IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF URINARY TRACT INFECTIONS (UTIs) IN PATIENTS PRESENTING AT ZVISHAVANE DISTRICT HOSPITAL

BY

BELOVED S. NYAKUNHUWA

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Department of Applied Biosciences and Biotechnology Faculty of Science and Technology
Midlands State University

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ABSRACT

Urinary tract infections (UTIs) are the most common type of infections encountered in medical practice today. Effective treatment of these infections is being hindered by antibiotic resistance of UTI pathogens. Prevalence of UTIs differs with geographical location, seasons, and gender. Therefore, it is important to isolate and identify the uro-pathogens causing UTIs in an area and determine their antibiotic susceptibility patterns. In this study, a total of 92 mid-stream urine samples from patients who were presenting at the Zvishavane District Hospital (ZDH), were examined for UTIs using standard microbiological techniques during the period May 2017January 2018. Of the 92 samples examined 12 (13%), comprising nine from females and three from males, were infected with UTIs. *Escherichia coli* was the most prevalent uro-pathogen isolated comprising 58% of the isolates, followed by *Klebsiella spp* (17%) while three other isolates, *S. saprophyticus, S. aureus*, and *P.aeruginosa* had the same prevalence of 8%. Chloramphenicol was the most effective antibiotic being effective against 10 out of the 12 (83%) isolated uro-pathogens, followed by Gentamicin which was effective against 8 out of 12 (67%). Fusidic acid was the least effective antibiotic because all isolates (100%) were resistant to it. Only 2 out of 12 isolates were sensitive to Nalidixic acid. Five out of 12 isolates were resistant to the drug combination of Nalidixic acid and Fusidic acid, (resistant pattern or antibiotype Nalidixic Acid^{R} Fusidic Acid^{R}), suggesting that, this treatment combination should not be prescribed for UTIs. Chloramphenicol and Gentamicin were equally effective against most isolates because there was no significant difference in the proportion of the isolates that were sensitive to both of them (Chisquare, $p = 0.001$). The take home message from this study is that the most common UTIcausing pathogen in patients presenting at ZDH was *E. coli* and most pathogens were resistant to Nalidixic acid and Fusidic acid but sensitive to Gentamicin and Chloramphenicol. Therefore, Gentamicin and Chloramphenicol are recommended for UTIs treatment. A relatively low prevalence of 13% suggests that UTIs are not a risk to the residents of Zvishavane.

DECLARATION FORM

I, **Beloved S. Nyakunhuwa**, do hereby declare that I am the sole author of this dissertation. I, therefore authorize Midlands State University to lend this dissertation to other institutions or individuals for the purpose of scholarly research.

Signature ……

Date ……

APPROVAL

This dissertation with title, ―Identifications and antibiotic Susceptibility patterns of Urinary Tract infections in patients presenting at the Zvishavane District Hospital‖ by **Beloved S. Nyakunhuwa,** meets the regulations governing the award of the degree of Applied Biosciences and Biotechnology of the Midlands State University, and is approved for its contribution to knowledge and literal presentation.

Supervisor.1 …… Supervisors 2……...

Date ……. Date…… ……

DEDICATION

To: my mom, (Etheli Nyakunhuwa), my dad, (Obedience Nyakunhuwa), my little brother, my sisters and my Christian family.

ACKNOWLEDGEMENTS

I would like to acknowledge the grace of God that took me this far. Had it not been for the Lord, I would not have managed at all. My deepest gratitude goes to my supervisors, Drs T. Muteveri and M. Muteveri for their hammering, guidance, support and patience, which brought everything unto perfection. Without them it would not to be possible. I am also grateful to the Zvishavane District Hospital Laboratory members, especially Mr B. Mudzingwa (Laboratory Head) and Mr T. Tinarwo (quality officer) for their support and permission grant, allowing me to carry out my experiment at their Laboratory using their resources, making the project a success, which I would not have funded myself. Also worth of appreciation is the KAHIM family, whom by their prayers and support made it a better path to walk on. I want to give thanks to my dad for the financial support, taking pride in me ensuring that I get the best out of the education system. Lastly, but not least, I want to also acknowledge every member of my family for their moral support and love, I found the strength to go on because of them. May God be praised.

Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 INTRODUCTION

1.1 Background

Urinary Tract Infections (UTIs) are microbial infections that affect different parts of the urinary tract. UTIs are caused by bacteria or, in rare cases, fungi, in the gastro-intestinal tract that colonize the periurethral area. Gram negative bacteria such as *Escherichia coli*, *Proteus* species, *Klebsiella* species, *Enterobacter* species, *Serratia* species and *Pseudomonas* species are usually detected in recurrent infections (Ogbukagu *et al*., 2016). Other bacterial pathogens also frequently associated with UTIs include *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis.* However the most common causative agent is *Escherichia coli*, a bacterium commonly absent in normal urine, such that, its presence would mean an infection (Dromigyn, 2002).

Urinary tract infections are the most common causes of morbidity and mortality in the hospital environment (Ogbukagu et al, 2016), meaning that it is important to find effective strategies to cure them. The prevalence of UTIs depends on the geographical location (Eriksson, *et al*, 2013). Due to poor hygiene and poverty, the UTIs are more prevalent in developing countries than the developed world (Foxman and Brown, 2003).

The identification of UTIs is done by following the gold standard methods which involve examination of the mid-stream urine sample by urine microscopy and culture Finch *et al*. (2006).

The presence of UTIs is confirmed by the presence of $>10^5$ microorganisms of a single strain of bacterium per milliliter in two consecutive midstream samples of urine (Loh and Sivalingam, 2007). The world is currently faced with widespread antibiotic resistance including resistance to drugs used for treating UTIs. Effective control of UTIs requires identification of antibiotics that are effective against UTIs (Habib, 2012).

UTIs are more prevalent in females than in males most probably owing to differences in anatomy. The urethra of men is much longer and the distance between the anus and urethral meatus is greater than that of women (Haider, *et al.*, 2010). After the post-infancy drop, the prevalence of urinary tract infection increases with age becoming higher in women by about five to twenty percent compared to that of men which ranges from 0.1 to 10% (Kunin, 1997).

1.2 Problem statement

Most serious UTIs cause significant economic burdens and morbidity especially in the adults because of different factors. Increasing multiple drug resistance in bacterial uro-pathogens is an important and emerging public health problem that requires regular monitoring of antibiotic susceptibility of the uro-pathogens in a particular area. To date the antibiotic resistance pattern of the uro-pathogens at Zvishavane District Hospital has not been documented. This knowledge gap is posing challenges for physicians because effective and efficient treatment of UTIs depends mostly on knowledge of bacterial spectrum and their susceptibility profile. Additionally, some antibiotics only have marginal activity against the given uro-pathogens resulting in a compromise or reduction of the effectiveness of other drugs in the same class or in other classes.

1.3 Justification

Since the bacterial spectrum and antibiotic sensitivity of UTIs differ with geographical location, a local hospital based study of bacterial spectrum and antibiotic sensitivity is important for effective treatment of UTIs. Therefore, this study seeks to justify the practice by identifying UTIs and finding their antibiotic susceptibility tests of patients presenting at this hospital. As the foundation of treatment of serious UTIs is by the use of the most effective antibacterial chemotherapy, an on-going surveillance is needed to minimize chances of antibiotic resistance. This will help to monitor the trends allowing for the recommendation of the most effective empiric regimens treatment.

Therefore, the use of the targeted therapy involving the antibacterial spectrum is most likely to reduce morbidity, emergence of resistance, associated cost and helps maintain the efficacy class (Foxman, 2002). Information obtained in this study will help responsible authorities in formulating policies and public awareness campaigns that safeguard the public from such infections to reduce morbidity and economic costs for UTIs treatment as they can be prevented. The study will form the basis of further research in the particular study area which upcoming students at the Midlands State University may use as reference.

1. 4 Objectives

The main objective of this study is to identify the causative agents of urinary tract infections and determine their antibiotic susceptibility patterns among patients presenting at Zvishavane District Hospital.

1.5 Specific objectives

- To isolate and identify bacteria causing UTIs at ZDH
- To determine the common types of microbes causing UTIs in patients coming to the

Zvishavane District Hospital.

- To determine the effect of gender on UTIs.
- To determine the susceptibility patterns of the uro-pathogens to antibiotics commonly used by doctors

CHAPTER 2 LITERATURE REVIEW

2.1. Definition of urinary tract infections

A urinary tract is the pathway in which the urine passes through until it is expelled out of the body. The urinary tract serves to remove wastes as well as excess water from the human body (Sibi *et al.*, 2011). It is a structure consisting of the kidneys, the bladder, the ureters and the urethra. Each organ contributes in the making of the urine within the human body. For example, the kidney functions to filter blood, removing waste production alongside excess water passing the latter to the organ known as the ureters which serves to empty the urine into the bladder where it can be stored until it can be passed out of the body through the urethra, a process known as urination (Hertzberg *et al.*, 2018). The urinary tract can be divided into two, that is, the lower and upper urinary tract with the upper urinary tract consisting of the kidneys and the lower urinary tract consisting of the bladder as well as the urethra (Hertzberg *et al.*, 2018). Foxman and Brown, (2003) define urinary tract infections as an infection of any part of a system. However if the urethra is the one infected, the infections would be referred to as urethritis, if it is the bladder, cystitis, if the ureters, urethritis, and if it is the kidney it will be pyelonephritis (Barber *et al.*, 2016).

The urinary tract infections can be symptomatic or asymptomatic. By symptomatic, the symptoms of the infections would generally be clear however the UTIs are asymptomatic, usually termed asymptomatic bacteriuria (ASB) a positive urine culture would have been collected from a patient without symptoms of a UTI (Loh and Sivalingam, 2007). UTIs can be classified as complicated and uncomplicated UTIs. Uncomplicated UTIs known as cystitis in

women, were found to be having no anatomical and functional abnormalities of the urogenital tract.

