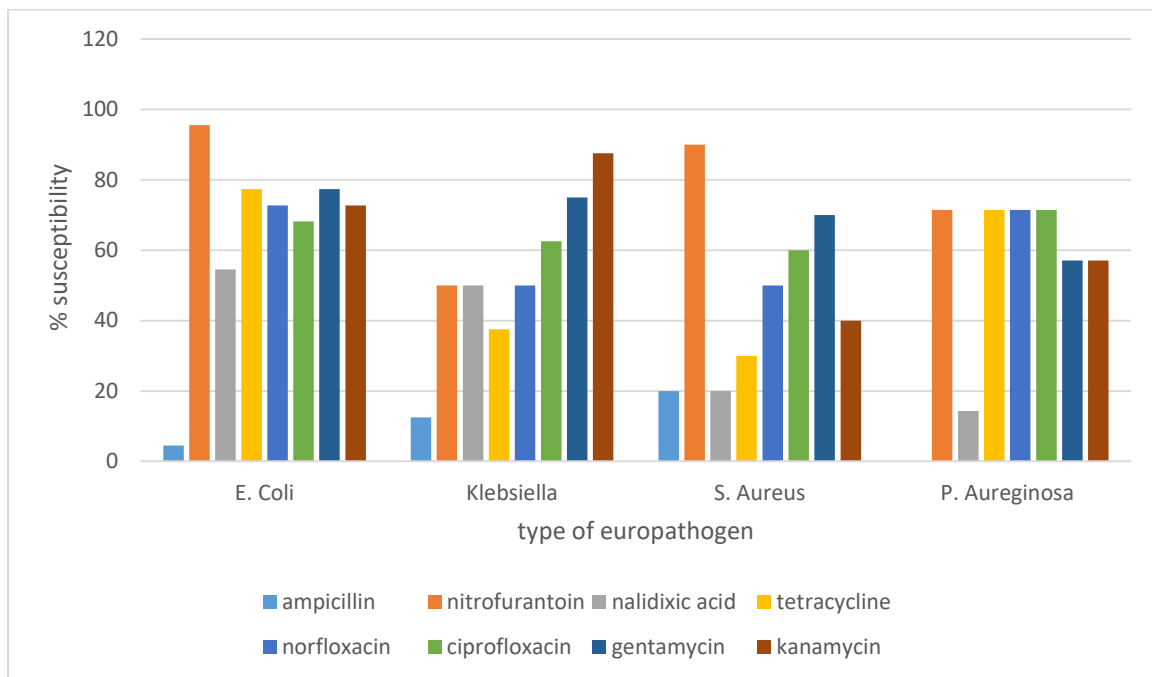


Fig 4.2: Susceptibility of the four bacteria strains to the antibiotics tested.

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Prevalence of Urinary Tract Infections

Prevalence of urinary tract infections among patients presenting at Masvingo General Hospital was 38%. This prevalence is similar to the one reported by Bitew *et al.* (2017) of 36 % though the sample size in this study was bigger. UTI prevalence from this study was higher than 17% reported by Jambo *et al.* (2005) and 20% reported by Oduyebo *et al.* (2001) in Nigeria, a developing country like Zimbabwe. The relatively high prevalence of UTIs found in this study suggests that the people in Masvingo Province are at high risk of UTIs. August and De Rosa (2012) opine that the high prevalence of UTIs in the developing world is caused by limited access to health care.

According to gender, female patients had the highest prevalence of UTIs (77%) than their male counter parts with a prevalence of 23%. This higher prevalence of UTIs in women (77%) than men (23%) observed in this study is similar with that reported in Gyansa-Lutterodt *et al.* (2014) where the prevalence of female patients was 67% and that of males was 33%. Also a study by Akram *et al.* (2007) shows a similar pattern. Females are at high risk of UTIs because women have a shorter urethra than men do, which shortens the distance that bacteria must travel to reach the bladder (Kahlmeter, 2003), Also Women who use diaphragms and spermicidal agents for birth control may be at higher risk (Stamm and Norrby, 2001). Furthermore, it is believed that after menopause, a decline in circulating estrogen causes changes in the urinary tract that make women more vulnerable to infection (Stamm and Norrby, 2001).

With respect to age, no statistically significant differences of prevalence were noticed across all the age groups when all the samples were considered. However when the males and females were analysed separately differences in prevalence across age groups were noticed. The prevalence was increasing with the increase in age for both males and females with the 31-40 years age group having the highest prevalence of UTIs. This age group is highly sexually active and this increases the risk of infection especially in the females (Fihn, 2003). This is similar to the findings of Linhares *et al.*, (2013) who postulated that even though it has been stated that factors such as age might influence the aetiology of urinary tract infection, in their study it was not observed significant differences among the bacteria responsible for these infections in the different age groups when all samples were considered. However, significant differences were

observed for all age groups when female and male were analysed separately and, the differences increasing with the patient age.

With regard to patient status there were no significant differences in prevalence between the outpatients and inpatients. The pattern noticed in this study differ from a study by Muzaaheed *et al.*, (2008) with a higher prevalence for outpatients and lower for inpatients. However another study with a high prevalence in inpatients than the outpatients was done by Archibald *et al.*, (1997) in the United States. The high prevalence in inpatients are usually increased by risk factors such the use of catheters leading to the occurrence of catheter associated UTIs (Wazait *et al.*, 2003).

5.1.2 Isolated uropathogens

Isolating and identifying the uropathogens causing UTIs in patients presenting at Masvingo General Hospital was one of the objectives of this study. The uropathogens that were isolated from the collected urine sample in this study were *E. coli*, *Klebsiella spp*, *P. aureginosa* and *S. aureus*. These same uropathogens were also found in a study in Kenya by Nyambane (2015). These four uropathogens are the most common causes of UTIs globally. However, besides these four bacteria there are others that are associated with UTIs, for example, *Proteus mirabilis*, *Streptococcus faecalis*, *Proteus vulgaris*, *Providencia stuartii*, *Staphylococcus epidermidis* (Abubakar, 2009).

