A STUDY ON THE DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCERS AND CLASS I INTERGRONS IN *E. COLI* ISOLATES FROM URINARY TRACT INFECTED PATIENTS AT UTH

BY

CHIRWA EMMANUEL

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DECLARATION

I, **Emmanuel Chirwa**, do solemnly declare that this dissertation is my own original work. It has been presented in accordance with the guidelines for masters' dissertation of the University of Zambia. I further declare that this work has not been submitted before for the award of any degree or examination at the University of Zambia or any other university.

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APPROVAL

The University of Zambia approves the dissertation submitted by EMMANUEL CHIRWA, as fulfilling the partial requirements for the award of the Master of Science Degree in Medical Microbiology by the University of Zambia.

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Examiner 3	Signature	Date
	……	……...
Chairperson	Signature	Date

Board of examiners

Dr G Mulundu.

	…….	…….....
Supervisor	Signature	Date

Prof B Hang'ombe

	……	…….....
Co-Supervisor	Signature	Date

ABSTRACT

Escherichia coli that produce extended spectrum beta lactamase (ESBL) enzymes pose a global challenge in the management of urinary tract infections (UTIs) caused by these organisms. Beta lactamases are the primary cause of resistance to beta lactam antibiotics. These enzymes confer multi drug resistance (MDR) thus limiting and complicating treatment options. The beta lactamase resistant genes are usually encoded on plasmids and often confer resistance to other classes of antibiotics, further underscoring their clinical significance. In Zambia there is paucity of data available in relation to UTIs caused by ESBL producing *E. coli.* There is little information about genes responsible for these enzymes and the organisms' susceptibility patterns to commonly used antibiotics.

This cross sectional study used urine samples from 327 consenting patients from the University Teaching Hospital in Lusaka, Zambia. The ESBL producing isolates were obtained by culturing urine samples on MacConkey agar supplemented with cefotaxime followed by polymerase chain reaction (PCR) to detect the resistant genes in positive isolates. A questionnaire was used to collect information from patients for assessment of risk factors relating to infection with ESBL producing *E. coli.* The antibiotic susceptibility pattern was determined by using the disc diffusion method.

Of a total 327 samples analyzed, 15 (4.6%) were positive for ESBL producing *E. coli.* Genes of resistance significance that included the blaINT *bla*CTXM, *bla*SHV and *bla*TEM were detected. There was 100% resistance of the isolates to Cotrimoxazole, ampicillin and cefotaxime while no resistance (0%) was detected to Nitrofurantoin. All the ESBL producers had MDR (at least 30%) patterns. None of the risk factors was statistically significant in this study.

This study detected the presence of ESBLs and class I integrons in MDR *E. coli* in UTIs at the University Teaching Hospital in Zambia. This prompts the need for antimicrobial resistance surveillance to monitor hospital resistance patterns for these microorganisms.

Key words: *E.coli*, ESBL, *bla*INT, MDR

DEDICATION

To God almighty for abundant favor. My dedication also extends to my parents and siblings for the moral support, prayers and encouragement throughout my studies.

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ABBREVIATIONS

AMP	Ampicillin
CHL	Chloramphenicol
CIP	Ciprofloxacin
CTX	Cefotaxin
E. coli	*Escherichia coli*
ESBL	Extended Spectrum Beta Lactamases
INT	integron
LUTI	Lower Urinary Tract Infection
MDR	Multi Drug Resistance
N	Number
NOR	Norfloxacin
PCR	Polymerase chain reaction
S. aureus	*Staphylococcus aureus*
SMART	Study for monitoring antimicrobial resistant trends
STR	Streptomycin
SXT	Trimethoprim-sulphamethoxazole
TET	Tetracycline
UTH	University Teaching Hospital
UTIs	Urinary Tract Infections
UNZA	University of Zambia

CHAPTER 1
INTRODUCTION

1.0 Background

Extended spectrum beta lactamases (ESBLs) are enzymes capable of hydrolyzing dependable antibiotics that include penicillins, broad-spectrum cephalosporins and monobactams (Rawat and Nair, 2010). These enzymes are produced by bacteria with genes that code for the same enzymes. The ESBLs are often located on plasmids that are transferable from bacterial strain to strain and between species. ESBLs productivity were first observed by Abraham and Chen even before the introduction of therapeutic penicillin as early as 1940 (Ramanan *et al*., 2013). It has been speculated that these enzymes might have evolved for a possible physiological role of peptidoglycan assembly or to protect bacteria against beta lactams produced by other bacteria and fungi (Justina *et al*., 2012). The enzyme hydrolyze the beta lactam ring of beta lactam drugs rendering them ineffective (Rawat and Nair, 2010).

In 1944 penicillinase, a beta lactamase was first detected in Gram positive *Staphylococcus aureus*. According to Kong *et al*., (2010) genes encoding for beta lactamase in bacterial chromosomes were initially inducible and expressed only in small amounts. A plasmid mediated beta lactamase was reported for the first time from an *E. coli* isolate in 1965 from a Greek patient called Temoneria. This enzyme was designated as TEM 1 and within a decade it quickly spread to other members of the *Enterobactereceae* (Drawz and Bonomo, 2010). *Enterobactereceae* is a family of gram negative bacteria that is non-spore forming and is found in the soil, in the water, on plants and in the gastro intestinal tract (GIT) vertebrate and invertebrate animals. Some of the genera of interest in this family includes *Escheria, Shigella, Klebsiela and Salmonella*. The TEM 1 enzyme within the same period spread to *Haemophillus influenza* and *Neiserria gonorrhea* (Drawz and Bonomo, 2010).

A closely related beta lactamase to TEM 1 was found in *Pseudomonas aeruginosa* in 1969 in Great Britain and was designated as TEM 2 (Doi *et al*., 2013). Another similar chromosomal enzyme with more activity against ampicillin was discovered in a single *Klebsiella* isolate. It was originally designated PIT 2 but was later changed to SHV 2- for the sulphyhydril variable of the

enzyme to which P- chloromercuribenzoate attaches (Draws and Bonomo, 2010). Most ESBLs are mutations of TEM 1, TEM 2 and SHV 2 (Karen and Jed, 2011). ESBLs have been observed in the members' species of *Enterobacteriaceae*, that include *Enterobacter, Salmonella, Proteus*, *Citrobacter, Morganella, Serratia, Shigella* as well as *Pseudomonas* and *Acinetobacter* (Draws and Bonono, 2010).

E. coli have been known to be the major causative agents of urinary tract infections (UTI). *E. coli* associated UTIs can be life threatening as they can lead to toxemia. For a pathogen to cause a UTI it must be endowed with virulence factors to enable it survive in the urinary tract (Bien *et al*., 2012). Such organisms should be able to resist the flushing effect of urine by adhering to the urinary tract using pili. These organisms should be motile and should have the ability to break down chemical waste products which are excreted in the urine that would otherwise be harmful to the pathogens.

E coli and *Klebsiella* species stand out as the two main pathogens that produce ESBL (Kylie *et al*., 2014). They have emerged as serious pathogens in health care facilities where they cause hospital acquired infections. The two pathogens are also mainly responsible for community acquired infections. The ESBL hydrolyzes the beta lactam ring in the beta lactam-containing drugs rendering the organism resistant to drugs containing the beta lactam ring (Karen and Jed, 2011). These drugs include penicillins, carbapens, cephalosporins and monobactams.

Escherichia coli hydrolyzes the beta lactam ring resulting in resistance which limits treatment options for UTIs caused by this organism (Downing, 2015). *E coli* has extended resistance spectrum to all the penicillin and cephalosporin antibiotics with a possibility that it may also carry resistance to other drugs such as ciprofloxacin (Rawat and Nair, 2010).

Antibacterial misuse has been one of the major factors for the emergency of ESBLs producing bacteria (Shaikh *et al*., 2015). Increased use of beta lactam antibiotics has resulted in the emergency, and subsequent spread and evolutionary mutations of ESBL bacteria (Paterson, 2010). This has been assisted by modification of structural resistance genes via mutation and recombination. These structural genes are encoded by type 1 integrons (Barrios *et al*., 2012).

Integrons are genetic structures in bacteria which express gene cassettes which can be acquired and exchanged freely among bacteria. These gene cassettes may also encode genes for antibiotic resistance according to shaikh *et al*., (2015).

The aim of this study was to investigate the presence of extended spectrum of beta lactamase productivity and type 1 integrons in *E. coli* causing UTIs at the University Teaching Hospital in Lusaka.

1.2 Statement of the Problem

Treatment of infections due to bacteria which produce extended spectrum beta lactamases is a growing concern among health care providers due to the serious threat they pose to patients. The microorganisms have increased capacity to cause serious hospital acquired infections often resulting in poor clinical outcomes. Patients easily acquire these pathogens and become reservoirs who then spread them in their communities (Baguma *et al.*, 2017). The spread may be done through contamination of water, food. The ESBL genes are located on self-transmissible plasmids which can easily be transmitted between bacteria of the same species or different species (Stadler *et al*., 2018)

ESBLs production by *E. coli* is a significant resistance-mechanism that impedes the treatment of UTIs and is a serious threat to the currently available antibiotic armory (Codjoe and Donkor, 2018). The carbapenems drugs that are used to treat UTIs due to ESBL producing bacteria are given parenterally e.g. imipenem and etapenem. There is growing concern for possible development of antibiotic resistance of ESBL producing bacteria to drugs currently used to treat infections caused by these pathogens (Rawat and Nair, 2010). These resistant genes can be acquired by other bacteria through horizontal gene transfer. The ESBLs inactivate the beta lactam rings of all cephalosporins, penicillins and the monobactam aztreonam (Karen and Jed, 2011). Some members of the *Enterobacteriaceae* such as *Acinetobacter* and *Pseudomonas* produce carbapenases and the genes which encode for these enzymes are plasmid mediated (Hu *et al*., 2014). These genes may be shared horizontally with the ESBL producing *E. coli* and this might make the ESBL producing *E. coli* resistant to carbapenems. Carbapenases are beta lactamases that hydrolyze the beta lactam ring of penicillins monobactams, carbapenems and

cephaolosporins (Codjoe and Donkor2018). Bacteria which produce ESBLs and carbapenases are resistant to aztreonam, third generation cephalosporins, carpapenems, aminoglycosides and quinolones (Melese *et al*., 2016). Worldwide, and particularly in resource-limited settings like Zambia, documented information on the mechanisms of virulence of these bacteria is very limited. One study was done by Chishimba *et al.*, (2014) detected ESBL producing *E. coli* in Lusaka, Zambia. There is development of drugs to curb possible emergence of resistance is slow or absent. This situation has become of concern enough to warrant need for research on the ESBL producing microorganisms.