This type does not exhibit signs of tissue invasion as well as systemic infection. However, in complicated UTI, it is possible to make a differentiation between UTI with systemic symptoms known as febrile UTIs. The febrile UTIs are uro-sepsis, pyelonephritis, and prostatitis (Geerlings *et al*, 2016). It was also noted that there is an increased risk for treatment failure in patients with complicated UTIs posing implications on the differentiation between the complicated and uncomplicated UTIs (Wolfson and Hooper, 1989). A host can be referred to as complicated when the host has an increased risk for complications. The following groups can be complicated host, i.e. men, pregnant women, immune-compromised patients, as well as those who have an anatomical or functional abnormality of the urogenital tract like that of the spinal cord-injury , renal kidney stones and urinary catheter (Llor and Bjerrum, 2014).

2.2. Pathogenesis of UTIs

Urinary Tract Infections are caused by wide range of microorganisms which typically multiply at the urethral opening and travel up to the bladder (Davis and Flood, 2002). Rarely, bacteria spread to the kidney from the bloodstream. The pathogenesis of uncomplicated urinary tract infection (UTI) is complex and influenced by many host biological and behavioral factors as well as by properties of the infecting uro-pathogens (Hooton, 2000: 2015). Therefore, pathogenesis of UTIs focuses on acute kidney injury, pyelonephritis, ascension, uro-epithelium penetrations and colonization. Acute pyelonephritis can lead to maternal sepsis and requires parenteral antibiotics (Hertzberg *et al.*, 2018). The pathogenicity of urinary tract infers that not all bacterial species are equally capable of inducing an infection as such the more compromised the patient’s natural defense mechanisms, the fewer the virulence needed by any bacterial strain to induce infection.

This is so because, some bacterial strains of a species are uniquely equipped with some specialized virulence factors.

For example, the different pilli type needed for the facilitation of ascension of bacteria from the fecal flora, periurethral area or the introitusvaginae up the urethra into the bladder (Davis and Flood, 2002). This can also allow the organisms to induce some systemic inflammation once they reach the kidneys. The organisms will then colonize, after the penetration of the uro-epithelium.

2.2.1 Etiology

The most members of these genera include some Extended- Broad Spectrum Î-Lactamaseproducing *Escherichia coli* and *Klebsiella pneumonea* and some *Enterobacter* as well as *Proteus* spp (Ogbukagu *et al.*, 2016). *E. coli* is regarded as the major etiological agent causing UTIs, accounting for up to 90% UTIs cases (Gunn and Davis, 1988). Enterococci and Urea-plasma urea-lyticum are also causative agents of UTIs although they are less common. Some UTIs causative agents are the gram positive organisms including the Group B *Streptococcus, Staphylococcus aureus, Staphylococcus saprophyticus and Staphylococcus haemolyticus* (Badri, 2017).

2.3 Epidemiology of UTIs

An estimate of 1-5% of healthy premenopausal women have got asymptomatic bacteriuria which increases to 4-19% in otherwise healthy elderly people of both sexes being about 0.7-27% in diabetics whilst it is about 2-10% in pregnant women, then about 15-50% in elderly populations at an institution, and 23-89% in spinal cord injury patients (Nicolle, 2000 ; Vosti, 1975). The asymptomatic bacteriuria is not common in younger men, however, when detected, it is encouraged that a chronic bacterial prostatitis must be considered (Oluwole and Victoria, 2016).

Moreover, the asymptomatic bateriuria spectrum is the same as that of species found in either the uncomplicated or complicated UTIs, although this is subject to whether or not there is a risk factor.

There are some neonatal complications which are associated with asymptomatic bacteriuria which include intrauterine growth restriction, low birth weight and pre-term premature rupture of membrane (Fadel *et al.*, 2004). Moreover, it has been found out that, some maternal complications which are associated with asymptomatic bacteriuria are hypertension, preeclampsia and maternal anemia (Bjork *et al.*, 2017). Without treatment, this condition leads to symptomatic cystitis in about 30% of pregnant mothers of whom about 50% will eventually develop acute pyelonephritis (Loh and Sivalingam, 2007).

In the non-obstructed and non-pregnant female adult, acute uncomplicated UTI is believed to be a benign illness with no long-term medical consequences (Delzell and Lefevre, 2000). However, UTI elevates the risk of pyelonephritis, premature delivery, and fetal mortality among pregnant women, and is associated with impaired renal function and end-stage renal disease among pediatric patients. Financially, the estimated annual cost of community-acquired UTI is significant, at approximately $1.6 billion (Ogbukagu *et al.*, 2016)

2.4 Risk factors associated with UTIs

Risk factors for UTIs can be classified into four groups. These include, the latrogenic, behavioral, anatomical and genetic factors (Alkhyat *et al.*, 2014). The latrogenic risk factors involve the use of antibiotics, now known as recent antimicrobial chemotherapy spermicides and some of the indwelling catheters all of which increase the chances of infections of the urinary tract. Behavioral risk factors involve, voiding dysfunction and frequent sexual intercourse. Foxman and Brown (2003), noted that, maternal history of UTI, young age at first UTI, as well as sexual

intercourse and spermicide use, are risk factors for recurrent UTI in young women. The anatomical or functional factors include the the vesicoureteral reflux, female sex as well as pregnancy (Badri and Mohammed, 2017). The majority of UTIs in women were found not to be associated with underlying functional or anatomical abnormalities of the urinary tract, a case in sexual intercourse, spermicide use, a history of recurrent UTI which are important risk factors (Burleigh *et al.*, 2013). UTIs can be influenced by some genetic factors like vaginal mucus properties, familial tendency and susceptible uro-epithelial cells. The association of recurrent UTI in certain age groups with the ABH blood group non-secretor phenotype, a maternal history of UTI and early age at onset of UTIs suggest a genetic predisposition (Badri and Mohammed, 2017). The virulence determinants of pathogens of the urinary tract are more important in the normal host than in the host who has a functional or anatomical abnormality of the genitourinary tract.

For some of young healthy women, it was found out that some features of pelvic anatomy appear to be associated with UTI risk. However, in postmenopausal women, anatomical and functional characteristics of the genitourinary tract are more strongly associated with UTI risk than in younger women (Hooton *et al.*, 1996).

2.5 Diagnosis of UTIs

The diagnosis of a UTI can be made based on a combination of symptoms and a positive urine analysis or culture with $> 10^5$ colony forming units (CFU)/ml of urine. It is known that the gold standard for diagnosing UTIs is running a urine culture and sensitivity test. Pyuria which is > 10 white blood cells per high power field (x400), in re-suspended sediment of a centrifuged aliquot of urine can be used for UTIs diagnosis. For routine assessment, a dipstick method, a leukocyte esterase test, and a nitrite reaction maybe used (Kibret and Abera, 2014).

2.6 Clinical presentation and Treatment options for UTIs

The clinical presentation of UTIs involves utheritis, cystisis, pyelonephritis, uro-sepsis and the male genetalia glands. The cystisis involves *E. coli* however these are often recurrent with susceptibility to standard antibiotics. It has been found out that *K. pneumonia* is associated with severe pyelonephritis characterized by high fever and vomiting.

This is often associated with urological diseases like the kidney stones due to *Klebsiella spp.* As for uro-sepsis, *Enterococcus spp,* that are sensitive to antibiotics are common causing severe uro-sepsin patients who use indwelling catheters (Ehlers and Merill, 2018).

Carbapenems are considered the most reliable treatment for infections caused by ESBLproducing bacteria. Despite their utility, resistance has emerged, placing a focus on finding alternative antibiotics for UTIs so that carbapenems can be reserved for more serious infections (Dominick and Beth, 2015). The complexity of these infections combined with a drastic rise in antibiotic resistant pathogens alarm the need for alternative therapies (Barber, *et al.,* 2016). There are other non-carbapenems which provide a potential alternative therapy. These include the aminoglycosides, quinolones and other bacteriostatic antibiotics which have proven to be better treatment options.

2.6.1 Antibiotic class and mode of action

2.6.1.1 Aminoglycoside

Gentamicin, a broad spectrum antibiotic falls under aminoglycosides (de Sousa *et al.*, 2013). To inhibit bacterial action, the antibiotic binds to the bacterial 30S ribosomal subunit, causing misreading of t-RNA. As such, the bacterium will be unable to synthesize proteins vital to its growth. Gentamicin can be primarily used in infections involving aerobic, gram-negative

bacteria, Acinetobacter, and Enterobacter (Eriksson *et al.*, 2013). It can also be used against gram-positive bacteria although it is less potent and more damaging to the host.

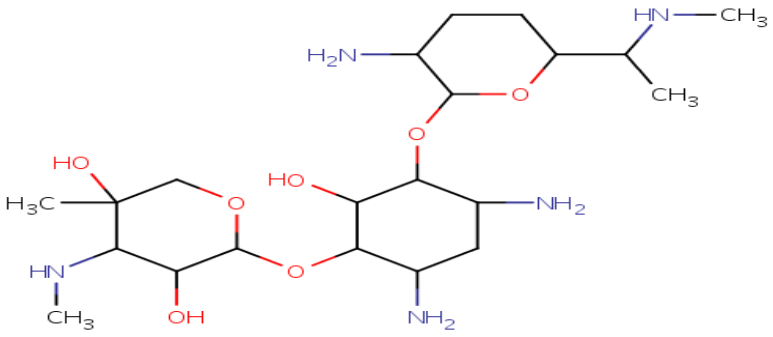


Fig 2. 1 Structure of Gentamicin

2.6.2 Bacteriostatic antibiotics

2.6.2.1 Chloramphenicol

Chloramphenicol is classified under the bacteriostatic drugs, it functions by binding to the 50S ribosomal subunit then inhibits the peptidyl-transferase step in protein synthesis (Rubin and Rajendra, 2012). Its resistance is by inactivating by chloramphenicol acetylate enzymes that acetylate chloramphenicol.