The most prevalent uropathogen among the four in this study was *E. coli* with a prevalence of (47%). This high prevalence of *E. coli* was also recorded by Sabir *et al.* (2014) with an *E. coli* prevalence of 80%. Enteric bacteria (in particular, *E. coli*) remain the most frequent cause of UTI, although the distribution of pathogens that cause UTI is changing (Kolawole *et al.*, 2010).

The second most prevailing uropathogen was *S. aureus* with a prevalence rate of about 21% followed by *Klebsiella spp* (17%) and *P. aureginosa* (15%). This correlates with results obtained in a European study were *E. coli* was first followed by *S. aureus*, *Klebsiella spp* and lastly *P. aureginosa* (Bouza *et al.*, 2001). This study findings are in agreement with a study by Amin *et al.*, (2009) with differences among the proportion of the uropathogens isolated. The least prevailing uropathogen in this study was *P. aureginosa* (15%).

5.1.3 Susceptibility profiles of the uropathogens

One of the objectives of this study was to determine the patterns of susceptibility among the uropathogens. *Escherichia coli* isolates showed high susceptibility to the antibiotic Nitrofurantoin with a percentage of 86%. Chomarat, (2000) postulated that nitrofurantoin and fosfomycin-trometamol remain highly active against urinary Enterobacteriaceae, with over 90% of *E. coli* being susceptible. However the *E. coli* isolates were less susceptible to the antibiotic ampicillin (5%). This correlates with the findings of Kurutepe *et al.* (2005) in Turkey where the resistance of *E. coli* to ampicillin was in the range of 47.8% to 64.6%. The high resistance of *E. coli* is due to the presence of R-TEM enzymes that degrades the ampicillin thus reducing its effectiveness (Chomarat, 2000).

With regards to *Klebsiella spp* the isolates were highly susceptible to the antibiotic Kanamycin (88%). This high susceptibility to Kanamycin is in agreement with the findings of Ullah *et al.* (2009) who found the susceptibility to gentamicin was (17.39%); kanamycin (63.04%); gatifloxacin (45.65%); ciprofloxacin (41.3%); enoxacin (43.48%); doxycycline (15.22%) and to co-trimoxazole only (6.52%). *Klebsiella spp* were less susceptible to ampicillin and norfloxacin both with a percentage susceptibility of 13%. The reasons for this could be the occurrence of ESBL-producing *Klebsiella spp*, which have a tendency for co-resistance to non- β -lactam classes of antimicrobials (Muzahed *et al.*, 2008).

Staphylococcus aureus isolates were most susceptible to Nitrofurantoin (90%). This closely relates to the findings of Jha and Bapat (2005) in which 78% of *S. aureus* isolates were sensitive to nitrofurantoin. Since nitrofurantoin had a high sensitivity percentage this proves that it can be effectively used for UTIs caused by *S. aureus*. Resistance of *S. aureus* to ampicillin and nalidixic acid is in agreement with Tambekar *et al.* (2006) in which all the uropathogens including *S. aureus* isolated showed resistance to ampicillin (87%) and nalidixic acid (88%).

In this study bacterial isolates of *P. aeruginosa* were highly susceptible to norfloxacin and nitrofurantoin (both 71%). This result disagrees with the findings of Farrell *et al.* (2003) who recorded that *Pseudomonas aeruginosa* was resistant to nitrofurantoin. This is because of the difference in geographical location and also the differences of the *Pseudomonas aeruginosa* species that might have been isolated from this study. However *P. aeruginosa* was less susceptible to ampicillin with a percentage susceptibility of 0%.

5.1.4 Recurrent UTIs

From the results of this study the number of recurrent cases was 18 out of the 47 UTI positive cases (38%). The recurrent cases were most prevalent in female patients (28%) than males (10%). According to Stamm and Norrby (2001), the increased risk of infection in women to UTIs is believed to be due to the makeup of their urinary tract system. Women tend to have a wider and shorter urethra that makes the passage of bacteria to the bladder very easy. Also the close proximity of the vagina to the anus is another factor that increases risk in women (Stamm and Norrby, 2001). The high percentage of recurrent cases suggests that high levels of antibiotic resistance are occurring at Masvingo General Hospital.

5.1.5 Drug effectiveness

The efficacy of the antibiotics used against the four isolated uropathogens in this study varied depending on the type of antibiotic. The most effective antibiotics against *Escherichia coli* caused UTIs were kanamycin, gentamycin, ciprofloxacin, norfloxacin and nitrofurantoin. There were no statistically significant differences observed in susceptibility of *Escherichia coli* for these five antibiotics. These antibiotics therefore can be used effectively in the treatment of UTIs being caused by *Escherichia coli*. This is so because according to studies by Costelloe *et al.* (2010) it was observed that agents which inhibit cell wall synthesis (ciprofloxacin and norfloxacin) or alter cell wall structure (nitrofurantoin) produce synergism against enterococci (in particular *Escherichia coli*) when combined with aminoglycoside antibiotics (kanamycin and gentamicin). Therefore, it seems that the aminoglycoside is important in actually producing the bactericidal effect when used in combination with agents which inhibit cell wall synthesis.

The most effective antibiotics against *Klebsiella spp* were nitrofurantoin, kanamycin, gentamycin and nalidixic acid. No significant differences in effectiveness were noticed for these antibiotics which implies that they can be used as a combination in the treatment of UTIs caused by *Klebsiella spp*. However Laxminarayan *et al.* (2013) noted that although these antibiotics can be used as effective treatment for UTIs continual, empirical and prolonged use of them will result in the emergence of resistance.

The four best antibiotics against *Pseudomonas aureginosa* were kanamycin, norfloxacin, nitrofurantoin and tetracycline. These drugs were noticed to be effective in the treatment of UTIs caused by the bacterial isolate *Pseudomonas aureginosa*. For the four best drugs there were no statistically significant differences of effectiveness that were noted.