1.3 Justification of the study

There has been a number of outbreaks of infections with ESBL producers which have been reported in many African countries e.g. Tunisia, South Africa and Tanzania (Stoneberg, 2015). In Zambia there are limited surveillance figures documenting data for outbreaks or prevalence of infections as a result of ESBL producing organisms. It is therefore necessary to investigate the burden of disease and risk factors associated with ESBLs producing pathogens and their related type I integrons in Zambia. This study seeked to document scientific information that would significantly contribute to effective antibacterial treatment choices and reduce possibility to multi-drug resistance in UTI.

1.4 Research Questions

1.4.1 Main Research Question

Are ESBL and class1 Integrons producing *Escherichia coli* contributing to UTIs in patients at the University Teaching Hospital?

1.4.2 Specific Research Questions

1. Are there ESBL producing *E coli* in urine specimens from UTI patients at the University Teaching Hospital?
2. Do ESBLs producing *E. coli* causing UTIs in patients at UTH encode type 1 integrons and other resistant genes?

3. What are the risk factors associated with ESBL infections among UTI patients at the University Teaching Hospital.
4. What antibiotics are ESBL producing *E. coli* at UTH resistant to?

1.5 Objectives

1.5.1 Main Objectives

The objective of this study was to investigate ESBL producing *E. coli* and class I integrons from urine specimens of UTI patients at the University Teaching Hospital.

1.5.2 Specific Objectives

(a) To investigate the presence of ESBL producing *E. coli* in urine samples of UTI patients at UTH.

(b) To detect type I integrons and other genes associated with ESBL producing *E. coli* at UTH.

(c) To assess risk factors associated with ESBL infections among UTI patients at the University Teaching Hospital.

(d) To determine antibiotic susceptibility patterns of ESBL producing *E. coli* isolated from urine specimens of patients of UTIs from University Teaching Hospital.

CHAPTER 2
LITERATURE REVIEW

2.1 History of Extended Spectrum Beta Lactamase Productivity

Extended spectrum beta lactamase enzymes have always been produced by bacteria in the environment in response to naturally environmental pressure and from β-lactam-producing soil organisms (Shaikh *et al*., 2015). Antibiotic resistance has always been in the environment even before the first penicillin antibiotics were used for treatment (Baquero *et al*., 2011). Bacteria have always expressed genes against naturally occurring chemicals in the environment. This is because bacteria have several intrinsic mechanisms which can be used to break down these chemicals such as the production of enzymes or the expression of efflux pumps that pump out these chemicals. The other mechanisms through which bacteria protect themselves is by forming communities made up of different species for example through biofilm formation and indirect resistance (Hughes and Andersson 2017). Bacteria in a biofilm have greater resistance to antibiotics than planktonic bacteria due to the combined effect of their altered growth physiology and physical protection. Reduced antibiotic susceptibility contributes to the persistence of biofilm infections. In biofilms, poor antibiotic penetration, nutrient limitation and slow growth, adaptive stress responses and formation of persister cells constitute a multi-layered defense system (Stewart, 2002). In indirect resistance, resistant bacteria secret enzymes in the environment that protect other susceptible bacteria from chemicals that are found in the environment. The secreted enzymes reduce the concentration of chemicals in the environment and hence the susceptible bacteria are not affected (Hughes and Andersson, 2017).

After the introduction of beta lactam penicillin antibiotics that were specifically designed to be resistant to the hydrolytic action of beta lactamases on the market, there was an increase in the emergency of ESBL producing bacteria as a result of natural selection pressure (Dan and Diarmaid, 2010). Selective pressure on the use and overuse of new antibiotics in the treatment of patients soon resulted in new variants of β-lactamases (Davies and Davies, 2010). Beta lactam drugs continue to be the drug of choice for bacterial infections. Persistent exposure of beta lactam drugs has induced dynamic and continuous production and mutation of β-lactamases in these bacteria, expanding their activity even against the newly developed β-lactam antibiotics

(Patterson and Bonono, 2005). Multi drug resistance particularly in gram negative bacteria soon developed, and hence, the term extended spectrum beta lactamases (Rawat and Nair, 2010). The mutated genes can spread from cell to cell by mobile genetic elements such as plasmids, transposons and bacteriophages. Resistant bacteria flourish in areas of heavy antibiotic use such as hospitals. For each new beta lactam antibiotic drug that was developed, resistance was soon established: for example, the world wide use of the beta lactam drug penicillin resulted in the increased frequency of penicillinase producing *Staphylococci* (Islam *et al*., 2014).

In general, bacterial genes are encoded on plasmids and chromosomes (Barrios *et al*., 2012). Many gram negative genera of bacteria have naturally occurring chromosomally mediated beta lactamases (George, 2009). These enzymes are thought to have evolved from penicillin-binding proteins, with which they show some sequence homology. These plasmids encoded genes could easily be transferred from one bacterium to another through conjugation, transformation and transduction and hence resistance quickly becomes widespread among bacteria (Ramanan *et al.,* 2013).

The first plasmid mediated ESBL isolated was from a woman who had *E. coli* septicemia (Temonieria) and hence the enzymes were named TEM 1 in the 1960s (Rawat and Nair 2010). This was just a single strain of *E. coli*. The TEM 1 is now found worldwide (Karen, 2010) and is now present in the following gram negative group: *Haemophilus influenzae, Psuedomonus aeruginosa* and *Neisseria gonorrhoeae*. Later other types of ESBLs were discovered called Sulphydryl 1 Variable (SHV), which are found in the majority of isolates of *E. coli* and *Klebsiella pneumonia* (Lascols *et al*., 2012). *E. coli and Klebsiella* have emerged as serious pathogens both in hospital and community acquired infections worldwide and account up to about 85% of UTIs caused by ESBL producing *Enterobacteriaceae* (Davies and Davies, 2010). These pathogens have multi drug resistant phenotypes which restricts the efficacy of many antimicrobial agents, thus reducing the therapeutic options significantly and making the provision of an appropriate antimicrobial therapy more challenging with consequent major public health concern.

There have been many reports by different researchers recently concerning the rise in the incidence of UTIs due to ESBL producing *E. coli* (Stefano *et al*., 2013). Few studies are available regarding community-onset UTIs caused by extended-spectrum β-lactamase (ESBL)-producing bacteria in children (Fan *et al*., 2014).

In one prospective study done by Stefano *et al*., (2014) between January 2008 and September 2011, concerning UTI infections due to ESBL producing *E. coli*, 49 patients needed hospitalization. Of these patients, 7 presented with positive blood and urine culture for ESBL positive *E. coli*. The median hospital stay of these patients was 23 days (range 13 to 45 days). Since these patients were reservoirs of ESBL producing *E. coli*, the prolonged hospital stay meant that the patients continued passing the bacteria in the hospital and other patients might have acquired the bacteria from them.

In other reviews found in Europe (1999-2008), records show an increase in the UTI infections caused by *E. coli*. For example, in the study for monitoring antimicrobial resistant trends (SMART) which was done between 2009 and 2010, it was concluded that in Europe ESBL prevalence among *E. coli* was 17.6%, in North America it was 8.5%, Asia had 5%, New Zealand and China had 67% and 61% respectively (Garaua *et al*., 2014). These findings show the significance of ESBL producing *E. coli* and their involvement in causing UTIs. The prevalence of ESBL producing *E. coli* ranges from 1% to 85 % depending on anti-biotic use (Reuland *et al*., 2016), age ≥ 65 years recent hospitalization, prolonged hospital stay, recent surgery, recurrent UTIs, travel to foreign countries, severe illness, immobilization and residence at nursing home (Andreas *et al*., 2016). Since China and New Zealand have aging population, they have higher prevalence compared to other countries.

In Africa, a retrospective study done by Storberg and reported in PubMed (2008-2012) on ESBL with the key words Africa, *Enterobaceriaceae* and ESBL found that the prevalence of ESBL was 16.4% in Kenyan hospitals, 38.8% in Rwanda and 56% in Kenya. Niger had 40%, Nigeria 27.5% and South Africa had 5% (Storbeg, 2014). These findings from studies carried out in other African countries, though not in Zambia are significant enough to prompt research about these microorganisms in our environment.

In Zambia studies on ESBL producing *E. coli* have been conducted more on animals (Chishimba *et al*., 2016) with very little information concerning infections in humans. ESBL-producing *E. coli* have gradually become co-resistant to other broad-spectrum antibiotics notably ciprofloxacin, norfloxacin and chloramphenicol (Fan *et al*., 2014). UTIs caused by such resistant organisms lead to a longer hospital stay and more antibiotic usage.

2.2 *E coli* Colonization and invasion

Uropathogenic *E. coli* causes 90% of infections in anatomically normal unobstructed UTIs. The bacterium usually begins by colonizing the perennial region before ascending to the upper urinary tract via the urethra. Because females have a shorter urethra, the frequency of UTIs is 14 times more in females than in men (Brusch, 2016). The ureter of pregnant women widens which results in impaired drainage and this increases the risk of bacterial colonization, women are also likely to get a UTI when they have a new sexual partner (Ho *et al*., 2010).

A number of virulence factors are responsible for the pathogenicity and invasion by *E. coli.* The bacterium has an adhesin called P. fimbria or pyelonephritis associated pilli which is used for attachment (Melican *et al*., 2011). The bacterium attaches to the uroepithelial cells when moving up the urinary tract. This attachment prevents the bacterium from being washed away by flow of urine. The P fimbria has the ability to specifically bind to the P blood group antigen which contains a D galactose residue. The fimbriae bind not only to red cells but to a specific galactose disaccharide that is found on the surfaces of uroepithelial cells in approximately 99% of the population (Antão *et al*., 2009).

The other virulence factor is the type 1 fimbrae which provides a supplementary mechanism of adherence and also plays a role in aggregating the bacteria to a specific manosyl-glycoprotein that occurs in urine (Melican *et al*., 2011).

In order to survive in the host, the bacterium requires iron. This iron has to be obtained from the host. The bacteria use siderophores to capture iron from the host during and after colonization (Hiten, 2010). This iron has to be captured by destruction of the host cells. The host cells are perforated by cytotoxic hemolysins which form trans-membranous pores in the host cells. Once

iron and other nutrients are released from cells which have been perforated, the bacteria capture these and use them for growth. The alpha-hemolysins of *E. coli* also lyse lymphocytes, and the beta-hemolysins inhibit phagocytosis and chemotaxis of neutrophils which allows the pathogen to proliferate (Antão *et al.*, 2009) host cells.