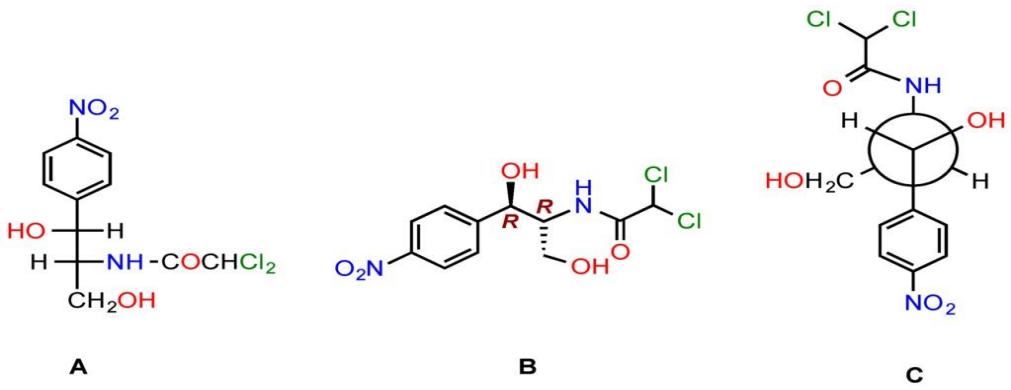


Fig 2. 2 Structure of chloramphenicol

2.7.2.2 Fusidic acid

Fusidic acid also falls under bacteriostatic antibiotics. The antibiotic is usually used for treatment against skin infections (Spelman, 1999). It functions by interfering with the elongation factor G (EF-G) by hydrolyzing GTP to GDP providing energy required for the translocation of peptidyl – tRNA from the A site to the P site on the 50S ribosomal subunit (Moorhouse *et al.*, 1996).

Hence, this causes protein synthesis inhibition.

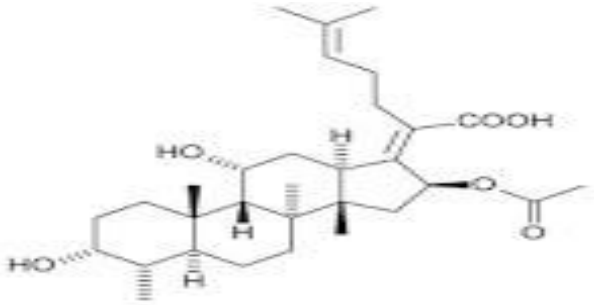


Fig 2. 3 Chemical Structure of Fusidic acid**.**

2.6.3Quinolones

Nalidixic acid is classified under quinolone antibiotics. It can be used in the treatment of uncomplicated UTIs caused by gram-negative microbes like *E. coli, Klebsiella spp and Proteus spp* (Röderova *et al.*, 2016) Its activity against gram-positive organisms like *P.aeruginosa*, is poor, it also lacks potency, has inadequate serum concentrations and has poor tissue distribution.

It functions by inhibiting the bacterial enzyme DNA gyrase.

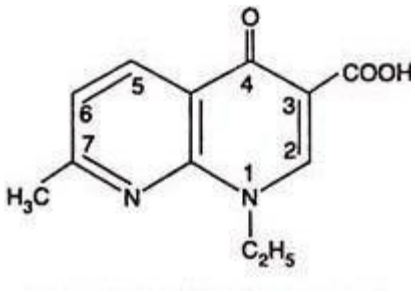


Fig 2. 4 Structure of Nalidixic Acid.

2.7 Multi Drug Resistance

As a result of the high empiric use of antibiotics for the treatment of UTI, there has been an emergence of antibacterial resistance by the Enterobacteriaceae (Sangamithra *et al.*, 2017). Theses gram negative Extended-Spectrum Beta Lactamase producing Enterobactreiaceae are an increasing concern in regards to antibiotic resistance since they have a potential cause of serious infections which are difficult to treat (Shaikh *et al.,* 2015). Once they survive treatment with an antibiotic, they can reproduce and create more resistant bacteria. The more often the antibiotics are used, the more the chances of resistance. UTIs are often incompletely resolved by antibiotic therapy leading to frequent recurrence. The uro-pathogenic bacteria go through a series of steps to achieve resistance i.e. invade, replicate, and persist within host epithelial cells (Barber *et al.*, 2013).

CHAPTER 3

MATERIALS AND METHODS

3.1. Study site

This study was done at Zvishavane District Hospital in Zvishavane, a small town that is in the Midlands Province of Zimbabwe. Zvishavane has got a population of about 42 300 people (Central Statistical Office, 2012). Analyses were done at the Zvishavane District Hospital Laboratory. The District hospital serves 11 clinics, which forward their samples for analysis to it.

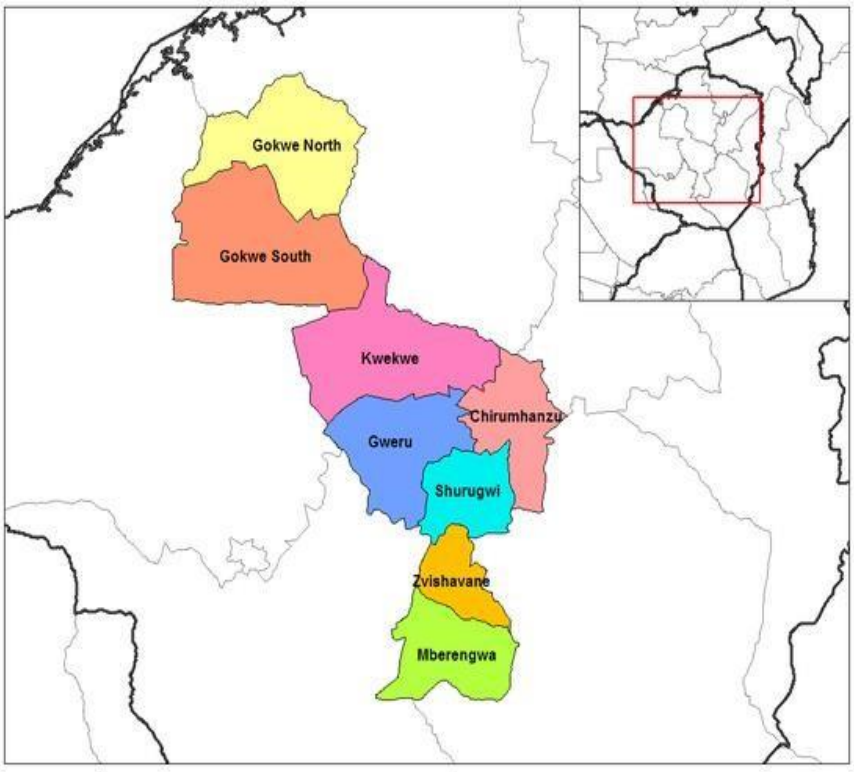


Fig 3. 1 . Location of Zvishavane town, (study site), in the Midlands Province of Zimbabwe.

3.2Laboratory analyses

3.2.1 Sample collection and primary procedures

Urine samples were collected using the mid-stream–clean-catch method as instructed by the physician. Samples were delivered to the laboratory soon after collection. Samples from other clinics were delivered to the laboratory in a vaccine carrier within a period of two hours. Upon arrival, the samples were recorded in the laboratory book, decoding the age and sex. Thereafter, a laboratory or sample number was allocated to the sample to avoid working with names and mixing up of the samples. Primarily, a urine chemistry test and a urine microscopy, which are screening tests used for information addition to the diagnosis, were done before proceeding.

3.2.2 Urine chemistry

Urine samples were tested for the abnormalities in their chemical composition using a dipstick method in which urine was transferred to a 10 ml plastic test tube to enable full immersion of the paper strip into the urine sample. Comparisons of the color changes on the strip and on the record on the strip container were used to identify presence of leucocytes or nitrites, which are not normally found in the urine infection, a primary indicator of UTIs.

3.2.3 Urine microscopy

The urine sample was put in an aliquot tube and centrifuged at 5000 rpm for five minutes to concentrate the organisms, if present, at the bottom of the test tube. The supernatant was discarded until a tiny drop of the sediment remained, which was taken for microscopy. The sediment drop was used to make a wet preparation which was examined under an electrical light microscope. The wet preparation was examined for budding yeasts which would clearly be seen as small round cells diving if present in the urine sample indicating an infection needing further examination processes. Microscopy was also used to determine leucocytes which were considered pyuric given that the leucocytes counted were 10 cells/mm^3 and above.

3.2.4 Bacterial identification

The collected urine samples in containers were shaken to re-suspending the organism present if any. A sterilized 1/500ml calibrated wire loop was used to inoculate a loopful of urine onto a shell fraction of the Blood Agar plate. The streaking method was used to spread the possible available organism. The technique involves sterilizing the wire loop, cooling the loop in a portion of the media, away from the inoculum, then make horizontal lines adjacent to each other. The loop was sterilized after use. The same procedure was repeated on the Cysteine-lactose-electrolyte-deficient media after the loop sterilization. The plates for each urine sample were placed in an incubator and incubated at 36^{o}C overnight for about 16-18 hour. The colony forming units were counted for significance, that is, if the bacterial concentration was above 10^5 colony forming units/ml of urine. Furthermore after growth, the bacterial colonies were confirmed by a gram status and further biochemical tests which included citrate tests, oxidase tests, coagulase test and indole tests.

3.2.5 Cultural observations

The incubated plates were observed for colony morphology. The plates were also observed for both the colony color and size.

3.2.6 Gram staining

The gram stain technique was done only on the cultures which had significant growth, i.e> 10^5 colony forming units (CFUs). A drop of saline was placed onto a labeled, clean glass slide, where, using a sterile wire loop, an individual colony was smeared onto the saline flooded portion of the glass slide. The slides were allowed to air dry and heat fixed by passing them through the flame thrice. The prepared slides were flooded with Gram's crystal violet (S012) and

left for a minute before being washed with water. Another stain known as Gram's Iodine (S013) was used to flood the smear on the slides and left for a minute.

Thereafter, the Gram's Iodine was washed off the slides using water and a Gram's decolorizer was flooded onto the smears on the slides and left for two minutes before being washed off using water. A counter stain known as 0.5% Safranin (S027), was flooded on to the slide, after a minute it was washed off using water and the slides were air dried and observed under a light microscope at 100X objective lenses under oil immersion.