The four best antibiotics against *Staphylococcus aureus* were gentamycin, ciprofloxacin, norfloxacin and nitrofurantoin. No statistically significant differences were noticed for the effectiveness of these drugs. These drugs were noticed to be effective in the treatment of UTIs caused by the bacterial isolate *S. aureus*. The antibiotics that were least effective for *S. aureus* are ampicillin, nalidixic acid, tetracycline and kanamycin. Statistically, the differences in proportions of sensitive isolates for these antibiotics were insignificant

When all the sensitivity result across the eight antibiotics used were combined it was noted that the best antibiotics that could be used against all the uropathogens isolated were gentamycin, kanamycin and nitrofurantoin. These antibiotics had the highest effects on all the bacterial isolates. No statistically significant differences were noted for the effectiveness of these antibiotics which implies that they can be used effectively as a combination in the treatment of UTIs caused by all the four isolated uropathogens in this study. These drugs were best because they are under the aminoglycosides class of antibiotics that are bactericidal and stop bacteria from producing proteins especially the gram negative bacteria that are the major cause of UTIs (*Escherichia coli*, *Pseudomonas aurigunosa* and *Klebsiella spp*) (Gelband *et al.* 2015).

The least effective antibiotics against all the four bacterial isolates were ampicillin and nalidixic acid. There were no statistically significant differences in the effectiveness of these antibiotics which implied that they both where not effective and could not be used for the treatment of UTIs caused by the four uropathogens. Ampicillin was not effective because it is not the agent of first choice for gram-negative infections, since the high inhibitory concentrations necessary are not always reached in the aqueous humor (Braykov *et al.* 2013). As for nalidixic acid it has the challenge that it has got specifics upon use in that full absorption has to be prevented and it works best when kept at a constant level of concentration (www.medicinenet.com). Also both ampicillin and nalidixic acid are both susceptible to uropathogens that produce β -lactamase which is an enzyme that degrades these antibiotics thereby reducing their effect on the uropathogens (Gelband *et al.* 2015).

5.2 Recommendations

Based on the results of this study the following recommendations can be made:

- There is need to monitor the profile of etiological bacteria of UTIs and the antimicrobial resistance regularly. This would show emergence of resistance to newer therapeutic agents as well as keep track of effectiveness of serving therapeutic agents.

- Health workers should mobilize patients to ensure that they have duly completed the prescribed antimicrobial therapy as not finishing it leads to resistance.
- Based on the prevalence results of this study the health council of Masvingo should organise awareness campaigns in order to educate the public on how they can prevent UTIs and also how the antibiotics can be used once one is infected.
- Also this study can be done on a larger scale in order to accommodate the prevalence of UTIs in the whole of Masvingo province and also to allow for a much bigger sample size.
- Also from the results of this study the antibiotic Ampicillin should not be used for the uropathogens isolated from this study (*E. coli*, *Klebsiella*, *S. aureus* and *P. aureginosa*) as they showed high resistance to the antibiotic.
- Nitrofurantoin should be used as a treatment option for the bacterial uropathogens isolated in this study as it was the most effective antibiotic against all the four uropathogens.
- Continual research should be made to monitor the prevalence and also to check for the efficacy of the antibiotics that will be currently in use at that time.

5.3 Conclusions

The prevalence of UTIs was 38% in patients presenting at Masvingo General Hospital with UTI symptoms. The UTIs were bacterial infections caused by four uropathogens namely *E. coli*, *Klebsiella*, *P. aureginosa* and *S. aureus*. *Escherichia coli* was the most common bacterial isolate with a prevalence of 47% and the uropathogen with the lowest prevalence was *P. aureginosa* (15%). Females were more susceptible to UTI with a prevalence of 77% than males (23%). Antimicrobial susceptibility test showed that *E. coli* is highly resistant to ampicillin but most sensitive to nitrofurantoin. *Klebsiella* species were highly resistant to Ampicillin but highly sensitive to Kanamycin. *Pseudomonas aureginosa* was totally resistant to ampicillin. There were four drugs that were effective for *P. aureginosa* with the highest sensitivity which are nitrofurantoin, tetracyclin, ciprofloxacin and norfloxacin. Lastly *S. aureus* species were highly sensitive to nitrofurantoin and the most resisted drugs were Nalidixic acid and Ampicillin. Overall, all the four uropathogens were generally most resistant to Ampicillin and Nalidixic acid while most sensitive to Nitrofurantoin, kanamycin and gentamycin. Therefore for effective treatment of UTIs there should be the use of these three best antibiotics hence reducing the recurrence of UTIs at Masvingo General Hospital.

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APPENDICES

APPENDIX A

Table A1: Chi square for Homogeneity of proportions for prevalence in different age groups

age_group * uti_status Crosstabulation

			uti_status		Total
			Positive	Negative	
age_group	0-10yrs	Count	0	1	1
		% within age_group	.0%	100.0%	100.0%
		% within uti_status	.0%	1.3%	.8%
		% of Total	.0%	.8%	.8%
	10-20yrs	Count	2	2	4
		% within age_group	50.0%	50.0%	100.0%
		% within uti_status	4.3%	2.6%	3.3%
		% of Total	1.6%	1.6%	3.3%
	20-30yrs	Count	11	20	31
		% within age_group	35.5%	64.5%	100.0%
		% within uti_status	23.4%	26.3%	25.2%
		% of Total	8.9%	16.3%	25.2%
	30-40yrs	Count	15	28	43
		% within age_group	34.9%	65.1%	100.0%
		% within uti_status	31.9%	36.8%	35.0%
		% of Total	12.2%	22.8%	35.0%
	40-50yrs	Count	8	16	24
		% within age_group	33.3%	66.7%	100.0%
		% within uti_status	17.0%	21.1%	19.5%
		% of Total	6.5%	13.0%	19.5%
	50+yrs	Count	11	9	20
		% within age_group	55.0%	45.0%	100.0%
		% within uti_status	23.4%	11.8%	16.3%
		% of Total	8.9%	7.3%	16.3%
Total		Count	47	76	123
		% within age_group	38.2%	61.8%	100.0%
		% within uti_status	100.0%	100.0%	100.0%
		% of Total	38.2%	61.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.783 ^a	5	.581
Likelihood Ratio	4.046	5	.543
Linear-by-Linear Association	1.170	1	.279
N of Valid Cases	123		

a. 4 cells (33,3%) have expected count less than 5. The minimum expected count is ,38.