E. coli are resistant to the compliment dependent bactericidal effect of serum. The presence of k-antigens is associated with upper UTIs (Hooton *et al.*, 2010). K antigens of *E. coli* are capsular antigens that may promote bacterial virulence by decreasing the ability of antibodies and/or complement to bind to the bacterial surface. K antigens also decrease ability of phagocytes to recognize and engulf bacterial cells (Bien *et al*., 2012).

2.3 Establishment of UTI caused by *E. coli*

E. coli is the most frequent bacterium that is found in human fecal flora. This bacterium colonizes the perineum before causing an ascending infection in the urinary tract (Tenaillon *et al.*, 2010). *E. coli* has special properties that enable it to overcome host defenses. It can move into a new niche devoid of other bacterial species (Slavchev *et al.*, 2009). According to Najar *et al.*, (2009), *E. coli* is the most common cause of UTI among virtually every patient group and accounts for 80 to 90% of cases of uncomplicated pyelonephritis and cystitis among otherwise healthy women.

Infections in children are often due to blockages in the urinary tract, resulting in pools of stagnant urine (Najar *et al*., 2009). *E. coli* that causes UTI is distinguished from related members of its genus and species by the presence of specific virulence determinants and microbial adaptations promoting success in the urinary tract (Bien *et al*., 2012). Virulence determinants allow uropathogenesis to overcome intrinsic urinary tract defense mechanisms that normally protect the host against UTIs according to Subashchandrabose and Mobley (2015). Such factors include the flow of urine that washes away bacteria in the urinary tract, variable pH which might decrease bacterial metabolism since different bacteria have different pH values at which optimum bacterial metabolism occurs, lack of nutrients which prevents bacterial proliferation, exfoliation of epithelial cells which results in bacteria being washed away by urine, and the

production of cytokines that result in recruitment of polymorphonuclear leukocytes to the bladder mucosa.

Infections resulting in inflammation can occur in the urethra (urethritis), bladder (cystitis) and kidneys (pyelonephritis). In urethritis there is moderately severe pain associated with burning on voiding urine. Suprapubic pain is associated with cystitis. Pyelonephritis is the most severe and is associated with fever, chills, and flank pain from renal inflammation (Flores-Mireles *et al.*, 2015).

2.4 Disease determinants

E. coli UTIs occur as a result of various combinations of a number of factors and determinants that include the following:

2.4.1 The role of the environment in the host parasite relationship

The influence of adverse environmental conditions on the outcome of the infections has been recognized for many centuries. Stress has been and is still found to frequently account for individual cases of disease (Louise *et al*., 2011).

During an ascending infection in the urinary tract, the bacterial cells come in contact with human urine prior to reaching the bladder or kidneys (Jonstone *et al*., 2006). The presence of significant amounts of inorganic ions and urea in urine imposes osmotic and denaturing stresses on bacterial cells (Withman *et al*., 2013). A study to determine the transcriptional adaptive responses of UPEC strain CFT073 to the presence of 0.3 M NaCl or 0.6 M urea in the growth medium was carried out by Withman *et al.*, (2013). The cell responses to these two osmolytes were drastically different. Although most of the genes of the osmotically inducible regulon were overexpressed in medium with salt, urea failed to stimulate osmotic stress response. At the same time, UPEC colonization genes encoding type 1 and F1C fimbriae and capsule biosynthesis were transcriptionally induced in the presence of urea but did not respond to increased salt concentration. It is speculated that urea can potentially be sensed by uropathogenic bacteria to initiate infection (Subashchandrabose and Mobley, 2015). In addition, several molecular chaperone genes were overexpressed in the presence of urea, whereas adding NaCl to the

medium led to an up-regulation of a number of anaerobic metabolic pathways (Withman *et al*., 2015).

2.4.2 The role of host factors in the host parasite relationship

A variety of host factors play an important role in disease establishment. Humans infected with HIV and those with AIDS tend to have a low lymphocyte count which results in a compromised immune system and hence are more susceptible to uropathogenic *E. coli* infections. T and B lymphocytes are responsible for the manufacturing of antibodies and memory cell which are vital components of the acquired immunity (Denise *et al*., 2004).

Metals such as Iron are required for microbial growth (Hoffmann *et al*., 1999). The majority of bacteria including *E. coli* will grow readily in a wide range of iron concentrations (from 1.0um to 1000 um). In order to limit microbial growth, the host must render iron non available. This is achieved by virtue of the presence of iron binding proteins which bind to the metal so tightly that there is no free iron for bacterial reproduction (Lewis, 2010). Another example of the influence of host factor is the effect on the administration of Vitamin A in inducing nonspecific resistance to experimental infection. The mechanism may be associated with an increase in the bacterial capacity of phagocytic cells (Villamor and Fawzi, 2005).

2.5. Pathogenicity of Uropathogenic *E. coli* (UPEC)

The pathogenicity of Uropathogenic *E. coli* **(**UPEC) differs from non-pathogenic *E. coli* and from other E. *coli* pathotypes. The UPEC produce specific virulence factors, which enable the bacteria to adhere to uroepithelial cells and establish UTIs (Bien *et al*., 2012). Besides adherence factors, toxins, modulins, capsules, and iron uptake systems contribute to the virulence of the strains (Lewis, 2010).

In order to colonize and establish a UTI, UPEC strains take advantage of an assortment of virulence properties (Drawz and Bonomo, 2010). Bacterial adherence to and colonization of the urinary tract by UPEC strains are mediated by the expression of several types of fimbriel and

nonfrimbriel adhesins according to Subashchandrabose (2015). Type I and P fimbrie, the most common fimbrie found in UPEC strains, enhance virulence and are involved in initial urethral colonization. By attaching to host structures, these microbial pathogens avoid being swept along by the normal flow of urine (Wiles *et al*., 2008).

E. coli strains secrete a cytolytic protein known as alpha hemolysin according to Garcia *et al*., (2011). Approximately half of UPEC strains that cause upper UTIs, about a third of those that cause lower UTIs, and only about 10 % of fecal isolates produce a hemolysin. These Hemolysin molecules insert into lipid-containing membranes producing cation-selective channels of large conductance with a diameter of 2 nm that increase the permeability of erythrocyte membranes to Ca2+, K+, mannitol, and sucrose. In addition to lysing erythrocytes, hemolysin is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury, and impaired host defenses Clara *et al*., 2011). Such toxic activity may contribute to the kidney damage seen in pyelonephritis.

Aerobactin, a bacterial siderophore, has recently been shown to be associated with *E. coli* strains which cause pyelonephritis and cystitis (Subashchandrabose and Mobley, 2015). It is an iron sequestration and transport system which enables *E. coli* to grow in iron poor environments such as dilute urine and complement-depleted serum. The aerobactin system is associated with *E. coli* isolates from serious UTI and other serious infections in humans and animals, probably because it promotes bacterial growth in the limiting iron concentrations encountered during infection (Porcheron *et al*., 2013).

Many of the virulence genes of pathogenic strains of *E. coli* are carried in large multigene chromosomal segments called pathogenicity islands (PAIs) that are absent from normal fecal and laboratory K-12 strain of this bacterium (Lloyd *et al*., 2007). The uropathogenic strain 536 contains two large unstable pathogenicity islands: PAI-In (70 kb) and PAI-II (190 kb) according to Samei *et al*., (2016). Both pathogenicity islands are flanked by short (16 - 18 bp) direct repeats, which are likely responsible for their deletion due to recombination at a frequency 10-3. PAI-I encodes the *hly* hemolysin operon. PAI-II encodes another *hly* operon and *prf* (P-related fimbriae) pilus operon. Uropathogenic strain J96 contains two pathogenic islands inserted in two

different tRNA genes (pheV at 64 min for PAI-IV and pheR at 94 min for PAI-V (Samei *et al*., 2006).

2.6 Control and Treatment

Control of ESBL organisms is challenging because of the rapidity with which resistance to the new drugs develop. Carbapenems (Imipenem, Ertapenem, Meropenem and Doripenem) are considered drugs of choice for the treatment of ESBLs producing *E. coli* infections (Souha *et al*., 2011). Ertapenem is given once a day and can be given intramuscularly unlike the other carbapenems which are given intravenously. This makes Ertapenem a better option in patients with intravascular abscesses. Carbapenems are however very expensive and very broad spectrum agents. The emergency of carbapenem resistance *Enterobacteriaceae* has made treatment and control of ESBL producing organism even more difficult and as such researchers and clinicians are now resorting to old antibiotics which were initially used for the tretamen of UTI like colistin which are nephrotoxic (Hughes and Anderson, 2017) Colistin can be used as a last resort since there are no new gram negative anti-biotics that are being made (Shaikh *et al*., 2015). Deanna *et al*., (2017) isolated *Psudomonas* resistant to carbapenems in a retrospective cohort study that was done in Pittsburgh Medical Centre (UPMC) between 1 January 2009 and 30 September 2014.

Fosfomycin is reported to have admirable in vitro activity against the ESBL-producing *E. coli* or *K. pneumoniae* (Ho *et al.,* 2015). Colistin is another antibiotic that can be considered even though it is nephrotoxic.

Although beta lactamases are inhibited by clavulanic acid, a combination of a beta lactam drug and clavulanic acid rarely gives optimum results since the majority of ESBL producing micro-organisms produce more than one type of beta lactamase (Rawat and Nair, 2010). The ESBL-producing organisms may also continue to harbor parent enzymes (for example, SHV-1 or TEM-1). Hyper production of these non–ESBL-producing β-lactamases or the combination of β-lactamase production and porin loss can also lead to a reduction in activity of β-lactamase

inhibitors. The advantages of using β-lactamase inhibitors is that they inhibit ESBLs and appear to impair the emergence and spread of *Klebsiella*-carrying resistance plasmids. The administration of inhibitors may exert *in vitro* pressure on ESBLs, thereby facilitating their reverse mutation to less harmful enzymes.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study design

The study was a cross sectional design involving the collection of urine samples from UTI patients and establishment of ESBL productivity and characterization of *E. coli* type I integrons. The samples were collected from February to June 2017.

3.2 Study area

This study was conducted at the University Teaching Hospital which is located along Nationalist road (joins Burma Road and Independence avenue road) opposite the central statistics office.

3.3 Sampling frame.

Urine samples were collected from patients who presented with UTIs at the University Teaching Hospital. Samples were collected from both in-patients and out patients at the Out Patient Department (both high and low cost), Gynecology ward, Clinic 7 and Clinic 3. Attending clinicians and nurses helped in the identification of patients who had UTIs.

3.3.1 Inclusion Criteria

The study recruited patients of both sexes between 6 years - 60 years who had UTIs and had given consent to take part in the study.