3.2.7 Biochemical tests

3.2.7.1 Citrate test

This test is based on the ability of an organism to use citrate as its only source of carbon.

Simmons citrate agar was prepared in bijou bottles according to manufacturer's instructions. The slopes were then stabbed and incubated at 37^{o}C for 48 hours. The test was used to differentiate between *Klebsiella spp* from *E. coli* colonies. Bromothymol blue, a pH indicator, was added to the agar slant and a colour change was observed and noted. A blue colour change would indicate positivity.

3.2.7.2 Oxidase test

A portion of a colony from the test organism was smeared by the inoculating needle onto an oxidase disk. A color change was observed and noted after about 5-10 seconds.

3.2.7.3 Indole test

The bacteria were sub-cultured in nutrient broth and incubated for 24 hours, three drops of Kovac's indole reagent were added to 1% Tryptone broth and mixed gently. This test was used in organisms suspected to be *E. coli* and *Klebsiella spp*. A red colour indicates positivity.

3.2.7.4 Coagulase test

About two similar colonies of an isolate were emulsified in two drops of physiological saline. A loopful of citrated human plasma was added and examined after 2 minutes,

3.2.8 Antibiotic sensitivity tests

Sensitivity tests were done on Mueller Hinton Agar prepared and sterilized following the manufacturer's instructions. The medium was poured in 90mm diameter sterile petri dishes according to Clinical and laboratory standards institute (CLSI, 2015) and international guidelines. A control of a strain of *E. faecalis* (ATCC 2921) and co-trimoxazole disc was used for each new batch of the Mueller Hinton Agar. The zone of inhibition that was used as standard was a diameter of 20mm or more. The media was then stored in a laboratory freezer at a temperature range of 2-8^{o}C. About 3-4 colonies of similar colonies of each organism were emulsified into an aliquot tube containing 2ml physiological saline until a turbidity equivalent to that of 0.5 McFarland's solution was obtained. The standard barium sulfate that was equivalent to McFarland's solution was prepared by adding 1ml of concentrated sulfuric acid to 99ml of distilled water, mixed well to give 1% v/v solution of sulfuric acid, 99.4ml of which were mixed with 0.6ml of barium chloride solution and stored at a range of 20-28^{o}C.

Using a sterile swab, a plate of Mueller Hinton was inoculated and excess fluids were removed from the swab by pressing and rotating the swab against the sides of the aliquot tube above the level of suspension. The swab was streaked evenly over the surface of the medium in three directions rotating the plate at an angle that is approximately 60^{o}. Using sterile forceps and a multidisc dispenser, four different antibiotics, which include, Gentamicin, Nalidixic Acid,

Fusidic Acid and Chloramphenicol, were evenly distributed on the inoculated plate, however being 15mm from the plate edge. Lightly, the antibiotic discs were pressed down the media to ensure their contact with the agar. The lid was placed back onto the plate, inverted and incubated aerobically at 35^{o}C overnight for 16-18 h. After overnight incubation, the inhibition zone diameter was measured, in mm, from the underside of plate using a ruler.

3.8. Data analyses

The prevalence of the UTIs from different perspectives, i.e. overall, gender based and specific organism prevalence were calculated using the formula:

Prevalence = number of positive X 100%

÷ Total number of samples (N)

Susceptibility percentage of each isolate was calculated using the formula:

Susceptibility=Number of sensitive isolates X 100%÷ Total Number of isolates

Data from different antibiotics were analyzed using the chi-square test for proportions in SPSS to test for the proportion of sensitive isolates across drugs. A statistical significance at a 95% confidence interval, a $p< 0.05$ was considered. One sample t-tests were performed in the Rsoftware to test whether the antibiotic zone of inhibition exceeded the threshold level or not for each antibiotic. Therefore, to determine whether the isolates were sensitive/not sensitive to the given antibiotic, the following hypotheses were tested against the CLSI threshold:

Ho: $\mu_1 \geq \mu_o$

Ha: $\mu_1 < \mu_o$, where μ_o =the threshold disc diffusion diameter and μ_1 is the recorded disc diffusion diameter.

CHAPTER 4 RESULTS

4.1 Identification of UTIs

From a total of 92 samples, comprising 61 from females and 31 from males, received from patients who were presenting at the Zvishavane District Hospital, only 12, 3 from males and 9 from females, were positive for UTIs (**Table 4.1)**

Table 4. 1 Distribution of UTIs amongst patients presenting at Zvishavane District Hospital

Type	Male		Female		Overall	
	Number	**%**	Number	%	Number	%
UTI	3	3.3	9	9.8	12	13.1
Non-UTI	28	30.4	52	56.5	80	86.9
Total	31	33.7	61	66.3	92	100

According to gender, 75% of the isolates were from females whilst 25% were from the males (**Fig 4.1**).

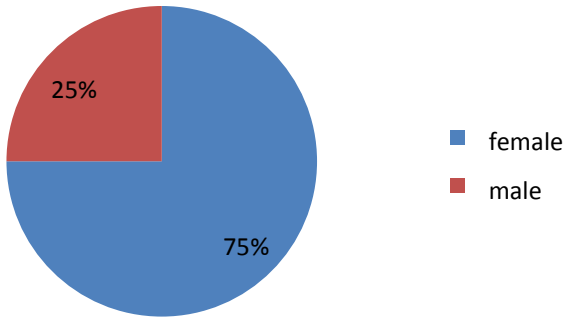


Fig 4.1 Proportions of female to male prevalence of UTIs.

4.2 Identification of etiological agents isolated from patients presenting with UTIs at the Zvishavane District Hospital

From the 12 urine samples positive for UTIs, five different types of etiological agents were isolated. *Escherichia coli* was the most common with a frequency of 58.3% followed by *Klebsiella spp* (16.7 %) and the other three etiological agents, *S. aureus, S. saprophyticus and P. aeruginosa*, had the same percentage frequency of 8.3% (**Table 4.2**). Microscopy was done to determine the yeasts however; no yeast cells were present in the samples.

Table 4. 2. Pathogens isolated from patients presenting with UTIs at the Zvishavane District Hospital

Microorganism	Number of isolates	Percentage (%)
Gram negatives		
E. coli	7	58.3
Klebsiella spp	2	16.7
P.aeruginosa	1	8.3
Gram positives		
S. saprophyticus	1	8.3
S. aureus	1	8.3
Total	**12**	**100**

TABLE 4. 3 Susceptibility profiles of isolates to the four Antibiotics

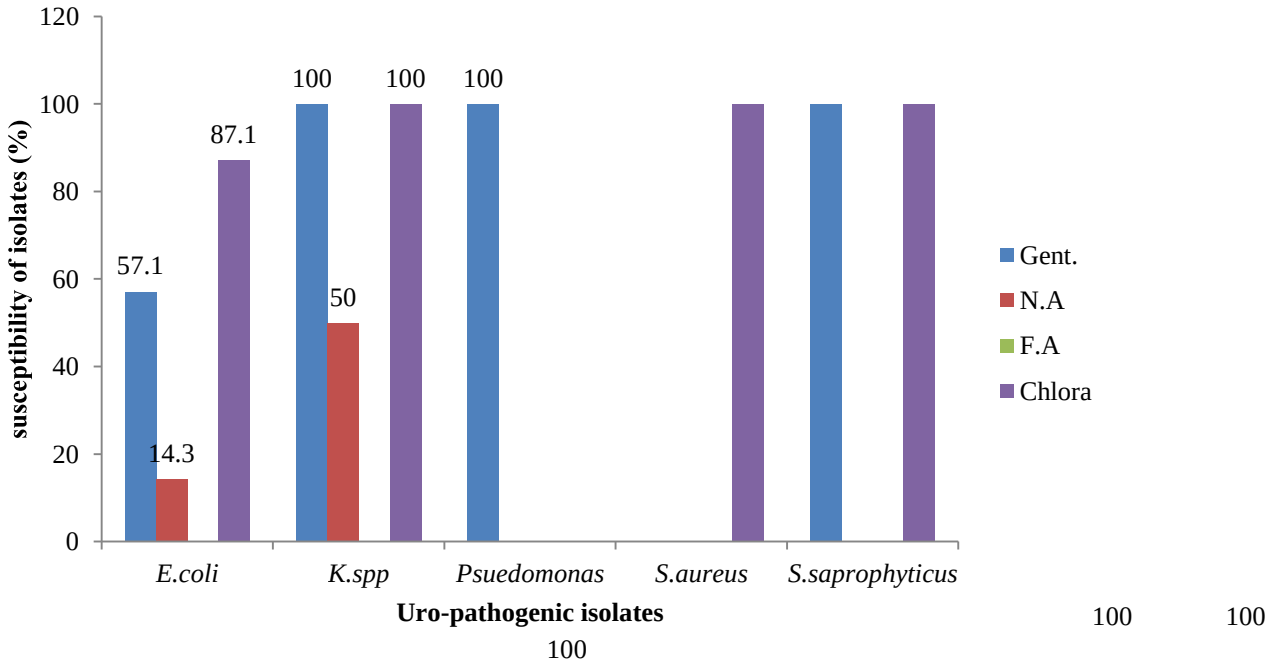
BACTERIAL ISOLATE	TOTAL	PATTERN	GENTAM Number	GENTAM %	N.ACID Number	N.ACID %	F.ACID Number	F.ACID %	CHLORA number	CHLORA %
		S	4	**57.1**	1	**14.3**	0	**0**	6	**85.7**
E. coli	**7**	**I**	0	**0**	0	**0**	0	**0**	1	**14.3**
		R	3	**42.9**	6	**85.7**	7	**100**	0	**0**
		S	2	**100**	1	**50**	0	**0**	2	**100**
Klebsiella spp	2	**I**	0	**0**	1	**50**	0	**0**	0	**0**
		R	0	**0**	0	**0**	2	**100**	0	**0**
S. saprophyticus	1	**S**	1	**100**	0	**100**	0	**0**	1	**100**
		I	0	**0**	0	**0**	0	**0**	0	**0**
		R	0	**0**	1	**0**	1	**100**	0	**0**
S. aureus		1	**S**	0	**0**	0	**0**	0	**0**	1	**100**	**I**
		1	**100**	0	**0**	0	**0**	0	**0**			
		R		0	**0**	1	**100**	1	**100**	0	**0**
P.aeruginosa	1	**S**		1	**100**	0	**0**	0	**0**	0	**0**
		I		0	**0**	0	**0**	0	**0**	0	**0**
		R		0	**0**	1	**100**	1	**100**	1	**100**

Key: GENTAM= GENTAMYCIN**, N.ACID=** NALIDIXIC ACID**, F.ACID** = FUSIDIC ACID**, CHLORA =** CHLORAMPHEMICOL, **S**= SENSITIVE, **I**=INTERMEDIATE, **R**=RESISTANT

4.3 Antibiotic sensitivity of the isolates

The *E. coli* isolates were most sensitive to Chloramphenicol (N=6, 87.5%), followed by Gentamicin (N=4, 57.1%), followed by Nalidixic acid (N=1, 14.3%) and the isolates were not sensitive to Fusidic acid, which was the least effective drug.