Chi square for Homogeneity of proportions of prevalence in different sexes

Table A2 Homogeneity of proportions of prevalence in all sexes

gender * uti_status Crosstabulation

			uti_status		Total
			positive	negative	
gender	male	Count	11	22	33
		% within gender	33.3%	66.7%	100.0%
		% within uti_status	23.4%	28.9%	26.8%
		% of Total	8.9%	17.9%	26.8%
	female	Count	36	54	90
		% within gender	40.0%	60.0%	100.0%
		% within uti_status	76.6%	71.1%	73.2%
		% of Total	29.3%	43.9%	73.2%
Total		Count	47	76	123
		% within gender	38.2%	61.8%	100.0%
		% within uti_status	100.0%	100.0%	100.0%
		% of Total	38.2%	61.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.455 ^a	1	.500		
Continuity Correction ^b	.216	1	.642		
Likelihood Ratio	.460	1	.498		
Fisher's Exact Test				.537	.323
Linear-by-Linear Association	.451	1	.502		
N of Valid Cases ^b	123				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 12,61.

b. Computed only for a 2x2 table

Chi square for Homogeneity of proportions of prevalence in different patient statuses

Table A3 Homogeneity of proportions of prevalence in all patient statuses

patients_status * uti_status Crosstabulation

			uti_status		Total
			positive	negative	
patients_status	in patients	Count	13	14	27
		% within patients_status	48.1%	51.9%	100.0%
		% within uti_status	27.7%	18.4%	22.0%
		% of Total	10.6%	11.4%	22.0%
out patients		Count	34	62	96
		% within patients_status	35.4%	64.6%	100.0%
		% within uti_status	72.3%	81.6%	78.0%
		% of Total	27.6%	50.4%	78.0%
Total		Count	47	76	123
		% within patients_status	38.2%	61.8%	100.0%
		% within uti_status	100.0%	100.0%	100.0%
		% of Total	38.2%	61.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
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Pearson Chi-Square	1.447 ^a	1	.229		
Continuity Correction ^b	.958	1	.328		
Likelihood Ratio	1.421	1	.233		
Fisher's Exact Test				.266	.164
Linear-by-Linear Association	1.435	1	.231		
N of Valid Cases ^b	123				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 10,32.

b. Computed only for a 2x2 table

Chi square for Homogeneity of proportions of prevalence in recurrent and non-recurrent cases

Table A4 Homogeneity of proportions of prevalence in recurrent and non-recurrent cases

recurrence * uti_status Crosstabulation

			uti_status		Total
			positive	negative	
recurrence	recurrent	Count	18	0	18
		% within recurrence	100.0%	.0%	100.0%
		% within uti_status	38.3%	.0%	14.6%
		% of Total	14.6%	.0%	14.6%
	non-recurrent	Count	29	76	105
		% within recurrence	27.6%	72.4%	100.0%
		% within uti_status	61.7%	100.0%	85.4%
		% of Total	23.6%	61.8%	85.4%
Total		Count	47	76	123
		% within recurrence	38.2%	61.8%	100.0%
		% within uti_status	100.0%	100.0%	100.0%
		% of Total	38.2%	61.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	34.096 ^a	1	.000		
Continuity Correction ^b	31.099	1	.000		
Likelihood Ratio	39.855	1	.000		
Fisher's Exact Test				.000	.000

Linear-by-Linear Association	33.819	1	.000	
N of Valid Cases ^b	123			

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6,88.

b. Computed only for a 2x2 table

Chi square for Homogeneity of proportions of prevalence in different types of uropathogens

Table A5 Homogeneity of proportions of prevalence in different types of uropathogens

uropathogen * uti_status Crosstabulation

			uti_status		Total
			positive	negative	
uropathogen	e coli	Count	22	0	22
		% within uropathogen	100.0%	.0%	100.0%
		% within uti_status	46.8%	.0%	17.9%
		% of Total	17.9%	.0%	17.9%
	klebsiella	Count	8	0	8
		% within uropathogen	100.0%	.0%	100.0%
		% within uti_status	17.0%	.0%	6.5%
		% of Total	6.5%	.0%	6.5%
	s aureus	Count	10	0	10
		% within uropathogen	100.0%	.0%	100.0%
		% within uti_status	21.3%	.0%	8.1%
		% of Total	8.1%	.0%	8.1%
	p aureginosa	Count	7	0	7
		% within uropathogen	100.0%	.0%	100.0%
		% within uti_status	14.9%	.0%	5.7%
		% of Total	5.7%	.0%	5.7%
	no growth	Count	0	76	76
		% within uropathogen	.0%	100.0%	100.0%
		% within uti_status	.0%	100.0%	61.8%
		% of Total	.0%	61.8%	61.8%
Total		Count	47	76	123
		% within uropathogen	38.2%	61.8%	100.0%

% within uti_status	100.0%	100.0%	100.0%
% of Total	38.2%	61.8%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.230E2 ^a	4	.000
Likelihood Ratio	163.612	4	.000
Linear-by-Linear Association	98.715	1	.000
N of Valid Cases	123		

a. 5 cells (50,0%) have expected count less than 5. The minimum expected count is 2,67.