3.3 2 Exclusion Criteria

i. Patients who were not suffering from UTIs
ii. Patients below 4 years
iii. Patients who did not consent to take part in the study were excluded.

3.3.3 Sample Size

The urine samples came from a finite population and hence Shafer et al's sample size calculation was used. The prevalence (p) of ESBLs producing *E. coli* bacteria in Zambia is not known and hence the prevalence was set at 50%. So P=0.5, Z=1.96, e= 0.04 and N= 10 000. The sample size

n= a/b were a= Z^2 P (1-P)/e and b= 1+ (Z^2. P (1-P)/e^2N. This gave n=297 as the minimum number of patients that were recruited.

3.4 Urine sampling

Patients who signed the consent forms were given labeled sterile, dry, leak-proof containers containing a measured amount of boric acid powder (0.1g/10ml of urine) in which they voided mid-stream urine (Alanazi *et al* 2018). For catheterized patients, the urine sample was collected from the sampling ports of the catheter bags (Nicolle, 2014) in order to avoid contamination of the urine. The specimens were sent to the microbiology laboratory within 48 hours of collection. Medical doctors from the sites were briefed about the study and assisted in the identification of UTI patients.

Specimen collection was carried out by the investigator with permission from the ward in-charge and attending clinician. Microbiological examination of specimens was done from the University Teaching Hospital bacteriology laboratory while PCR was done from the Centre for Zoonosis Control, University of Zambia, School of Veterinary medicine.

3.5 Ethical consideration

Ethical clearance was obtained from UNZABREC and a written consent and assent form was obtained from each patients or bed-sider after the study was explained fully accompanied with a hand-out information sheet. Patients were told how the specimen they provided was to be used, decontaminated and then disposed of. Some participants feared that their results would be made known to other people. The study participants were however assured of privacy and strict confidentiality of all findings. Another concern by participants was the fear that once they agreed to participate in the study they would not be able to withdraw. However, all participants were assured that they were free to withdraw from the study at any point without giving any reasons and with no compromise to their treatment. Participants were assured that they would still receive the best treatment available that is always offered by UTH.

3.6 Laboratory procedures for screening of ESBL production

Initial screening was done by inoculating each sample on MacConkey agar (Oxoid, UK) supplemented with cefotaxime at a screening concentration of 2µg/ml and incubated at 37ºC for 24 hours. Growth on this screening media with the antibiotic was suggestive of ESBL production (Patterson and bonomo, 2005). The positive cultures were then sub cultured on MacConkey agar and pure colonies were then obtained. Phenotypic confirmatory test for ESBL production was done with a cefotaxime disk (30µg) alone and in combination with clavulanic acid (30µg /10 µg) (Rawat and Nair 2010). Phenotypic confirmatory test was based on 5mm increase in zone diameter for cephalosporin and its cephalosporin/clavulanic disk combination. To ensure that there was quality in the work that was done, positive and negative control samples were tested on the media. The ESBL positive control samples grew on the media and the negative samples did not grow. To ensure that there was no contamination of the media used, one plate was not inoculated and no organisms grew on that plate.

For genetic detection, ESBL producing *E. coli* were cultured in BHI (Brain Heart Infusion, HiMedia laboratories, India) at 37ºC for 18 hours. After incubation, 1ml of bacterial suspension was centrifuged at 5800 *x g* for 5 minutes (Patterson amd Bonomo, 2005). After centrifuging, the supernatant was discarded. The remaining cell pellet was washed with 500µl of normal saline, centrifuged at 13000 x g for 5 minutes and the supernatant discarded (Fatemeh *et al*., 2012). After washing, 500µl of TE buffer (pH 8.0) was added to the cell pellet and then heat treated until boiling, then immediately transferred to ice for 10 minutes. The cell debris was removed after centrifuging at 13000 *x g* for 5 minutes, while the supernatant was transferred into a new microfuge tube and stored at -20ºC until use (Changkaew *et al*., 2015).

The master mix per reaction tube was made with 5µl of Phusion Flash, 2µl sterile water, 1µl of reverse primer, 1µl of forward primer and 1µl of DNA template, giving total volume of 10µl per reaction tube, which was mixed using a vortex mixer (Shawa, 2015). The PCR primers used for the detection of *bla*TEM, *bla*SHV and *bla*CTX-M are indicated in Table 3.1. The PCR conditions were set as 98℃ for 30 seconds, 98℃ for 0 seconds, 60℃ for 5 seconds (35 cycles), 72℃ for 15 seconds, 72℃ for 2 minutes, and holding at 4℃ infinitely (Jorgenson *et al*., 2010).

After PCR, the products were visualized on a trans-illuminator machine following staining with ethidium bromide (Patterson and bonomo, 2005)

Table 3.1 Primers used in this study

Gene	Primer	Sequence	Expected amplicon size
bla$_{TEM}$	*bla*$_{TEM}$ –F	5'TCGGGGAAATGTGCG3'	1074
	bla$_{TEM}$ –R	5' TGCTTAATCAGTGAGGCACC3'	1074
bla$_{SHV}$	*bla*$_{SHV}$ –F	5'GCCGGGTTATTCTTATTTGTCGC 3'	1016
	bla$_{SHV}$ –R	5' ATGCCGCCGCCAGTCA 3'	1016
bla$_{CTX-M}$	*bla*$_{CTX-M}$– F	5' CGATGTGCAGTACCAGTAA 3'	585
	bla$_{CTX-M}$– R	5' TAAGTGACCAGAATCAGCGG 3'	585
bla$_{INT}$	*bla*$_{INT-F}$	5′-CCTCCCGCACGATGATC-3′	280
	bla$_{INT-R}$	5′- TCC ACG CAT CGT CAG GC-3′	280

The bla*CTX-M* positive isolates were subjected to antimicrobial sensitivity test using the Kirby-Bauer agar disk diffusion method. Antimicrobial discs were placed on growth plates of Muellar-Hinton agar (Becton Dickinson and company, USA) and incubated at 37ºC for 24 hours. The following commercially available antimicrobial discs (Becton Dickinson and company, USA) were used: ampicillin (AMP), trimethoprim-sulphamethoxazole (SXT), streptomycin (STR), tetracycline (TET), gentamycin (GEN), nalidixic acid (NAL), ceftazidime (CAZ),

chloramphenicol (CHL), norfloxacin (NOR), ciprofloxacin (CIP) and cefotaxime (CTX). The inhibition zone diameters were measured and interpreted according to the manufacturers' instructions based on guidelines laid down in the Clinical and Laboratory Standards Institute (2009).

All resistant isolates were tested for the presence of class 1-integrone genes by PCR using primers INT-1U and INT-1D. PCR reaction mixture (total 20 µl) consisted of 1 µl of DNA template, 1× Green GoTaq reaction buffer, each dNTP (0.2 mM), 1 µl of each primer (0.4 µM) and 0.5 U of GoTaq DNA polymerase (Kotlarska *et al*., 2015). The reaction parameters were 35 cycles at 98, 56 and 72 °C for 10 sec, 10 sec and 1 min, respectively, followed by a final extension at 72°C for 4 min. PCR products were analysed by electrophoresis using 1% agarose gel and the sizes of the PCR products were determined using a 100-bp DNA ladder and lambda *Hind* III digest as size markers (Barrios *et al.,* 2012).

All class 1 integrase PCR-positive strains were further analysed for their gene cassette patterns by amplifying the variable region between the 5'-conserved segment and 3'-conserved segment. The following four primer sets were designed and employed: 5'CS2 (F) with 3'CS2 (PCR reaction mixture (total 20 µl) consisted of 1 µl of DNA template, 1× Ex Taq buffer, each dNTP (0.25 mM), 1 µl of each primer (0.5 µM) and 0.5 U of Ex Taq (Takara Biomedicals, Tokyo 3.5.6, Japan). The reaction parameters were 35 cycles at 98, 56 and 72°C for 30 sec, 30 sec and 2 min, respectively, followed by a final extension at 72°C for 7 min.

3.7 Data analysis.

Culture and PCR were used to detect the presence of ESBL producing *E.coli* (objective 1). PCR was used to detect the resistance genes of interest (objective 2). For the third objective the dependence of the categorical response variable (ESBL result) on the hypothesized explanatory variables (which was categorical) was modeled using logistic regression. Statistical significance was set at $P \leq 0.05$.

Susceptibility patterns of non-beta lactamase antibiotics among ESBL producers and non-ESBL from producers were determined using the anti-microbial sensitivity test method. The rate of

antimicrobial resistance and ESBL-producing isolates were analysed using SPSS version 20. A comparison of antimicrobial resistance phenotype between ESBL-producing isolates and non ESBL-producing isolates was determined by the chi-square test. Differences were considered significant at $P < 0.05$.

3.8 Summery of work flow

The summery of the work flow has been provided in figure 3 below. The urine was cultured followed by phenotypic identification of the bacteria. Bacterial resistance genes of interest were detected using PCR