The two *Klebsiella spp* isolates were sensitive to only Gentamicin and Chloramphenicol (N=2, 100%) moderately to Nalidixic acid, (N=1, 50%) and not sensitive at all towards Fusidic acid, (N=0), (from considering appendix D: Table **4.3** and Fig **4.2**). *S. aureus* and *S. saprophyticus* showed sensitivity to only Gentamicin and Chloramphenicol drugs with the *P. aeruginosa* isolate being sensitive to only Chloramphenicol.



100 100 100

Key: Genta= Gentamicin, N.A= Nalidixic acid, F.A= Fusidic acid and Chlora= Chloramphenicol.

Fig 4.2 Percentage antibiotic sensitivity patterns of the isolated etiological agents

4.4 The antibiotic resistance of the isolates

The *E. coli* isolates were mostly resistant to the antibiotic Fusidic acid (100%), followed by Nalidixic acid (N=6, 85.7%) and lastly to Gentamicin. These isolates had an intermediate

response (N= 1, 14.3%) towards Chloramphenicol. The two *Klebsiella spp* isolates were resistant to only Fusidic acid whilst the *S. saprophyticus* and *S. aureus* isolates were resistant to both Nalidixic acid and Fusidic acid. However *P. aeruginosa* was resistant only to the Chloramphenicol antibiotic (**Table 4.3**; **Fig 4.3**)

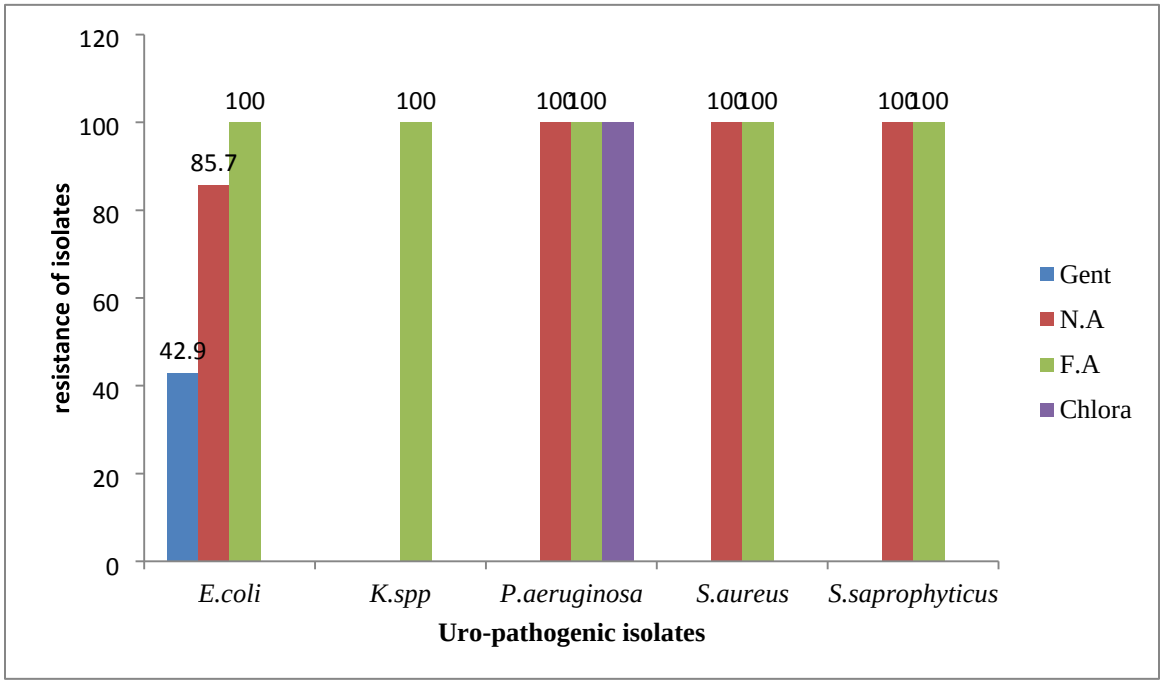


Fig 4. 3 Percentage antibiotic Resistance Patterns of isolates

4.5. Antibiotypes/ resistant patterns of the Isolates

The different UTIs causing uro-pathogens had a total of 5 different antibiotypes or resistant patterns (Appendix B, Table B3). Four of *E. coli* isolates, and the *S. saprophyticus* isolate had the most common antibiotype Nalidixic Acid^{R} Fusidic Acid^{R} with a frequency of 41.7% whilst 2 of them showed resistance to the antibiotics Gentamicin and Fusidic acid of antibiotype Gentamicin^{R} Fusidic Acid^{R} with the frequency 17%.

P. aeruginosa showed a unique resistant pattern Nalidixic Acid^{R} Fusidic Acid^{R} Chloramphenicol^{R} with a frequency of 8%. Both *Klebsiella* isolates had a unique antibiotype Fusidic Acid^{R} being resistant to only 1 type of antibiotic Fusidic acid, with a frequency of 17%. The antibiotype Gentamicin^{R} Nalidixic Acid^{R} Fusidic Acid^{R} was unique for the single *S. aureus* isolate with a frequency of 8% (Appendix B, Table B3).

4.6. Drug efficacy on the isolates

The isolates responded differently to the four antibiotics tested. For *E. coli* isolates, the proportion of sensitive isolates was significantly different across all the four antibiotics (Chi-square $p< 0.05$, **Appendix C, Table C1**). The proportion of sensitive isolates was significantly different for the three antibiotics Gentamicin, Nalidixic acid and Chloramphenicol used against the *E. coli* isolates (chi-square, $p< 0.05$, **Appendix C, TableC2**). The proportion of the sensitive *E. coli* isolates was insignificantly different for the two antibiotics Fusidic acid and Nalidixic acid, (Chi-square, $p>0.05$, **Appendix C, Table C4**). The proportion of sensitive isolates was insignificantly different for the two antibiotics, Gentamicin and Chloramphenicol, which the *E. coli* isolate were most sensitive to, (Chi-square $p> 0.05$, **Appendix C, Table C3**). *Klebsiella spp* were sensitive to Gentamicin and Chloramphenicol and moderately sensitive to Nalidixic acid. The *S. saprophyticus* isolate was sensitive to both Gentamicin and Chloramphenicol. The only isolate of *S. aureus* was sensitive to only Chloramphenicol and the *P.aeruginosa* isolate was sensitive to Gentamicin (**Table 4.3, Fig 4.2**).

CHAPTER 5 DISCUSSION

5.1 Prevalence and identification of bacteria causing UTIs

One of the study objectives was to isolate and identify the bacteria causing UTIs in patients presenting at the Zvishavane District Hospital. Five bacterial uro-pathogens were isolated including *E. coli, Klebsiella spp, S. aureus, S. saprophyticus and P. aeruginosa*. The relatively low prevalence (13%), suggest that the UTIs are not a risk to the Zvishavane residents. Three different studies had higher prevalence frequencies comparatively. These include studies done in Ethiopia, (90%), by Seifu and Gebissa, (2018), Tamilnadu, (32%) by Manisha, *et al.* (2015), and in Nigeria, (32%) by Mustafa, *et al*. (2013). However these studies had a different sample size from that of this study, i.e. 384, 7 868 and 100 respectively.

Females had a higher prevalence (10%) of UTIs than males (3%) (**Table 4.1**). This correlates with findings of Kibret and Abera (2014). The differences in these prevalence frequencies owe to differences in the anatomical features between the females and the males. The urethra in males is located at a distance from the anus unlike in the females where the distance is very short such that the gastro-internal microorganisms can be easily introduced to the urethra upon wiping if not done correctly (de Sousa *et al.*, 2013). The other possible reasons are that: due this physiological advantage, men's urethra is kept dry preventing the optimal bacterial growth and there are some prostate secretions which offer antimicrobial activities (Eriksson *et al.*, 2013). To reduce infections in the females as they have a short urethra, females should wipe their genitalia from the front to the back to reduce the introduction of gastro-internal microorganisms into the urinary tract (Eriksson *et al.*, 2013). Contary to this study

5.2 Common UTI pathogen

Another objective of this study was to determine the most common type of pathogen causing UTIs. *Escherichia coli* was the most common pathogen causing UTIs with a prevalence of about 58%. This correlates to the findings of Eriksson *et al*. (2013), who observed that the Enterobacteriaceae is predominant UTI pathogen. This is so because the family generally is ubiquity in both the environment as well as the animal host. The predominance of Enterobactreiaceae family as UTI pathogen is not surprising given that they easily acquire and transfer the genetic determinants which confer resistance towards most antibiotic classes (Vranic *et al.*, 2017).