Chi square for Homogeneity of proportions of E Coli isolates

Table A6 Homogeneity of proportions of all 8 drugs

antibiotic * e_coli Crosstabulation

			e_coli			Total
			sensitive	intermediate	resistant	
Antibiotic	ampicillin	Count	1	4	17	22
		% within antibiotic	4.5%	18.2%	77.3%	100.0%
		% within e_coli	.9%	30.8%	30.4%	12.5%
		% of Total	.6%	2.3%	9.7%	12.5%
	nitrofurantoin	Count	19	2	1	22
		% within antibiotic	86.4%	9.1%	4.5%	100.0%
		% within e_coli	17.8%	15.4%	1.8%	12.5%
		% of Total	10.8%	1.1%	.6%	12.5%
	nalidixic acid	Count	12	0	10	22
		% within antibiotic	54.5%	.0%	45.5%	100.0%
		% within e_coli	11.2%	.0%	17.9%	12.5%
		% of Total	6.8%	.0%	5.7%	12.5%

tetracycline	Count	12	5	5	22
	% within antibiotic	54.5%	22.7%	22.7%	100.0%
	% within e_coli	11.2%	38.5%	8.9%	12.5%
	% of Total	6.8%	2.8%	2.8%	12.5%
norfloxacin	Count	15	1	6	22
	% within antibiotic	68.2%	4.5%	27.3%	100.0%
	% within e_coli	14.0%	7.7%	10.7%	12.5%
	% of Total	8.5%	.6%	3.4%	12.5%
ciprofloxacin	Count	15	1	6	22
	% within antibiotic	68.2%	4.5%	27.3%	100.0%
	% within e_coli	14.0%	7.7%	10.7%	12.5%
	% of Total	8.5%	.6%	3.4%	12.5%
gentamycin	Count	17	0	5	22
	% within antibiotic	77.3%	.0%	22.7%	100.0%
	% within e_coli	15.9%	.0%	8.9%	12.5%
	% of Total	9.7%	.0%	2.8%	12.5%
kanamycin	Count	16	0	6	22
	% within antibiotic	72.7%	.0%	27.3%	100.0%
	% within e_coli	15.0%	.0%	10.7%	12.5%
	% of Total	9.1%	.0%	3.4%	12.5%
Total	Count	107	13	56	176
	% within antibiotic	60.8%	7.4%	31.8%	100.0%
	% within e_coli	100.0%	100.0%	100.0%	100.0%
	% of Total	60.8%	7.4%	31.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	54.199 ^a	14	.000
Likelihood Ratio	62.180	14	.000
Linear-by-Linear Association	9.783	1	.002
N of Valid Cases	176		

- a. 8 cells (33,3%) have expected count less than 5. The minimum expected count is 1,63.

Table A7 Homogeneity of proportions of the 5 best drugs

			antibiotic * e_coli Crosstabulation			Total
			e_coli			
			Sensitive	intermediate	resistant	
Antibiotic	nitrofurantoin	Count	19	2	1	22
		% within antibiotic	86.4%	9.1%	4.5%	100.0%
		% within e_coli	23.2%	50.0%	4.2%	20.0%
		% of Total	17.3%	1.8%	.9%	20.0%
	norfloxacin	Count	15	1	6	22
		% within antibiotic	68.2%	4.5%	27.3%	100.0%
		% within e_coli	18.3%	25.0%	25.0%	20.0%
		% of Total	13.6%	.9%	5.5%	20.0%
	ciprofloxacin	Count	15	1	6	22
		% within antibiotic	68.2%	4.5%	27.3%	100.0%
		% within e_coli	18.3%	25.0%	25.0%	20.0%
		% of Total	13.6%	.9%	5.5%	20.0%
	gentamycin	Count	17	0	5	22
		% within antibiotic	77.3%	.0%	22.7%	100.0%
		% within e_coli	20.7%	.0%	20.8%	20.0%
		% of Total	15.5%	.0%	4.5%	20.0%
kanamycin	Count	16	0	6	22	
	% within antibiotic	72.7%	.0%	27.3%	100.0%	
	% within e_coli	19.5%	.0%	25.0%	20.0%	
	% of Total	14.5%	.0%	5.5%	20.0%	
Total	Count	82	4	24	110	
	% within antibiotic	74.5%	3.6%	21.8%	100.0%	
	% within e_coli	100.0%	100.0%	100.0%	100.0%	
	% of Total	74.5%	3.6%	21.8%	100.0%	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.100 ^a	8	.424

Likelihood Ratio	10.532	8	.230
Linear-by-Linear Association	2.063	1	.151
N of Valid Cases	110		

a. 10 cells (66,7%) have expected count less than 5. The minimum expected count is ,80.

Chi square for Homogineity of proportions of Klebsiella spp isolates

Table A8 Homogineity of proportions of 4 best drugs

antibiotic * klebsiella_spp Crosstabulation

			klebsiella_spp			Total
			sensitive	intermidiate	resistant	
Antibiotic	nitrofurantoin	Count	4	1	3	8
		% within antibiotic	50.0%	12.5%	37.5%	100.0%
		% within klebsiella_spp	19.0%	100.0%	33.3%	25.8%
		% of Total	12.9%	3.2%	9.7%	25.8%
	nalidixic acid	Count	4	0	4	8
		% within antibiotic	50.0%	.0%	50.0%	100.0%
		% within klebsiella_spp	19.0%	.0%	44.4%	25.8%
		% of Total	12.9%	.0%	12.9%	25.8%
	gentamycin	Count	6	0	2	8
		% within antibiotic	75.0%	.0%	25.0%	100.0%
		% within klebsiella_spp	28.6%	.0%	22.2%	25.8%
		% of Total	19.4%	.0%	6.5%	25.8%
	kanamycin	Count	7	0	0	7
		% within antibiotic	100.0%	.0%	.0%	100.0%
		% within klebsiella_spp	33.3%	.0%	.0%	22.6%
		% of Total	22.6%	.0%	.0%	22.6%
Total		Count	21	1	9	31
		% within antibiotic	67.7%	3.2%	29.0%	100.0%
		% within klebsiella_spp	100.0%	100.0%	100.0%	100.0%
		% of Total	67.7%	3.2%	29.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.242 ^a	6	.221
Likelihood Ratio	9.810	6	.133
Linear-by-Linear Association	4.393	1	.036
N of Valid Cases	31		

a. 9 cells (75,0%) have expected count less than 5. The minimum expected count is ,23.