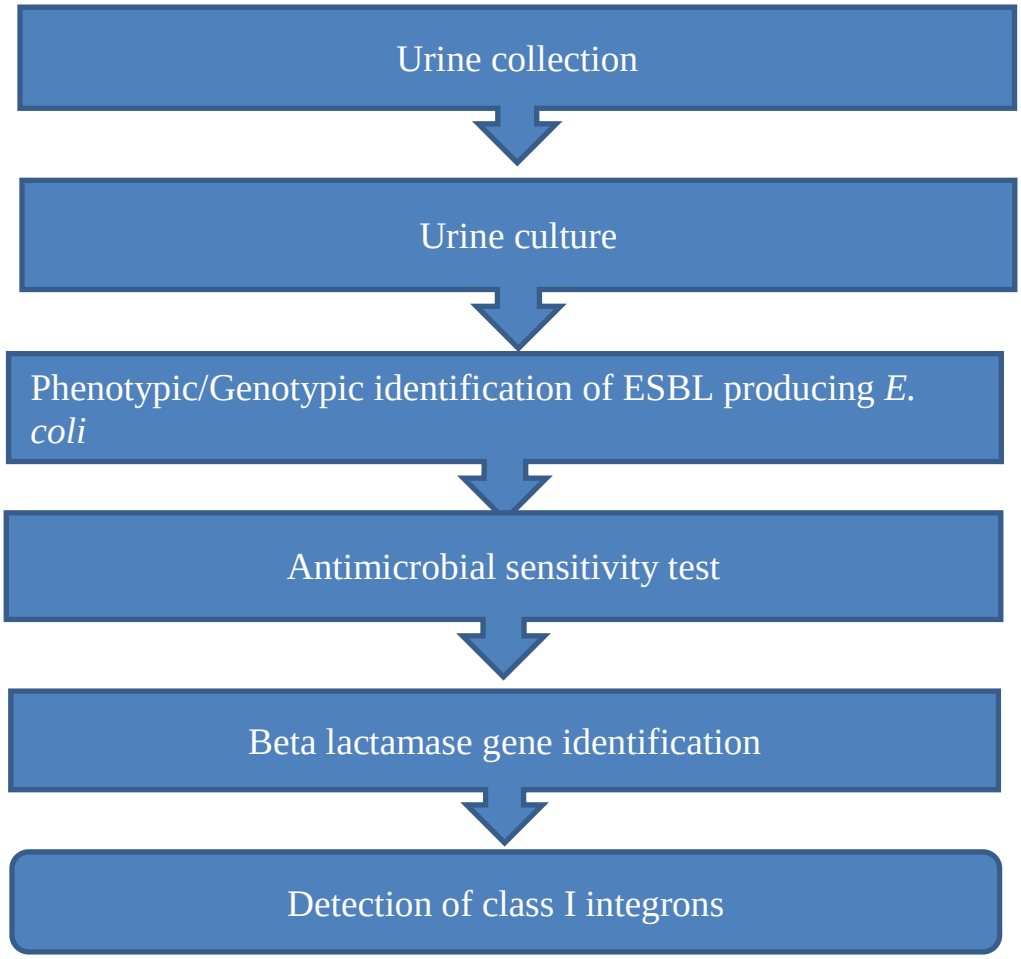

Figure 3.1 Summary of work flow

CHAPTER 4
RESULTS

4 .1.0 Demographic Profiles

This was a cross sectional study which involved the collection of urine samples from patients who were diagnosed with UTIs suspected to be caused by ESBL producing *E. coli*. The research sought to detect the presence of ESBL producing *E. coli* and class I integrons in urine specimens from infected people. To achieve this aim, the bacteria had to be isolated and the genes of interest were then detected using PCR. The information on the risk factors associated with ESBL infection was collected from patients using questionnaires. Antibiotic susceptibility patterns of the isolated bacteria was then done in order to establish sensitivity patterns.

4.1.1 Gender

This study recruited 327 participants over four months. Of these 54% (176) were male and 46% (151) were female. The study had more males than females (Figure 4.1)

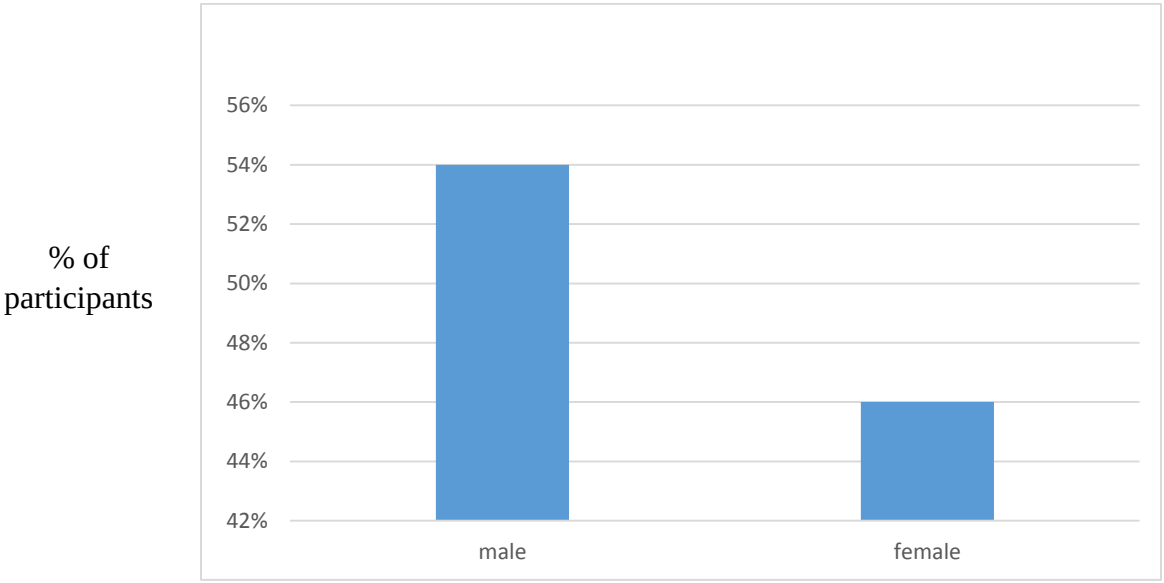

Figure 4.1: Gender distribution of Participants

4.1.2 Marital status

A total of 144 (44%) of the participants were single, 160 (49%) were married, 23 (7%) were divorced while 19 (6%) were widowed (Table 4.1).

Table 4.1: Marital status

Marital status	Frequency	Percentage
Married	144	49%
Divorced	160	7%
Single	23	44%
Total	327	100%

4.1.3 Age of participants

A total of 164, (50%) of the participants in this study were in the age range of 20-40 years. 111 (34%) were above 40 years while 52 (16%) were below 20 years (Table 4.2).

Table 4.2: Age of participants

Age of participants	Frequency	Percentage
Below 20 years	52	16%
20-40 years	164	50%
Above 40 years	111	34%
Total	327	100

4.1.4 Education

Of all the participants in this study, 110 (33%) had no formal education or they only went up to grade 7, 85 (26%) made it up to junior high school, 78 (24%) finished grade 12, 42 (13%) had a diploma while 13 (4%) had a degree (Table 4.3).

Table 4.3: Education level of participants

Education level	Frequency	Percentage
No formal education – Grade 7	110	33%
Grade 9	85	26%
Grade 12	78	24%
Diploma	43	13%
Degree	13	4%
Total	327	100%

4.1.5 Hand washing after using the toilet

A total of 167 (51%) of the respondents said they wash their hands with soap after using the toilet, 147 (45%) without soap while 13 (4%) did not wash their hands at all after using the toilet (Table 4.4)

Table 4.4: Hand washing after using the toilet

Handwashing after using the toilet	Frequency	Percentage
Absent	13	4
Without soap	147	45
With soap	167	51
Total	327	100

4.1.6 History of Hospitalisation

A total of 98 (30%) of the study participants were currently hospitalised while 29 (9%) had been hospitalised in the past 3 months, 105 (32%) had been hospitalised more than 3 months ago at the time that the study was being conducted and 95 (29%) had never been hospitalised. Of the participants that were currently hospitalised, 66 (67%) had been hospitalised for less than a week, 21 (21%) for 2-3 weeks while 12 (12%) were hospitalised for more than 3 weeks (Table 4.5)

Table 4.5: History of hospitalization

History of hospitalization	Frequency	Percentage%
Never hospitalized	95	29%
Hospitalized in the past three months	29	9%
Hospitalized more than three months ago	105	32%
Currently hospitalized	98	30%
Total	327	100%

4.1.7 Medical history of study participants

A total of 209 (67%) of the respondents had used antibiotics in the past 3 months while 108 (33%) had not used antibiotics in the past three months. Of these participants 226 (69%) had a history of Surgery while 101 (31%) had no history of surgery. A total of 234 (82%) of the respondents had never had underlying urinary system infection while 234 (28%) of the patients had an underlying urinary system infection. Of the total respondents in this study 52 (16%) were currently catheterised, 7 (2%) were catheterised less than three months ago, 52 (16%) were catheterised more than 3 months ago while 216 (66%) had never been catheterised, 52 (16%) at the time that this study was being conducted.

4.1.8 **Summary of data for the medical history of all participants**

The figure below (figure 4.1b) shows the summery of the medical data of all patients that participated in this study. The medical history includes history of antibiotic usage in the past three (3) months, history of surgery, presence of underlying UTI and history of catheterization in the past 3 months.

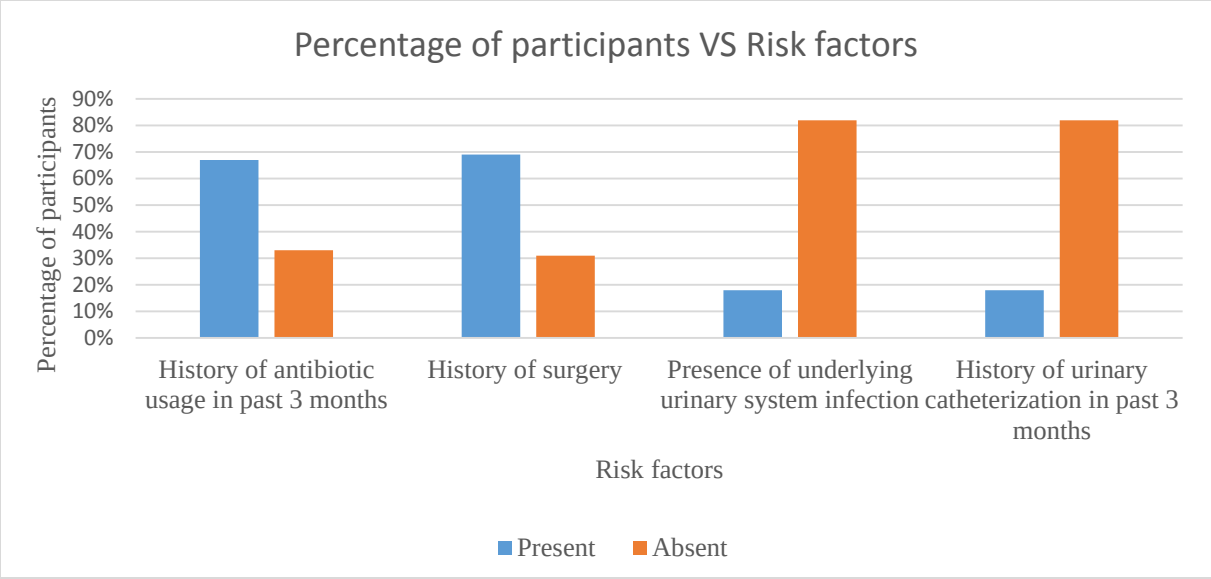

Figure 4.2: Medical history of study participants n=327

4.1.9 **Summary of data for patients who had UTIs caused by ESBL producing *E. coli***

Figure 4.3 below shows the summery of data for patients that had ESBL producing *E. coli* which includes antibiotic usage, history of surgery, history of UTIs and use of a urinary catheter.