The second highest pathogen for UTIs isolated in this study was *Klebsiella* (17%). This is also in agreement with findings of Leisy-azar and Ebadi (2017) who noted that *Klebsiella spp* were second common after *E. coli spp*. However, this trend differs from the findings of Prakasam *et al*. (2012) that, *Klebsiella spp* were even less prevalent not second, as *E. coli* had 83.8% UTIs prevalence and Klebsiella had only 9.6%. The other uro-pathogens in the study, namely, *P. aeruginosa*, *S. saprophyticus* and *S. aureus* had the same prevalence of 8.3% which was relatively lower. The low *P.aeruginosa* prevalence (8.3%) obtained in this study correlates with findings of Alkhyat *et al*. (2014) that it was 12.5% prevalent. A lower prevalence of *S. aureus*, (8.3%), is in agreement with the observation of Demuth *et al*. (1979), Barett *et al*. (1999) that *S. aureus* is relatively an uncommon cause of UTIs within the general population. The same can be said for *S. saprophyticus*.

5.3. Antibiotic susceptibility patterns of UTIs

Another objective of this study was to determine, the antibiotic susceptibility patterns of the uropathogens identified. There was a varied response to all the four antibiotics by the five uropathogens showing that the antibiotics had different drug efficacies. *E. coli* isolates were most sensitive to Chloramphenicol (86%) (**Table 4.3**). This is so because of some features that the chloramphenicol drug has. In a study conducted by Potrykus and Wegrzyn (2001), they found out that after using Chloramphenicol as the selective agents in an attempt to introduce some plasmids, they were no *E. coli* transformants that were identified. This means that the Chloramphenicol managed to inhibit the *E. coli* to transform, therefore, it can be used as treatment against the *E. coli*, hence the sensitivity. The *E. coli* isolates had an intermediate response towards Gentamicin (57%) (**Table, 4.3**). In other studies by Kibret and Abera (2011), however, *E. coli* had a higher sensitivity towards the antibiotic Gentamicin (80%) (**Table, 4.3**). Enterobacteriacaea that have emerged to cause UTIs have developed resistance to most antibiotics commonly used for UTIs and those reserved for severe infections which also include gentamicin and fluoroquinolones. As such this explains the differences in the sensitivity by E. coli to the same antibiotic used in this particular study and for other studies.

The study found out that, the *E. coli* isolates had a very low response towards Nalidixic acid (14%). This was expected because Nalidixic acid is a Quinolone, a class of antibiotics that are mostly used for as antimicrobial agents in UTIs treatment. However the extensive use has led to the increase in *E. coli* isolate rate of resistance as noted by (Roderova *et al.*, 2017).

The two *Klebsiella spp* were most sensitive to the antibiotics, Chloramphenicol and Gentamicin. This correlates with what was found out by Kumar (2013) when he noted that chloramphenicol and gentamicin among other drugs should be used as preferred treatment for *Klebsiella spp*. This is almost the same with what Azar and Ebadi, (2017), found out that the organism was resistant

to gentamicin by 33%, a lower resistance. To Nalidixic acid, the sensitivity was intermediate. This was so because previous studies done by Kyabaggu *et al.* (2007) showed that the *Klebsiella spp*, displayed massive resistance to Nalidixic acid among many other first line antibiotics, Cotrimoxazole and Erythromycin.

The *P. aeruginosa* isolate was only sensitive to Gentamicin, and least sensitive to the other 3 antibiotics because some time back in the 90's Lazaravic *et al*. (1998), found out that *P.aeruginosa* was most prominent to Aminoglycosides, hence, the sensitivity to this particular antibiotic.

S. aureus was only sensitive to Chloramphenicol because as noted by Rubin and Rajendra (2012*), S. aureus* was seen to have managed to develop resistance to many available antibiotics. As such chloramphenicol was discovered to be able to inhibit growth of *S. aureus* which is even resistant to methicillin antibiotics (Fayyaz et al, 2013).

S. saprophyticus was most sensitive to chloramphenicol and gentamicin in correlation with Trivedi, *et al.*, 2015) mentioned, this organism's strain are susceptible to gentamicin and chloramphenicol amongst the other antibiotics mentioned. Hovelius (1977), reported resistance of *S. saprophyticus* towards Nalidixic acid, as such the results of this study towards Nalidixic acid are expected.

The antibiotics Chloramphenicol and Fusidic acid are from the same class of drug but on the four uro-pathogenic organisms, i.e., *E. coli, S. aureus, S. saprophyticus* and *Klebsiella spp*, they have different efficacies because Fusidic acid is now usually used for skin infections caused by *Stapphylococcus spp* (Spelman, 1999). This antibiotic was once used for the treatment of most gram-negative bacteria causing UTIs but most of these organisms evolved some antibiotic

resistance mechanisms towards the drug such use against UTIs is no longer relevant (Llor and Bjerrum, 2014: Leisy-azar and Ebadi, 2017). Chloramphenicol drug efficacy is still being valued as it is a synthetic antibiotic with a broad spectrum that is active against most of the gram positive and negative bacteria, either aerobic or anaerobic (WHO, 2016).

5.4 Antibiotypes of UTIs

Another objective of this study was to identify the resistance patterns of the uro-pathogenic isolates to the four different drugs. The antibiotypes show antibiotics to which the individual isolates were resistant to. Four of the *E. coli* isolates, had the most common antibiotype Nalidixic $Acid^{R}$Fusidic $Acid^{R}$ showing that these isolates were resistant to the regimens Nalidixic acid and Fusidic acid. Therefore, this treatment combination should not be considered as treatment for UTIs caused by *E. coli*. The same applies for and *S. saprophyticus.* However, for *E. coli*, two more isolates had a different antibiotype, $Gentamicin^{R}$ Nalidixic $Acid^{R}$ Fusidic $Acid^{R}$, there is need to scrutinize the option to prescribe depending on the sensitivity of the strain. Both *Klebsiella isolates,* showed resistance towards one antibiotic as shown by the antibiotype Fusidic $Acid^{R}$. This particular antibiotype does not suggest that the *Klebsiella* isolates were sensitive to all three remaining antibiotics since one of them was intermediately responsive to Nalidixic acid. This difference in could be due to the fact that there are different strains of the organism which have got different responsive mechanisms towards certain antibiotics.

The *P. aeruginosa* isolate showed a certain different resistant pattern Nalidixic $Acid^{R}$ Fusidic $Acid^{R}$ $Chloramphenicol^{R}$ with a frequency of 8%. This suggests that the particular drug combination is less effective for *P.aeruginosa* isolate. This could be so because the organism developed some resistant mechanisms to the Quinolones and the bacteriostatic drugs despite

being effective in the past. The antibiotype Gentamicin^{R} Nalidixic Acid^{R} F.A^{R} was unique for the only *S. aureus* isolate with a frequency of 8% showing that this treatment combination is not effective against *S. aureus* UTIs. This could be because of the less potent aspects of the drugs making the isolate more resistant to the effects of the antibiotics.

5.5 Drug efficacy

The proportion of sensitive *E. coli* isolates was significantly different across all the four antibiotics (chi-square p< 0.05, Appendix C, Table 1). This is so because, *E. coli* these drug efficacies are so different Llor and Bjernum, (2014): Laxminarayan *et al.* (2013), noted that, although these antibiotics are used as the effective treatment for UTIs, continual, empirical and prolonged use of them will result in the emergence of resistance. The study observed that the proportion of sensitive *E. coli* isolates was significantly different for the three antibiotics Gentamicin, Nalidixic acid and Chloramphenicol used against the *E. coli* isolates (chi-square, p< 0.05, Appendix C, **Table C2**). This could be so because of the features chloramphenicol has against the *E. coli* as explained in **5.3** which the aminoglycoside Gentamicin doesn't have. Therefore Gentamicin is less effective against *E. coli* as compared to Chloramphenicol. The study observed that, the proportion of sensitive isolates was insignificantly different for the two antibiotics, Gentamicin and Chloramphenicol, which the *E. coli* isolates were most sensitive to,

(chi-square p> 0.05, **Appendix C, Table 4**). As such the most effective drugs for treating the

UTIs caused by *E. coli* are Chloramphenicol and Gentamicin (**Table** 4.6; **Fig**, 4.2)

The least performing drugs against *E. coli* isolates were Nalidixic acid and Fusidic acid. The isolates were not sensitive to Fusidic acid at all, as there was no zone of inhibition. They were however sensitive to Nalidixic acid by 14%. However, the proportion of the sensitive *E. coli* isolates was insignificantly different for these two antibiotics, (chi-square p>0.05, **Appendix C,**

Table 3). Therefore, Nalidixic acid and Fusidic acid should not be considered as treatment options as they are statistically not different, having less drug efficacies. Nalidixic acid has got a challenge in that it has got specifics upon medication consumption in that, full absorption has to be prevented and it works best when at kept at a constant level (www.medicinenet.com). As such the low efficacy in Nalidixic acid could be that the patient had not followed the instructions of consumption well such that *E. coli* developed resistance. The study observed that, the two Klebsiella *spp* were sensitive to Gentamicin and Chloramphenicol and moderately sensitive to Nalidixic acid. This means that only Chloramphenicol and Gentamicin had the required efficacies, and can be used as treatment options for UTIs with regard to this study. Moreover, the *S. saprophyticus* isolate was sensitive to both Gentamicin and Chloramphenicol. These two antibiotics can also be used as treatment for UTIs caused by *S. saprophyticus*. The only isolate of *S. aureus* was sensitive to only Chloramphenicol, this makes sense because of the efficacy Chloramphenicol has against *S. aureus*, as described by Fayyaz *et al*. (2013), making suitable for use as S. aureus treatment. Gentamicin had a considerable efficacy against *P. aeruginosa* in correlation with what Lazaravic *et al*. (1998), found out. Hence, the antibiotic Gentamicin can be used to treat UTIs caused by *P.aeruginosa*.

There were some *E. coli* isolates which were considered sensitive to Gentamicin which were statistically not sensitive $p<0.05$ R tests (Appendix D, **D1.1.6**). This means that, the prescription of this antibiotic can potentially result in the emergence of drug resistant if consumption is continued as it would less drug efficacy on the some *E. coli* isolates. The drug will not be effective enough anticipated, hence it should not be prescribed as it is difficult to tell its significance in UTIs treatment.

The differences and similarities between the findings of this study and other studies may be due to the locations and time factors. This is supported by El-Mahmood *et al*. 2009; Demile, *et al*. 2012, when they noted that the UTI bacterial etiologies can show some geographic variations and time variations in a population. This is also supported by Grabe *et al*. (2015) when they noted that bacterial spectrum of these pathogens may vary with time as well as from one hospital to another.