Table A9 Homogeneity of proportions of all drugs

antibiotic * klebsiella_spp Crosstabulation

			klebsiella_spp			Total
			sensitive	intermediate	resistant	
Antibiotic	ampicillin	Count	1	0	7	8
		% within antibiotic	12.5%	.0%	87.5%	100.0%
		% within klebsiella_spp	3.4%	.0%	26.9%	12.5%
		% of Total	1.6%	.0%	10.9%	12.5%
	nitrofurantoin	Count	4	1	3	8
		% within antibiotic	50.0%	12.5%	37.5%	100.0%
		% within klebsiella_spp	13.8%	11.1%	11.5%	12.5%
		% of Total	6.2%	1.6%	4.7%	12.5%
	nalidixic acid	Count	4	0	4	8
		% within antibiotic	50.0%	.0%	50.0%	100.0%
		% within klebsiella_spp	13.8%	.0%	15.4%	12.5%
		% of Total	6.2%	.0%	6.2%	12.5%
	tetracycline	Count	3	2	3	8
		% within antibiotic	37.5%	25.0%	37.5%	100.0%
		% within klebsiella_spp	10.3%	22.2%	11.5%	12.5%
		% of Total	4.7%	3.1%	4.7%	12.5%
	norfloxacin	Count	1	3	4	8
		% within antibiotic	12.5%	37.5%	50.0%	100.0%
		% within klebsiella_spp	3.4%	33.3%	15.4%	12.5%
		% of Total	1.6%	4.7%	6.2%	12.5%
	ciprofloxacin	Count	3	2	3	8
		% within antibiotic	37.5%	25.0%	37.5%	100.0%
		% within klebsiella_spp	10.3%	22.2%	11.5%	12.5%
		% of Total	4.7%	3.1%	4.7%	12.5%

gentamycin	Count	6	0	2	8
	% within antibiotic	75.0%	.0%	25.0%	100.0%
	% within klebsiella_spp	20.7%	.0%	7.7%	12.5%
	% of Total	9.4%	.0%	3.1%	12.5%
kanamycin	Count	7	1	0	8
	% within antibiotic	87.5%	12.5%	.0%	100.0%
	% within klebsiella_spp	24.1%	11.1%	.0%	12.5%
	% of Total	10.9%	1.6%	.0%	12.5%
Total	Count	29	9	26	64
	% within antibiotic	45.3%	14.1%	40.6%	100.0%
	% within klebsiella_spp	100.0%	100.0%	100.0%	100.0%
	% of Total	45.3%	14.1%	40.6%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	25.144 ^a	14	.033
Likelihood Ratio	30.110	14	.007
Linear-by-Linear Association	9.065	1	.003
N of Valid Cases	64		

a. 24 cells (100,0%) have expected count less than 5. The minimum expected count is 1,13.

Table A10 Homogeneity of proportions of 2 least performing drugs

antibiotic * klebsiella_spp Crosstabulation

			klebsiella_spp			Total
			sensitive	intermediate	resistant	
Antibiotic	ampicillin	Count	1	0	7	8
		% within antibiotic	12.5%	.0%	87.5%	100.0%
		% within klebsiella_spp	50.0%	.0%	63.6%	50.0%
		% of Total	6.2%	.0%	43.8%	50.0%
	norfloxacin	Count	1	3	4	8
		% within antibiotic	12.5%	37.5%	50.0%	100.0%
		% within klebsiella_spp	50.0%	100.0%	36.4%	50.0%
		% of Total	6.2%	18.8%	25.0%	50.0%

Total	Count	2	3	11	16
	% within antibiotic	12.5%	18.8%	68.8%	100.0%
	% within klebsiella_spp	100.0%	100.0%	100.0%	100.0%
	% of Total	12.5%	18.8%	68.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.818 ^a	2	.148
Likelihood Ratio	4.988	2	.083
Linear-by-Linear Association	1.063	1	.303
N of Valid Cases	16		

a. 4 cells (66,7%) have expected count less than 5. The minimum expected count is 1,00.

Chi square for Homogeneity of proportions of P. aureginosa isolates

Table A11 Homogeneity of proportions of all drugs

antibiotic * p_aureginosa Crosstabulation

			p_aureginosa			Total
			sensitive	intermediate	resistant	
Antibiotic	ampicillin	Count	0	0	7	7
		% within antibiotic	.0%	.0%	100.0%	100.0%
		% within p_aureginosa	.0%	.0%	28.0%	12.5%
		% of Total	.0%	.0%	12.5%	12.5%
	nitrofurantoin	Count	5	0	2	7
		% within antibiotic	71.4%	.0%	28.6%	100.0%
		% within p_aureginosa	20.0%	.0%	8.0%	12.5%
		% of Total	8.9%	.0%	3.6%	12.5%
	nalidixic acid	Count	1	0	6	7
		% within antibiotic	14.3%	.0%	85.7%	100.0%
		% within p_aureginosa	4.0%	.0%	24.0%	12.5%
		% of Total	1.8%	.0%	10.7%	12.5%
	tetracycline	Count	4	1	2	7
		% within antibiotic	57.1%	14.3%	28.6%	100.0%