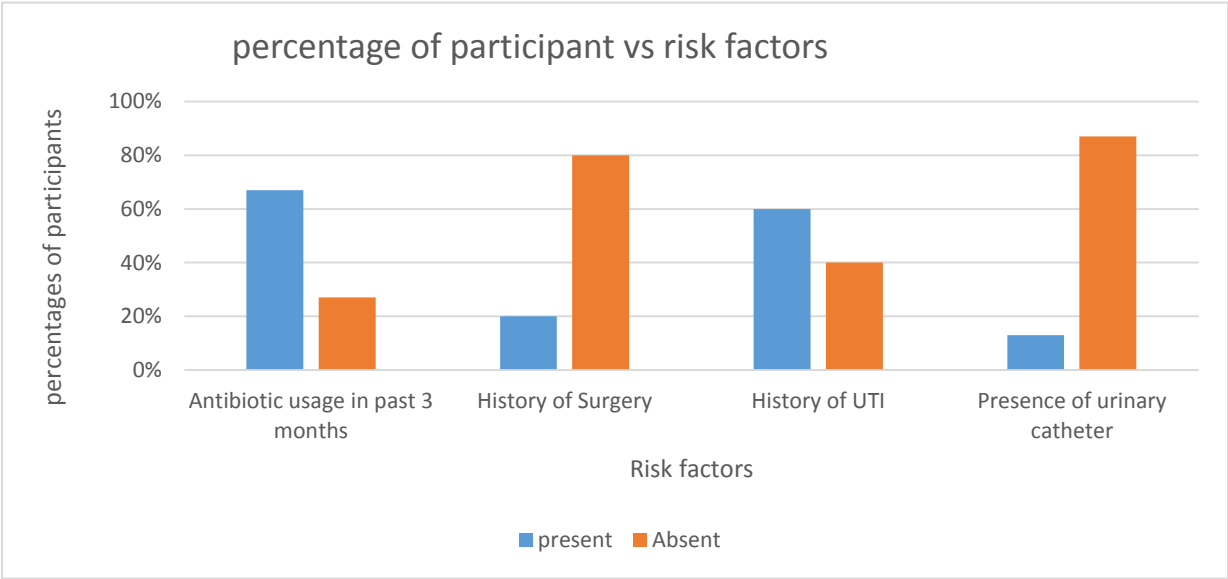

Figure 4.3: Data distribution for patients who had UTIs caused by ESBL producing *E. coli* n=15

4.1.10 Summery of risk factors of patients who UTIs caused by ESBL producing *E. Coli*

Table 4.6 below shows the risk factors patterns for patients that had ESBL producing *E. coli*. The majority of the patients (9/15) had a history of UTI with catheterization or hospitalization.

Table 4.6: Table of risk factors n=15

Risk factor	No of patients
History of UTI + Catheterization or Hospitalization	9
History of UTI + No catheterization AND Hospitalization	2
No history of UTI +Catherised + Hospitalized	1 (More than 3 weeks)
No history of UTI + No cather + hospitalised Hospitalized	1 (1 Week)
No History + no catheter + no hospitalization	1
No history + No catheter + Hospitalized < 3 months ago	1

4.2 Genotypic characteristics

A total of 327 urine samples were collected and out of these 15 (4.5%) samples were confirmed to be ESBL producers. Almost all the 15 isolates (93%) ESBL producers were positive for the *bla*CTXM gene. The PCR picture below (Figure 4.4) confirms the results for the *bla*CTXM gene result. Lanes 1-2 and 4-15 were positive while lane 3 was negative.

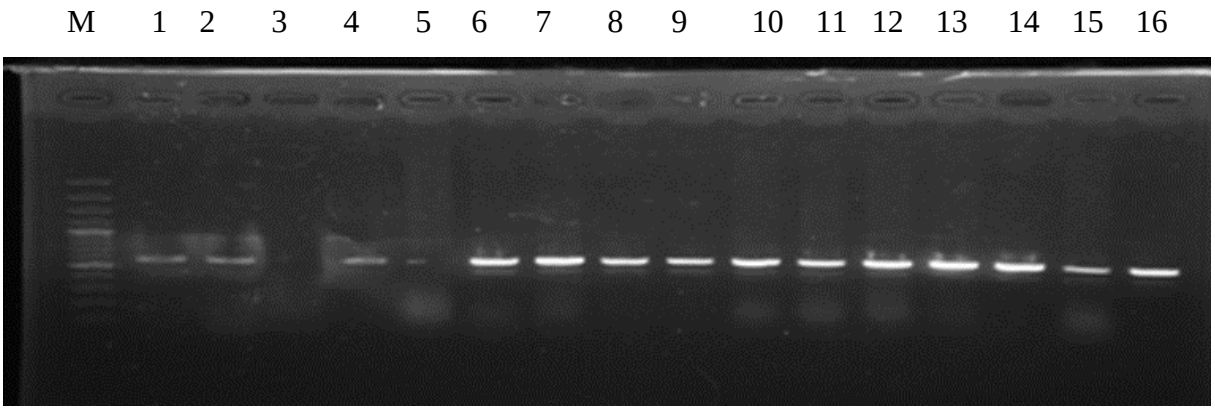

Figure 4.4: PCR results for *bla*CTX-M gene. M; DNA ladder, Lane 1 to 15 are samples, while Lane 16 is the positive control.

The picture below (Figure 4.5) shows results for the *bla*SHV gene. Lanes 4, 8 and 13 were the only positive lanes.

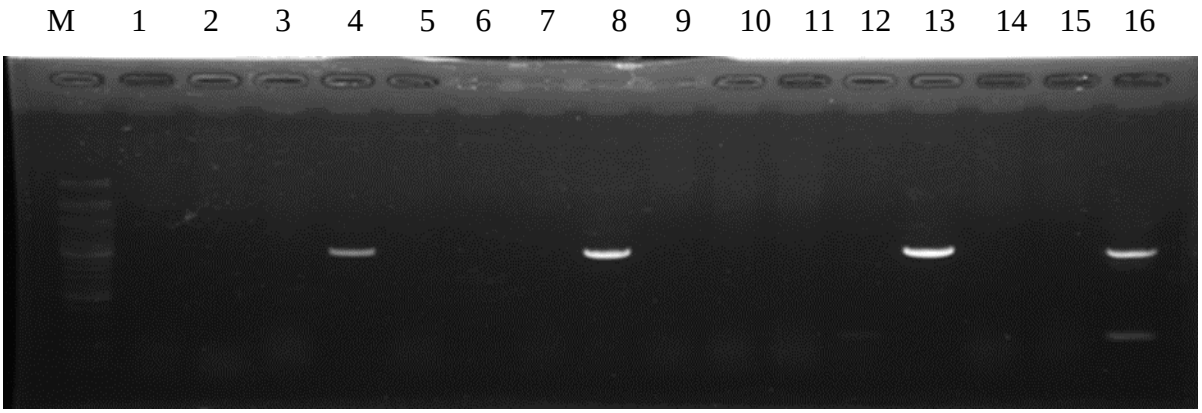

Figure 4.5: PCR results for *bla*SHV gene. M is DNA ladder, Lane 1 to 15 are samples, while Lane 16 is the positive control.

The picture below (Figure 4.6) confirms results for the *bla*TEM. Lanes 4, 8 and 13 were the only positive lanes.

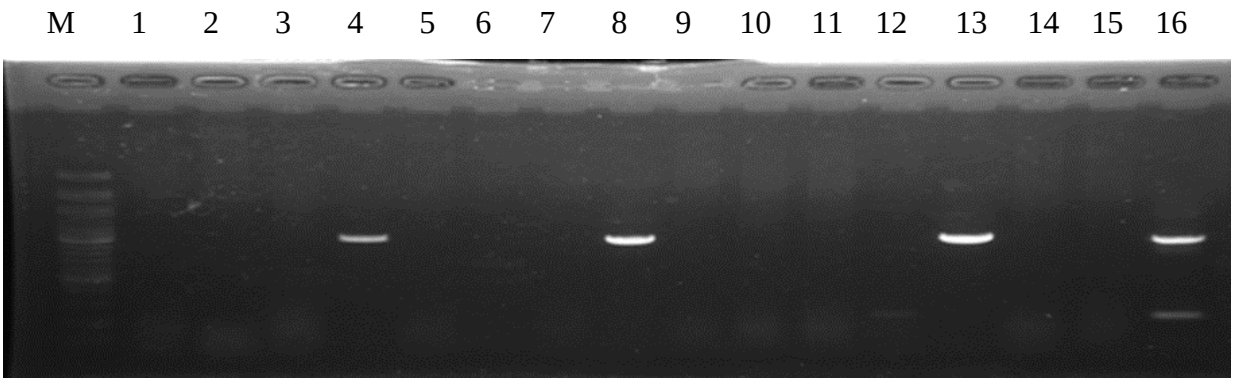

Figure 4.6: PCR results for *bla*TEM gene. M is DNA ladder, Lane 1 to 15 are samples, while Lane 16 is the positive control.

PCR analysis revealed that 7 (47%) ESBL producers had the INT class I integrin (figure 4.7). The picture below comfirms this. The only positive lanes were lane 4,6,8,9,12 and 13.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

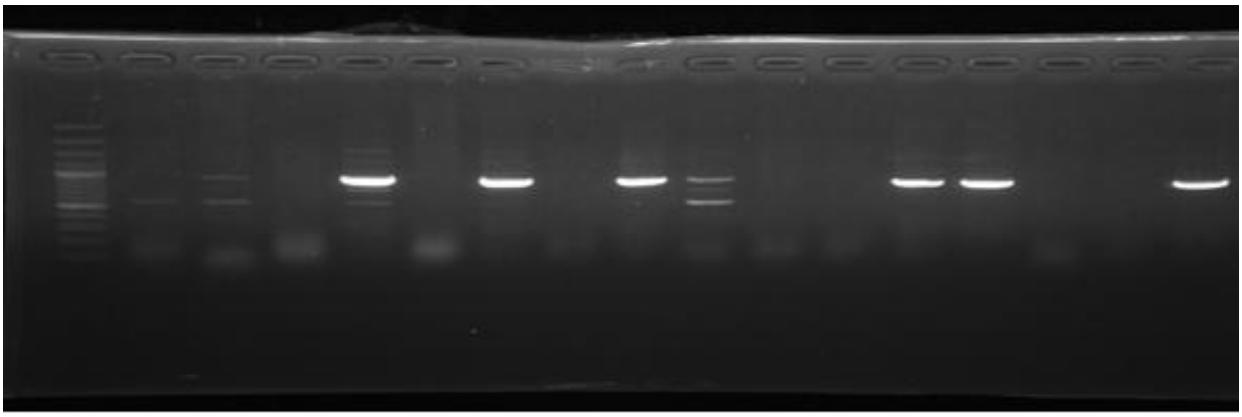

Figure 4.7: PCR results for *bla*INT gene. M is the DNA ladder, Lane 1 to 15 are samples, while Lane 16 is the positive control.