Grabe *et al*. (2015) mentioned that sometime in 1960, a biologist named Kass developed the significant bacteriuria> 10^5cfu/ml concept, which is currently recognized as standard quantity for urine cultures. However, despite the introduction of quantitative microbiology into infectious diseases diagnosis based on this concept, it has been found recently that, there is no fixed bacterial count indicative of the significant bacteriuria that ultimately can be applied to all UTIs types.

Recommendations

Since UTIs were more prevalent in female than in males in this study and that UTIs can be asymptomatic, it is encouraged that most women get screened for UTIs in case of asympomatic bacteria. There is also need to educate these women through awareness campaigns on how they can prevent UTIs as their physiology makes them more prone to infections than their male counterparts. Fusidic acid should not be used as a treatment option as the uro-pathogens isolated from this study showed resistance towards the antibiotic. Chloramphenicol and Gentamicin should be prescribed as a treatment combination for UTIs. Further studies should be done to monitor the prevalence as well as to check on the effectiveness of the antibiotic drugs that will be currently in use.

The studies should also be done in a longer time frame to accommodate more samples as the collection was based on the patient hospital visits. The different antibiotypes of the UTIs-causing uro-pathogens give an insight of the effectiveness of the drugs.

Conclusions

UTIs in Zvishavane District were bacterial infections caused by *E. coli, Klebsiella spp, S. aureus, S. saprophyticus* and *P.aeruginosa*, with *E. coli* being the most common followed by *Klebsiella* and the other three being rare. Females were more vulnerable (with a prevalence of about 10%) than their male counterparts (with a prevalence of about 3%). Chloramphenicol was the most effective drug for treatment of UTIs being effective against all isolates except for that of *P. aeruginosa*, which was susceptible only to Gentamicin. Therefore, Chloramphenicol and Gentamicin regiments can be used against UTIs interchangeably. The resistant patterns showed that most pathogens are resistant to the antibiotic pattern Nalidixic Acid^{R} Fusidic Acid^{R}, i.e., Nalidixic acid and Fusidic acid regimens. Hence this combination should not be considered for UTIs treatment. The isolates showed no particular resistance to the combination of Gentamicin and Chloramphenicol, suggesting that this treatment combination may be used for treating most UTIs.

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APPENDICES

Appendix A

Media, Antibiotics and Identification Tests

Table A1. Manufacturer's Product and Detail

Manufacturer's name	**Product Detail**
Mast Group	Mast Antibiotic Discs
Mast Mueller Hinton Agar (IDM170)	
Mast Indole (IDM34/A)	
Mast Citrate Agar (IDM23)	
Mast Oxidase strip	

Table A2. Antibiotics used in susceptibility tests

Drug	Antibiotic Class	Disk Potency
Gentamicin	Aminoglycoside	10µg
Nalidixic acid	Quinolone	30µg
Fusidic acid	Bacteriostatic	10 µg
Chloramphenicol	Bacteriostatic	30 µg

Table A3. Tests used to identify microorganisms.

Mic	Gram	Ind	Ox.	Cit	Coa	Cat	Microorganism
N.Y	- rod	+	-	-		+	*E.coli*
N.Y	-rod	+		+		+	*K.oxytoca*
N.Y	-rod	-	+	+		+	*P.aeruginosa*
N.Y	-rod	+	-	-		+	*E.coli*
N.Y	+cocci		-		-	+	*S.saprophyticus*
N.Y	- rod	+	-	-		+	*E.coli*
N.Y	-rod	+	-	-		+	*E.coli*
N.Y	-rod	+	-	-		+	*E.coli*
N.Y	-rod	+		+		+	*K.oxytoca*
N.Y	-rod	+	-	-		+	*E.coli*
N.Y	-rod	+	-	-		+	*E.coli*
N.Y	+cocci	-	-	+	+	+	*S.aureus*

Key: Nit = nitrites, Leu= lecucocytes, Mic= microscopy, Gram= gram status, Ind= Indole, Ox. =oxidase, Cit= citrate, Coa= coagulase, Cat= Catalase, + = positive and - = negative

Table A 4 Antibiotic diameter zone

Antibiotic	Sensitive	Intermediate	Resistant
Gentamicin	≥15	13-14	≤12
Nalidixic Acid	≥19	14-18	≤13
Fusidic Acid	≥22	18-21	≤17
Chloramphenicol	≥18	13-17	≤19

APPENDIX B

Table B1. Results and patient gender

Sample	Sex	Genta	N.A	F.A	Chlora
1	29F	S (16)	R (0)	R (0)	S (26)
2	34M	R (11)	S (20)	R (0)	S (26)
3	42F	R (10)	S (20)	R (0)	I (14)
4	75M	S(15)	R (0)	R (0)	S (26)
5	77F	S (17)	I (18)	R (0)	S (25)
6	60M	S (17)	S(19)	R (0)	S (26)
7	40F	I (13)	R (0)	R (0)	S (26)
8	23F	R (0)	R (0)	R (0)	S (26)
9	39F	S (15)	R (0)	R (0)	S (27)
10	38F	S (16)	R (0)	R (0)	S (7)
11	27F	S (17)	R(12)	R (0)	R (11)
12	33F	S (16)	R (0)	R (0)	S (25)

Key: F=female, M= male, Genta= Gentamicin, N.A=Nalidixic acid, F.A= Fusidic acid, Chlora=Chloramphenicol, S=Sensitive, R= Resistant and I= Intermediate

Table B2. Antibiotic Susceptibility Patterns

Antibiotic	**Number**		**n=12**
	Sensitive	**Intermediate**	**Resistant**
Gentamicin	8	1	3
Nalidixic acid	3	1	8
Fusidic acid	0	0	12
Chloramphenicol	10	1	1

Table B.3.Antibiotypes of UTIs causing uro-pathogens.

Antibiotic number	pattern	Number (%)
A1.a	$F.A^R$	1(8.3%) **A2.**a
$N.A_RF.A_R$	5(41.7%)	
b.	$Genta_RF.A_R$	2(16.6%)
A3.a	$N.A^R F.A^R Chlora^R1$	1(8.3%)
c.	$Genta^R N.A^R F.A^R$	2(16.6%)

key: Genta= Gentamicin, N.A= Nalidixic acid, F.A= Fusidic acid Chlora= Chloramphenicol and R= Resistant

APPENDIX C

Chi-Square: Homogeneity of proportions of *E. coli* isolates

Ho: the proportion of sensitive isolates is equal across all 4 antibiotics Ha: at least two proportions are different.

Table C1. Homogeneity of proportions of all 4 drugs.

Chi-Square Tests

	Value	df	Asymp. Sig. (2sided)
	16.349[a]	3	.001
Pearson Chi-Square	21.196	3	.000
Likelihood Ratio	1.182	1	.277
Linear-by-Linear Association			
N of Valid Cases	27		

a. 8 cells (100.0%) have expected count less than 5. The minimum expected count is 2.44.

Table C2.Homogeneity of proportion of 3 drugs (Genta,N.A,andChlora)

Chi-Square Tests

	Value	df	Asymp. Sig. (2sided)
Pearson Chi-Square	9.610[a]	2	.008
Likelihood Ratio	12.223	2	.002
Linear-by-Linear Association	3.556	1	.059
N of Valid Cases	20		

a. 6 cells (100.0%) have expected count less than 5. The minimum expected count is 2.70.

Table C3 Homogeneity of proportions of best 2drugs(Genta and Chlora)

profile * antibiotic Crosstabulation

			Antibiotic		Total
			Gentamicin	Chloramphenicol	
Profile	Susceptible	Count	4	6	10
		Expected Count	5.0	5.0	10.0
		% within profile	40.0%	60.0%	100.0%
	Resistant	Count	3	1	4
		Expected Count	2.0	2.0	4.0
		% within profile	75.0%	25.0%	100.0%
Total		Count	7	7	14
		Expected Count	7.0	7.0	14.0
		% within profile	50.0%	50.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2sided)	Exact Sig. (2sided)	Exact Sig. (1sided)
Pearson Chi-Square	1.400[a]	1	.237		
Continuity Correction[b]	.350	1	.554		
Likelihood Ratio	1.449	1	.229		
Fisher's Exact Test				.559	.280
Linear-by-Linear Association	1.300	1	.254		
N of Valid Cases	14				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.00.

b. Computed only for a 2x2 table

Table C4 Homogeneity of proportions of the least performing drugs N.A and F.A

Chi-Square Tests

	Value	df	Asymp. Sig. (2sided)	Exact Sig. (2sided)	Exact Sig. (1sided)
	1.077[a]	1	.299		
Pearson Chi-Square	.000	1	1.000		
Continuity Correction[b]	1.463	1	.226		
Likelihood Ratio					.500
Fisher's Exact Test		1	.317	1.000	
Linear-by-Linear Association	1.000				
N of Valid Cases	14				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .50.

b. Computed only for a 2x2 table

APPENDIX D

R-OUTPUT

D1.Output for GENTAMICIN

D1.1.1E.coli isolates1

```
> x=c(10,11,12,10)
>t.test(x,alternative="less",mu=15)
        One Sample t-test
data:  x t = -8.878, df = 3, p-value = 0.001507
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 11.87659
sample estimates: mean of x
  10.75
```

D1.1.2E.coli isolates2

```
> x=c(10,11,12,10)
>t.test(x,alternative="less",mu=15)
        One Sample t-test
data:  x t = -8.878, df = 3, p-value = 0.001507
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 11.87659
sample estimates: mean of x
  10.75
```

D1.1.3E.coli isolates3

```
> x=c(17,16,15,14)
>t.test(x,alternative="less",mu=15)
        One Sample t-test

t = 0.7746, df = 3, p-value = 0.7525 alternative
hypothesis: true mean is less than 15 95 percent
confidence interval:
-Inf 17.01909
sample estimates: mean
of x
   15.5
```