	% within p_aureginosa	16.0%	16.7%	8.0%	12.5%
	% of Total	7.1%	1.8%	3.6%	12.5%
norfloxacin	Count	5	0	2	7
	% within antibiotic	71.4%	.0%	28.6%	100.0%
	% within p_aureginosa	20.0%	.0%	8.0%	12.5%
	% of Total	8.9%	.0%	3.6%	12.5%
ciprofloxacin	Count	3	2	2	7
	% within antibiotic	42.9%	28.6%	28.6%	100.0%
	% within p_aureginosa	12.0%	33.3%	8.0%	12.5%
	% of Total	5.4%	3.6%	3.6%	12.5%
gentamycin	Count	3	1	3	7
	% within antibiotic	42.9%	14.3%	42.9%	100.0%
	% within p_aureginosa	12.0%	16.7%	12.0%	12.5%
	% of Total	5.4%	1.8%	5.4%	12.5%
kanamycin	Count	4	2	1	7
	% within antibiotic	57.1%	28.6%	14.3%	100.0%
	% within p_aureginosa	16.0%	33.3%	4.0%	12.5%
	% of Total	7.1%	3.6%	1.8%	12.5%
Total	Count	25	6	25	56
	% within antibiotic	44.6%	10.7%	44.6%	100.0%
	% within p_aureginosa	100.0%	100.0%	100.0%	100.0%
	% of Total	44.6%	10.7%	44.6%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	25.173 ^a	14	.033
Likelihood Ratio	29.033	14	.010
Linear-by-Linear Association	5.122	1	.024
N of Valid Cases	56		

a. 24 cells (100,0%) have expected count less than 5. The minimum expected count is ,75.

Table A12 Homogeneity of proportions of 4 best drugs

antibiotic * p_aureginosa Crosstabulation

			p_aureginosa			Total
			sensitive	intermediate	resistant	
Antibiotic	nitrofurantoin	Count	5	0	2	7
		% within antibiotic	71.4%	.0%	28.6%	100.0%
		% within p_aureginosa	27.8%	.0%	28.6%	25.0%
		% of Total	17.9%	.0%	7.1%	25.0%
	tetracycline	Count	4	1	2	7
		% within antibiotic	57.1%	14.3%	28.6%	100.0%
		% within p_aureginosa	22.2%	33.3%	28.6%	25.0%
		% of Total	14.3%	3.6%	7.1%	25.0%
	norfloxacin	Count	5	0	2	7
		% within antibiotic	71.4%	.0%	28.6%	100.0%
		% within p_aureginosa	27.8%	.0%	28.6%	25.0%
		% of Total	17.9%	.0%	7.1%	25.0%
kanamycin	Count	4	2	1	7	
	% within antibiotic	57.1%	28.6%	14.3%	100.0%	
	% within p_aureginosa	22.2%	66.7%	14.3%	25.0%	
	% of Total	14.3%	7.1%	3.6%	25.0%	
Total	Count	18	3	7	28	
	% within antibiotic	64.3%	10.7%	25.0%	100.0%	
	% within p_aureginosa	100.0%	100.0%	100.0%	100.0%	
	% of Total	64.3%	10.7%	25.0%	100.0%	

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.317 ^a	6	.634
Likelihood Ratio	5.205	6	.518
Linear-by-Linear Association	.006	1	.940
N of Valid Cases	28		

a. 12 cells (100,0%) have expected count less than 5. The minimum expected count is ,75.

Table A13 Homogeneity of proportions of 2 the least performing drugs

antibiotic * p_aureginosa Crosstabulation

			p_aureginosa			Total
			sensitive	intermediate	resistant	
Antibiotic	ampicillin	Count	0	0	7	7
		% within antibiotic	.0%	.0%	100.0%	100.0%
		% within p_aureginosa	.0%	.0%	58.3%	50.0%
		% of Total	.0%	.0%	50.0%	50.0%
	nalidixic acid	Count	1	1	5	7
		% within antibiotic	14.3%	14.3%	71.4%	100.0%
		% within p_aureginosa	100.0%	100.0%	41.7%	50.0%
		% of Total	7.1%	7.1%	35.7%	50.0%
Total		Count	1	1	12	14
		% within antibiotic	7.1%	7.1%	85.7%	100.0%
		% within p_aureginosa	100.0%	100.0%	100.0%	100.0%
		% of Total	7.1%	7.1%	85.7%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.333 ^a	2	.311
Likelihood Ratio	3.107	2	.211
Linear-by-Linear Association	1.918	1	.166
N of Valid Cases	14		

a. 4 cells (66,7%) have expected count less than 5. The minimum expected count is ,50.

Chi square for Homogeneity of proportions of S. aureus isolates

Table A14 Homogeneity of proportions of all drugs

antibiotic * s_aureus Crosstabulation

			s_aureus			Total
			sensitive	intermediate	resistant	
antibiotic	ampicillin	Count	2	0	8	10
		% within antibiotic	20.0%	.0%	80.0%	100.0%
		% within s_aureus	5.6%	.0%	25.8%	12.5%
		% of Total	2.5%	.0%	10.0%	12.5%
	nitrofurantoin	Count	9	1	0	10
		% within antibiotic	90.0%	10.0%	.0%	100.0%
		% within s_aureus	25.0%	7.7%	.0%	12.5%
		% of Total	11.2%	1.2%	.0%	12.5%
	nalidixic acid	Count	2	4	4	10
		% within antibiotic	20.0%	40.0%	40.0%	100.0%
		% within s_aureus	5.6%	30.8%	12.9%	12.5%
		% of Total	2.5%	5.0%	5.0%	12.5%
	tetracycline	Count	3	2	5	10
		% within antibiotic	30.0%	20.0%	50.0%	100.0%
		% within s_aureus	8.3%	15.4%	16.1%	12.5%
		% of Total	3.8%	2.5%	6.2%	12.5%
	norfloxacin	Count	5	1	4	10
		% within antibiotic	50.0%	10.0%	40.0%	100.0%
		% within s_aureus	13.9%	7.7%	12.9%	12.5%
		% of Total	6.2%	1.2%	5.0%	12.5%
	ciprofloxacin	Count	6	2	2	10
		% within antibiotic	60.0%	20.0%	20.0%	100.0%
		% within s_aureus	16.7%	15.4%	6.5%	12.5%
		% of Total	7.5%	2.5%	2.5%	12.5%
	gentamycin	Count	5	2	3	10
		% within antibiotic	50.0%	20.0%	30.0%	100.0%
		% within s_aureus	13.9%	15.4%	9.7%	12.5%
		% of Total	6.2%	2.5%	3.8%	12.5%
	kanamycin	Count	4	1	5	10
		% within antibiotic	40.0%	10.0%	50.0%	100.0%
		% within s_aureus	11.1%	7.7%	16.1%	12.5%
		% of Total	5.0%	1.2%	6.2%	12.5%
Total		Count	36	13	31	80