Of the 15 samples that were analysed, 3 samples had the CS2 genes (Figure 4.8). The lanes that had the CS2 were lanes 4, 6 and 12.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

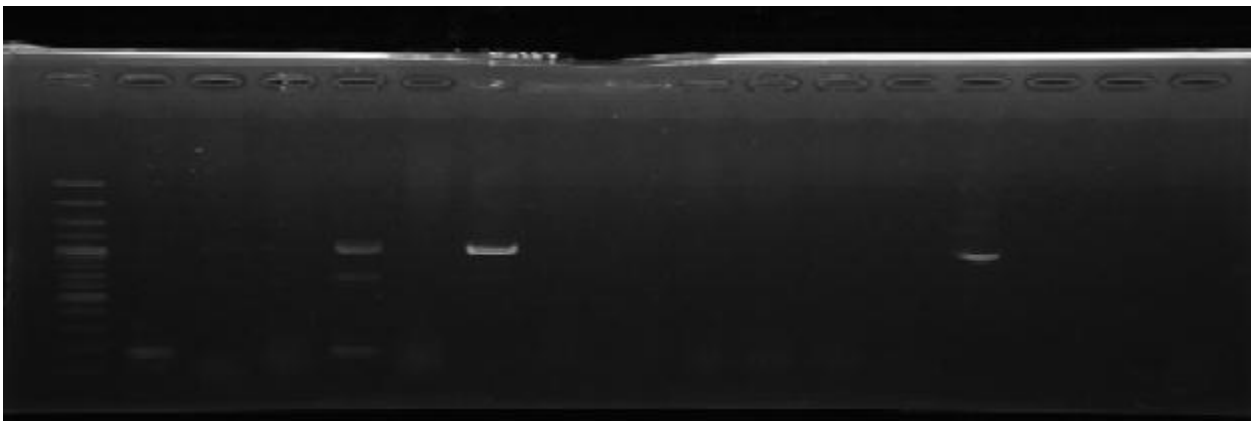

Figure 4.8: PCR results for *bla*CS2 gene. M is the DNA ladder, Lane 1 to 15 are samples, while Lane 16 is the positive control.

Table 4.2 below shows the proportion of the resistance genes that were detected in the bacterial isolates. The *bla*CTX gene had the highest percentage (93%) according to Table 4.7.

Table 4.7 ESBL resistance genes

Genes	Present	Absen
*bla*INT	4 (27%)	11 (73%)
*bla*CTX	14 (93%)	1 (7%)
blaSHV	7 (47%)	8 (53%)
*bla*TEM	3 (20%)	12 (80%)
*bla*CS2	3 (20%)	12 (80%)

4.3 Antimicrobial susceptibility results of *E.coli* isolates

All the 15 ESBL producers were cultured on Mueller Hinton agar for the antimicrobial susceptibility tests. A total of 10 antibiotics were used. The antibiotics that were used in this study are listed in Table 4.8.

Table 4.8 Antimicrobial susceptibility results in CTX-M producing *E. coli* isolates (n=15)

Antibiotic	Resistant		Sensitive	
	Number of isolates	Percentage	Number of isolates	Percentage
Ampicillin (A)	15	100	0	0
Cotrimoxazole (Co)	15	100	0	0
Teyracycline (TE)	8	53	7	47
Getamycin (G)	2	13	13	87
Nitrofurantoin (NIT)	15	0	0	100
Chloramphenicol (C)	6	40	9	60
Nalidixic acid (NA)	7	47	8	53
Cephotaxin (CTX)	13	87	2	13
Norfloxacin (NX)	7	47	8	53
Ciprofloxacin (CIP)	7	47	8	53

All the isolates had multi drug resistant patterns (resistant to at least 3 of the 15 antibiotics used). Three (3) isolates were resistant to ampicillin, cotrimoxazole and cefotaxime. Four isolates were

resistant to ampicillin cotrimoxazole cefotaxime and tetracycline. The table below (Table 4.9) shows the antibiotic susceptibility patterns.

Table 4.9: Antimicrobial susceptibility pattern in CTX-M producing *E. coli* isolates (n=15)

Resistance Combination	Number of isolates
A, CO, CTX	13
AMP, CO, CTX, TE	3
AMP, CO, CTX, CIP	7
AMP, CO, CTX, TE, C	3
AMP, CO, CTX TE,	5
AMP, CO, CTX, C, NA	3
AMP, CO, CTX,TE, NA	2
AMP, CO, CTX,TE, G	2
AMP, CO, CTX, TE, G, CTX	2
AMP, CO, CTX, TE, G, C, NA, NX, CIP	1
AMP, CO, CTX, TE, C, NA, NX, CIP	1

4.4 Risk factors

Marriage, History of surgery, Use of antibiotics in the past three month's presence of a urinary catheter were not significant predictors for the development of urinary tract infections. Marriage, history of surgery, use of antibiotics and presence of a catheter had the odds of 0.060, 0.111, 0.611 and 0.439 less likely to cause UTIs respectively (Table 4.10)

Tabl4 4.10 risk factors

Variable	Odds ratio	95% confidence interval for odds ratio	
		Lower	upper
Marriage	.713	.060	8.485
History of surgery	.555	.111	2.018
Use of antibiotics	2.406	.611	9.480
Presence of catheter	1.732	.439	6.837

CHAPTER 5
DISCUSSION

5.1 Prevalence of ESBL producing *E.coli*

The study isolated ESBL producing *E. coli* from the urine samples of the participants. The isolates had the following genes *bla*INT, *bla*CTXM, *bla*SHV, *bla*TEM and the *bla*CS2. All the isolates were resistant to at least three (3) of the ten (10) antibiotics that were used in the study.

The study recruited 327 participants. Out of this total number only 15 had positive ESBL *E.coli* producers, a prevalence of 4.6%. Of the 15 positive samples 12 (80%) were from inpatients while 3 (20%) were from out-patients. This finding shows that ESBL producing *E. coli* are present in our hospitals and environment since the study participants were both in- patients and out- patients. *E coli* has several virulent genes (Clara *et al*., 2011) which include pili which are used for the spread of extended spectrum beta lactam genes. These genes encoding for sex pilli are plasmid mediated and can be spread through conjugation. The integrons can also capture resistance genes from the environment and incorporate them in their plasmids (Barriors *et al*., 2012) and this also contributes to the spread of virulent genes in the environment (Rawat and Nair, 2010). If control measures are not put in place, prevelance may rise. These ESBL genes may be spread to other bacteria that are none ESBL producers. Microorganisms are widespread in nature and resistance might become even bigger problem than it is now (Wiles *et al*., 2008). This prevalence found in this study closely agrees with findings of similar studies which have been done in other African countries. In a study done by Storberg (2014) in Morocco, it was established that the prevalence of ESBL in UTI ranged from 1.3% to 7.5%. A similar study conducted in Rwanda resulted in a prevalence of 5.9%. In South Africa the prevalence was found to range from 8.8% to 13.1%. (Bien *et al*., 2012). Poverty appears to be a common denominator in these studies and could be a major player in contributing to the observed pattern in these resource-constrained countries. A number of factors associated with poverty may contribute to UTIs arising from ESBL producing *E.coli*. These factors include lack of access to clean drinking water, lack of toilet facilities linked to improper treatment of sewage, lack of community surveillance systems, lack of screening for ESBL producers by hospitals, underutilised or absent

health care systems and indiscriminate use of antibiotic in these countries (Stoberg, 2014). This is in addition to both host and agent factors in addition to the environment being able to contribute to the development of UTIs

5.2 Demographic data and risk factors

From the information that was collected from the questionnaire, 9/15 (60%) of the participants had a history of UTIs and were either hospitalized or were also catheterised during their hospitalization. Most hospital-acquired UTIs are associated with urinary catheters, a commonly used device among hospitalized patients. Up to 25% of hospitalized patients have a urinary catheter placed during their stay (Sanjay *et al.,* 2008). Presence of a urinary catheter causes UTI because the catheter helps inoculate organisms into the urinary tract. This urinary tract device is moist and it promotes bacterial proliferation (Hooton *et al*., 2010). It additionally provides a surface for bacterial adhesion and this can lead to mucosal irritation. The presence of a catheter is one of the most important risk factors for the development of bacteria. The daily incidence of bacteriuria is 3-10% once the catheter becomes inserted (Brusch, 2017). Between 90% and 100% of patients who undergo long-term catheterization develop bacteriuria. About 80% of nosocomial UTIs are related to urethral catheterization; only 5-10% are related to genitourinary manipulation. The presence of potentially pathogenic bacteria and an indwelling catheter predisposes to the development of a nosocomial UTI. The bacteria may gain entry into the bladder during insertion of the catheter, during manipulation of the catheter or drainage system, around the catheter, and after removal (Brusch, 2017).

Of the patients that presented with UTIs caused by ESBl producing *E.coli* 2 (13%) had a history of UTIs, were hospitalized but not catheterized. These patients may have acquired the UTIs from the hospital since they had been admitted for at least 1 week. Research which was done by Shawa (2015) shows that ESBL producing *E.coli* is endemic at UTH and thus necessitating the need to put in preventive measures which will reduce the risk of nosocomial infections. UTIs are the most common hospital acquired infections. They account for about 40% of all nosocomial infections (Sanjay *et al*., 2008).

Some of the patients who participated in this study had no immediate history of hospitalization and had no indwelling catheter but still presented with UTIs caused by ESBl producing *E coli.* This group of patients might have acquired the ESBL producing *E. coli* from the community. Community-acquired urinary tract infections are the most common infection caused by extended-spectrum *β*-lactamase (ESBL)-producing *Enterobacteriacea* particularly *E.coli* and *Klebsiella* (Ben-Ami *et al*., 2008).

Of the 15 patients that had UTIs caused by ESBL producing *E. coli*, 12 (80%) had taken antibiotics in the past 3 months before the study was conducted (Figure 4.1c). Bacterial infections are commonly treated by beta lactam antibiotics worldwide (Baguma and Bazira, 2017). Mutations in beta lactamases in bacteria has been due to persistent exposure of bacteria to beta lactam drug. This mutation has resulted in resistant even to the newly developed beta lactam drugs (Shaikh *et al*., 2015). Use of antibiotics especially 3rd generation cephalosporins results in selection pressure which leads to the proliferation of ESBL producing *E. coli*. (Rawat and Nair, 2010). A study that was done by Alanazi *et al*., (2018)

Over half of the participants (53%) who were confirmed to have ESBL producing *E. coli* were above 40. The incidence of ESBL producing *E. coli* is higher in older people compared to younger people. People who are very old and the infants tend to soil themselves and this promotes colonization of the perineum by ESBL *E. coli* which causes an ascending infection in the urethra. The elderly people in nursing homes tend to have a higher incidence of catheterization and hospitalization rates as well as having underlying infections of the urinary tract which are all risk factors for the development of UTIs caused by *E. coli* (Sanjay *et al*., 2008).