D1.1.4E.coli isolates4

> x=c(8,8,12,10)
>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = -5.7446, df = 3, p-value = 0.005239
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 11.75317
sample estimates: mean of x
9.5

D1.1.5E.coli isolates5

> x=c(17,15,16,17)
>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = 2.6112, df = 3, p-value = 0.9602
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 17.37659
sample estimates: mean of x
16.25

D1.1.6E.coli isolates6

=c(15,14,14,13)
>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = -2.4495, df = 3, p-value = 0.04586
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 14.96076
sample estimates: mean of x
14

D1.1.7 Ecoli isolate 7

x=c(11,9,10,10)
>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = -12.247, df = 3, p-value = 0.0005861 alternative hypothesis: true mean is less than 15 95 percent confidence interval: -Inf 10.96076 sample estimates: mean of x
10

D.1.2S.aurues

> x=c(12,13,14,11)

>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = -3.873, df = 3, p-value = 0.01523
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 14.01909
sample estimates: mean of x
12.5

D1.3.1 Klebsiella isolate 1

> x=c(15,18,18,17)
>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = 2.8284, df = 3, p-value = 0.9669
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 18.66408
sample estimates: mean of x
17

D1.3.2klebsiella isolate 2

> x=c(15,17,18,16)
>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = 2.3238, df = 3, p-value = 0.9486
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 18.01909
sample estimates:
mean of x
16.5

D.1.4. S.saprophyticus isolate

x=c(17,15,18,16)
>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = 2.3238, df = 3, p-value = 0.9486
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 18.01909
sample estimates: mean of x
16.5

D.1.5 P.aeruginosa isolate

> x=c(15,18,16,15)
>t.test(x,alternative="less",mu=15)

One Sample t-test

data: x t = 1.4142, df = 3, p-value = 0.8739
alternative hypothesis: true mean is less than 15
95 percent confidence interval: -Inf 17.66408
sample estimates: mean of x
16

D2.outpout NALIDIXIC ACID

D2.1.1 Ecoli isolate1

x=c(0,0,0,0)
>t.test(x,alternative = "less",mu=19)

One Sample t-test

data: x t = -Inf, df = 3, p-value < 2.2e-16
alternative hypothesis: true mean is less than 19
95 percent confidence interval:
NaNNaN
sample estimates: mean
of x
0

D2.1.2Ecoli isolate 2

> x=c(20,21,20,19)
>t.test(x,alternative = "less",mu=19)

One Sample t-test

data: x t = 2.4495, df = 3, p-value = 0.9541
alternative hypothesis: true mean is less than 19
95 percent confidence interval: -Inf 20.96076
sample estimates: mean of x

D3.Ecoli isolate 3 x=c(0,0,0,0)
>t.test(x,alternative = "less",mu=19)

One Sample t-test

data: x t = -Inf, df = 3, p-value < 2.2e-16
alternative hypothesis: true mean is less than 19
95 percent confidence interval:

NaNNaN
sample estimates: mean
of x
0

D2.1.4.Ecoli isolate 4

> x=c(20,20,21,21)
>t.test(x,alternative = "less",mu=19)

One Sample t-test

data: x
t = 5.1962, df = 3, p-value = 0.9931 alternative hypothesis: true mean is less than 19 95 percent confidence interval: -Inf 21.17936 sample estimates: mean of x
20.5

D2.1.5 Ecoli isolate5

x=c(0,0,0,0)
>t.test(x,alternative = "less",mu=19)
One Sample t-test
data: x t = -Inf, df = 3, p-value < 2.2e-16 alternative hypothesis: true mean is less than 19 95 percent confidence interval:
NaNNaN
sample estimates: mean
of x 0 **D2.1.6.Ecoli isolate 6**

x=c(0,0,0,0)
>t.test(x,alternative = "less",mu=19)
One Sample t-test
data: x t = -Inf, df = 3, p-value < 2.2e-16 alternative hypothesis: true mean is less than 19 95 percent confidence interval:
NaNNaN
sample estimates: mean
of x
0

D2.1.7.Ecoli isolate 7

x=c(0,0,0,0)

>t.test(x,alternative = "less",mu=19)

One Sample t-test

data: x t = -Inf, df = 3, p-value < 2.2e-16
alternative hypothesis: true mean is less than 19
95 percent confidence interval:
NaNNaN
sample estimates: mean
of x
0

D2.2.1 Klebsiella isolate 2 >
x=c(17,1719,17)
>t.test(x,alternative = "less",mu=19)

One Sample t-test

data: x t = 0.99647, df = 2, p-value = 0.788
alternative hypothesis: true mean is less than 19
95 percent confidence interval:
-Inf 2240.938
sample estimates:
mean of x
584.3333

D2.2.2 Klebsiella isolate 2
> x=c(19,20,18,17)
>t.test(x,alternative = "less",mu=19)

One Sample t-test

data: x t = -0.7746, df = 3, p-value = 0.2475
alternative hypothesis: true mean is less than 19
95 percent confidence interval: -Inf 20.01909
sample estimates: mean of x
18.5

D.2.3 S.aureus isolate x=c(0,0,0,0)
>t.test(x,alternative = "less",mu=19)

One Sample t-test

data: x t = -Inf, df = 3, p-value < 2.2e-16
alternative hypothesis: true mean is less than 19
95 percent confidence interval:
NaNNaN
sample estimates: mean
of x
0

D2.4 S.saprophyticus x=c(0,0,0,0)
>t.test(x,alternative = "less",mu=19)

One Sample t-test
data: x t = -Inf, df = 3, p-value < 2.2e-16 alternative hypothesis: true mean is less than 19 95 percent confidence interval:
NaNNaN
sample estimates: mean
of x
0

D2.5 P.aeruginosa isolate

> x=c(11,12,11,13)
>t.test(x,alternative = "less",mu=19)
One Sample t-test
data: x t = -15.145, df = 3, p-value = 0.0003125 alternative hypothesis: true mean is less than 19 95 percent confidence interval: -Inf 12.87659 sample estimates: mean of x
11.75

D3.Output for CHLORAMPHENICOL

D3.1.1 Ecoli isolate 1 x=c(24,27,27,26)
>t.test(x,alternative="less",mu=18)
One Sample t-test
data: x t = 11.314, df = 3, p-value = 0.9993 alternative hypothesis: true mean is less than 18 95 percent confidence interval: -Inf 27.66408 sample estimates: mean of x
26

D3.1.2 Ecoli isolate 2

> x=c(13,14,14,15)
>t.test(x,alternative="less",mu=18)
One Sample t-test
data: x t = -9.798, df = 3, p-value = 0.00113 alternative hypothesis: true mean is less than 18 95 percent confidence interval: -Inf 14.96076 sample estimates: mean of x
14

D3.1.3 Ecoli isolate 3

> x=c(24,28,25,26)
>t.test(x,alternative="less",mu=18)
One Sample t-test

data: x t = 9.0759, df = 3, p-value = 0.9986
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 27.75957
sample estimates:
mean of x
25.75

D3.1.4 E.coli isolate 4

```
> x=c(25,26,25,26)
>t.test(x,alternative="less",mu=18)
```

One Sample t-test

data: x t = 25.981, df = 3, p-value = 0.9999
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 26.17936
sample estimates: mean of x
25.5

D3.1.5 Ecoli isolate 5

```
> x=c(27,25,24,24)
>t.test(x,alternative="less",mu=18)
```

One Sample t-test

data: x t = 9.8995, df = 3, p-value = 0.9989
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 26.66408
sample estimates: mean of x
25

D3.1.6 Ecoli isolate 6

```
> x=c(28,8,25,7)
>t.test(x,alternative="less",mu=18)
```

One Sample t-test

data: x t = -0.18107, df = 3, p-value = 0.4339
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 29.99687
sample estimates: mean of x
17

D3.1.7 Ecoli isolate 7

```
> x=c(28,28,25,27)
>t.test(x,alternative="less",mu=18)
```

One Sample t-test

data: x t = 12.728, df = 3, p-value = 0.9995
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 28.66408
sample estimates: mean of x
27

> x=c(25,27,27,27)
>t.test(x,alternative="less",mu=18)

One Sample t-test

data: x t = 17, df = 3, p-value = 0.9998
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 27.67668
sample estimates: mean of x
26.5

D.3.2 S.aureus isolate

> x=c(26,27,26,23)
>t.test(x,alternative="less",mu=18)

One Sample t-test

data: x t = 8.6603, df = 3, p-value = 0.9984
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 27.53807
sample estimates: mean of x
25.5

D.3.3.1 Klebsiella isolate 1

> x=c(24,25,25,26)
>t.test(x,alternative="less",mu=18)

One Sample t-test

data: x t = 17.146, df = 3, p-value = 0.9998
alternative hypothesis: true mean is less than 18
95 percent confidence interval:
-Inf 25.96076 sample
estimates: mean of x
25

D.3.3.2 Klebsiella isolate 2

> x=c(26,26,27,24)
>t.test(x,alternative="less",mu=18)

One Sample t-test

data: x t = 12.318, df = 3, p-value = 0.9994
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 27.23063
sample estimates: mean of x
25.75

D.3.4 S.saprophyticus isolate

> x=c(23,27,27,26)
>t.test(x,alternative="less",mu=18)

One Sample t-test

data: x t = 8.1882, df = 3, p-value = 0.9981
alternative hypothesis: true mean is less than 18
95 percent confidence interval: -Inf 27.97742
sample estimates: mean of x
25.75

D.3.5 P.aeruginosa isolate

> x=c(12,11,12,12)
>t.test(x,alternative="less",mu=18)

One Sample t-test data: x
t = -25, df = 3, p-value = 7.017e-05 alternative hypothesis: true mean is less than 18 95 percent confidence interval: -Inf 12.33834 sample estimates: mean of x
11.75

D4.output for FUSIDIC ACID

> x=c(0,0,0,0)
>t.test(x,alternative="less",mu=22)

One Sample t-test

data: xt = -Inf, df = 3, p-value < 2.2e-16
alternative hypothesis: true mean is less than 22
95 percent confidence interval:NaNNaN
sampleestimates:mean of x 0