% within antibiotic	45.0%	16.2%	38.8%	100.0%
% within s_aureus	100.0%	100.0%	100.0%	100.0%
% of Total	45.0%	16.2%	38.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	24.554 ^a	14	.039
Likelihood Ratio	27.981	14	.014
Linear-by-Linear Association	.311	1	.577
N of Valid Cases	80		

a. 24 cells (100,0%) have expected count less than 5. The minimum expected count is 1,63.

Table A15 Homogeneity of proportions of 4 best drugs

antibiotic * s_aureus Crosstabulation

			s_aureus			Total
			sensitive	intermediate	resistant	
antibiotic	nitrofurantoin	Count	9	1	0	10
		% within antibiotic	90.0%	10.0%	.0%	100.0%
		% within s_aureus	36.0%	16.7%	.0%	25.0%
		% of Total	22.5%	2.5%	.0%	25.0%
	norfloxacin	Count	5	1	4	10
		% within antibiotic	50.0%	10.0%	40.0%	100.0%
		% within s_aureus	20.0%	16.7%	44.4%	25.0%
		% of Total	12.5%	2.5%	10.0%	25.0%
	ciprofloxacin	Count	6	2	2	10
		% within antibiotic	60.0%	20.0%	20.0%	100.0%
		% within s_aureus	24.0%	33.3%	22.2%	25.0%
		% of Total	15.0%	5.0%	5.0%	25.0%
	gentamycin	Count	5	2	3	10
		% within antibiotic	50.0%	20.0%	30.0%	100.0%
		% within s_aureus	20.0%	33.3%	33.3%	25.0%

	% of Total	12.5%	5.0%	7.5%	25.0%
Total	Count	25	6	9	40
	% within antibiotic	62.5%	15.0%	22.5%	100.0%
	% within s_aureus	100.0%	100.0%	100.0%	100.0%
	% of Total	62.5%	15.0%	22.5%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.276 ^a	6	.393
Likelihood Ratio	8.148	6	.227
Linear-by-Linear Association	3.644	1	.056
N of Valid Cases	40		

a. 8 cells (66,7%) have expected count less than 5. The minimum expected count is 1,50.

Table A16 Homogeneity of proportions of 4 least performing drugs

antibiotic * s_aureus Crosstabulation

			s_aureus			Total
			sensitive	intermediate	resistant	
antibiotic	ampicillin	Count	2	0	8	10
		% within antibiotic	20.0%	.0%	80.0%	100.0%
		% within s_aureus	18.2%	.0%	36.4%	25.0%
		% of Total	5.0%	.0%	20.0%	25.0%
	nalidixic acid	Count	2	4	4	10
		% within antibiotic	20.0%	40.0%	40.0%	100.0%
		% within s_aureus	18.2%	57.1%	18.2%	25.0%
		% of Total	5.0%	10.0%	10.0%	25.0%
	tetracycline	Count	3	2	5	10
		% within antibiotic	30.0%	20.0%	50.0%	100.0%
		% within s_aureus	27.3%	28.6%	22.7%	25.0%
		% of Total	7.5%	5.0%	12.5%	25.0%
	kanamycin	Count	4	1	5	10

	% within antibiotic	40.0%	10.0%	50.0%	100.0%
	% within s_aureus	36.4%	14.3%	22.7%	25.0%
	% of Total	10.0%	2.5%	12.5%	25.0%
Total	Count	11	7	22	40
	% within antibiotic	27.5%	17.5%	55.0%	100.0%
	% within s_aureus	100.0%	100.0%	100.0%	100.0%
	% of Total	27.5%	17.5%	55.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.636 ^a	6	.266
Likelihood Ratio	8.542	6	.201
Linear-by-Linear Association	1.281	1	.258
N of Valid Cases	40		

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.636 ^a	6	.266
Likelihood Ratio	8.542	6	.201
Linear-by-Linear Association	1.281	1	.258

- a. 8 cells (66,7%) have expected count less than 5. The minimum expected count is 1,75.

APPENDIX B

PREPARATION OF CLED (BEVIS) MEDIUM (CYSTEIN LACTOSE ELECTROLYTE DEFICIENT)

This is a differential medium for the enumeration of urinary tract pathogens.

Formulation	Gram/Litre
Balanced peptone No.1	4.0
Beef extract	3.0
Tryptone	4.0
Lactose	10.0
L-cysteine	0.128
Bromothymol blue indicator	0.02
Andrade's indicator	0.08
Agar No.1	15.0

Directions

1. Weigh 36 grams of powder to a conical flask; disperse in 1 litre of deionised water.
2. Allow soaking for 10 minutes; swirl to mix.
3. Sterilize by autoclaving for 15 minutes at 121oC.
4. Cool to 47oC and mix well before pouring to the sterile petri plates.

PREPARATION OF MUELLER HINTON AGAR

It is used for determination of susceptibility of microorganisms to antimicrobial agents.

Ingredients	Grams/Litre
Beef infusion	300.0
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.0

Directions

1. Suspend 38.0 Grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121o C) for 15 minutes.
4. Mix well before pouring onto sterile petri plates.