Of the 15 patients that had ESBL producing *E. coli,* 67% had a history of UTIs. Recurrent UTIs could be an indication of presence of an underlying risk factor for acquiring UTIs such as blockage of the urinary system, social habits that promote development of UTIs or frequent visits to the hospital as patients continuously seek medical intervention. Such patients are most likely have a history of antibiotic usage which promotes colonization by ESBL producing *E. coli.* A study that was done by Rodríguez-Baño *et al* (2004) confirmed that previous treatment with

fluoroquinolones, ciprofloxacin and cotrimoxazole was a risk factor for the colonization of patients with ESBL producing *E. coli* since the plasmids that encode ESBL encode the genetic determinants of cotrimoxazole. A history of UTIs may be an indicator of treatment failure (Sanjay *et al*., 2008) since the causative microorganism isolated in this study had multi drug resistant phenotypes.

None of the risk factors included in the study appeared statistically significant. The results obtained in the study however show that there was correlation between antibiotic use and development of UTIs caused by ESBL producing *E. coli.* Other studies however found different risk factors that include prolonged hospital stay with invasive devices and sharing a room with a patient who has ESBL producing *E. coli* and a history of surgery. Such findings may not be entirely surprising considering that surgery is usually followed by increase in interleukin 10 (IL-10) and a depression in antigen presentation by macrophages (Scholl *et al*., 2012). These immune responses to surgery are responsible for the increase in susceptibility to bacterial infections which may include ESBL-producers.

5.3 Genes involved in antibiotic resistance

The types and frequencies of extended-spectrum beta-lactamases produced by commonly encountered members of the *Enterobacteriaceae* have changed significantly since the early part of this decade. SHV- and TEM-derived ESBLs have arisen from mutations in the very common genes that encode TEM-1 and SHV-1 enzymes (Rawat and Nair, 2010). Most of these classic ESBLs have caused nosocomial infections in hospitalized patients or perhaps those residing in long-term-care facilities. However, a new group of ESBLs, the CTX-M family, has rapidly emerged and become the predominant ESBL type in most parts of the world. CTX-M extended-spectrum beta-lactamases (ESBLs) have emerged as the most common type of ESBL globally, their incidence easily surpassing those of SHV and TEM ESBLs according to Jorgensen *et al*., (2010). A similar pattern was observed in this study (Table 4.2), with most of the ESBL positive isolates having the CXT-M gene (93%). The high prevalence was attributed to the fact that cefotaxime was initially used for screening and all the isolates grew in the presence of this drug. The presence of the CTX-M gene resulted in 100% resistance to penicillin, ampicillin and the sulphonamide antibiotic cotrimoxazole. This gene is located on plasmids which can be shared

horizontally across strains, species and even genera. Other members of *Enterobactericeae* which are ubiquitous in nature can therefore easily acquire the genes and become resistance (Canton *et al., 2008).* This could explains why there is rapid emergency of CTX-M genes (Ben Ami *et al., 2009*). Both in patients (80%) and out patients (20%) had *E.coli* harbouring the CTX-M gene. This may make treatment more challenging because of the limited antibiotics which are available in health care centres. Great emphasis should therefore be put on the use of accurate and convenient testing methods for routine detection of CTX-M producing strains.

TEM and SHV genes were only found in 3 (20%) samples out of the 15 samples. All the samples that had the TEM gene also had the SHV gene and CTX-M gene. This therefore confirms that ESBL-mediated plasmids are capable of carrying more than one beta-lactamase gene (Fatemeh *et al.,* 2010) and this result in high-level presence of beta-lactam resistant phenotypes as was observed in the antimicrobial susceptibility test. Two (2) of the samples had a similar antibiotic resistance pattern the only difference being that one sample was susceptible to chloramphenicol while the other sample was resistant. This could be indicative that these samples had resistant alleles. When non-ESBL-producing beta-lactamases are hyper produced by an isolate there may be resistance to beta-lactamase inhibitors according to Paterson and Bonono (2005)**.** Acquired resistance to penicillin-β-lactamase inhibitor combinations in *Escherichia coli* is caused by the over production of penicillinase due to the presence of the $bla_{\text{TEM-1}}$ gene in plasmids or strong promoters. It is also caused by overproduction of the AmpC cephalosporinase and OXA-type inhibitor-resistant TEM (IRT) β-lactamases. Inhibitor resistance TEM enzymes emerge via mutational events from TEM-1 or TEM-2 β-lactamases that affect substrate affinity for β-lactamase inhibitors (Canton *et al*., 2008).

According to the PCR amplification of INT I results of the detection of class 1 integrons in this study, 46% of the isolates contained class 1 integrons which is indicative of very high frequency of occurrence of class 1 integrons in *E. coli* strains. This is the first study in Zambia which has looked at class I integrons at UTH in UTIs and hence it can be used as baseline data for other future studies. Integrons are genetic structures in bacteria that that are capable of expressing, acquiring and exchanging gene cassettes (Phongpaichit *et al*., 2011). Gene cassettes are mobile genetic elements that carry antibiotic resistant genes which confer multi drug resistance (Chen *et*

al., 2013). Integrons are transmitted vertically and horizontally and this contributes to growing antibiotic resistance (Barriors *et al*., 2012). All the isolates that had integrons were resistant to three (3) to seven (7) antibiotics out of the ten (10) antibiotics that were used in this study. These are the commonly used antibiotics at UTH and other health care facilities worldwide.

The CS2 gene was observed in 27% of the isolates. This gene is responsible for bacteria conjugation. Conjugation is one of the methods that bacteria use for horizontal transfer of genetic material by direct cell to cell contact (Wiles and Mulvey, 2008). Resistant bacteria transfer resistant genes to susceptible bacteria which become resistant upon acquisition of the resistant genes. This has resulted in the rapid spread of these genes were responsible for the observed antibiotic resistant phenotypes.

5.4 Antibiotic susceptibility pattern

The bacterial isolates had the highest resistant rate (100%) to cotrimoxazole which is a non-beta lactam antibiotic, ampicillin (100%) and cefotaxin (87%) both of which are beta lactam antibiotics. Ampicillin and cotrimoxazole are commonly used as empirical treatment for UTIs (Alanazi *et al*., 2018) and this high resistance limits their clinical use. Other studies done by other researchers in different parts of the world found similar results: study done by Karlosky *et al*., (2002) in the USA showed that ESBL producing *E. coli* exhibited 97.8% resistance to ampicillin and 92.8% to cotromoxazole while high resistance rates of 55% to ampicillin and 40% to cotrimoxazole were observed in the UK (Bean *et al*., 2008).

All the 15 isolates used in this study were resistant to these 3 antibiotics (Table 4.3). There was multi drug resistance to all the cepholosporins (Tables 4.3 and 4.4) because ESBL mediated plasmids may carry more than one beta lactamase gene and may account for a high-level of beta lactam resistant phenotypes (Karen, 2010). According to a study carried out by Shawa (2015) at UTH, cephalosporins and penicillins account for about 79% of antibiotics used at UTH. The high resistant rates to the beta lactam antibiotics complicates the treatment of UTIs using beta lactam drugs at UTH since cephalosporins are the most readily available drugs. The isolates were also resistant to other classes of drugs apart from beta lactams. This observation could be explained by the fact that large plasmids that harbour ESBL genes also harbour genes for resistance to

other antimicrobials (fluoroquinolones, aminoglycosides, and cotrimoxazole), leading to multidrug resistance (Patterson, 2006). This explained the high resistance that was observed in these classes of antibiotics that were used in the study.

The high resistant rate observed in ampicillin (100%) is due to the fact that many ESBL producing organisms also express AmpC β-lactamases and may be co-transferred with plasmids mediating aminoglycoside resistance (Rawat and Nair, 2010). This may explain why a high resistance rate was observed in Gentamycin (53%). In addition, there is an increasing association between ESBL production and fluoroquinolone resistance (norflocaxacin and ciprofloxacin both had resistance rates of 47% while Nalidixic acid had resistance rates of 40%) according to George (2008). Although in *in vitro* tests ESBLs are inhibited by β-lactamase inhibitors such as clavulanic acid, the activity of β-lactam/β-lactamase inhibitor combination agents may be influenced by the bacterial inoculum, dose administration regimen and specific type of ESBL present. For this combination to be effective, isolates should only produce a single ESBL. This however was not the case with results which were obtained in this study, the isolates had more than one type of ESBL (Rawat and Nair 2010).

From the results obtained in this study, Aminoglycosides and floroquinolones may not be recommended for the treatment of UTIs caused by *E. coli* at UTH because of their high resistance rates. This high resistance may be as a result of the use of aminoglycosides and fluoroquinolones in the treatment of UTIs.

Nitrofurantoin had the lowest susceptibility rate (100%) compared to other antibiotics that were used in this study. Studies which were carried out by Tasbakan *et al.*, (2012) to evaluate the effect of nitrofurantoin in the treatment of extended-spectrum β-lactamase (ESBL) producing *E coli* related lower urinary tract infection (LUTI) had a success rate of 68%. A similar study done by Analazi *et al* (2018) found a high suscpetibility rate of 97% collaborating the findings in this study. Resistance rates in developing countries range from 6.6% to 31.6% (Bean *et al*., 2008). These results suggest that nitrofurantoin may be an alternative in the treatment of ESBL-producing *E. coli*-related LUTI**.** Other studies have however found a persisting low prevalence resistance of 1.9% - 7.7% to nitrofurantoin in urinary *E.coli* isolates (Tasbakan *et al*., 2012). The

drug is bacteriostatic at lower concentrations and bactericidal at higher concentrations. The oral form is absorbed well. Approximately 60% is bound to plasma and is metabolised in the liver. About 30-50% is excreted via urine which makes it a good option in the treatment of UTIs. This drug is effective in both in-vitro and clinical studies against ESBL producing *E.coli* (Taskaban *et al*., 2012). More studies need to be carried out to determine the exact effectiveness of this drug.

Most of the antibiotics used in this study had a high resistant rate possibly due to selective pressures of antimicrobial usage in the treatment of UTIs since these antimicrobials can readily be accessed over the counter in Zambia without need for a prescription (Shawa 2015). The data obtained in this study found a high correlation of resistance (86.7%) to ampicillin, cotrimoxazole and cefotaxime (Table 4). The second highest correlation of resistance was observed in ampicillin cotrimoxazole cefotaxime and tetracycline (25%). Burow *et al*. (2013) emphasised that oral administration of antimicrobials can increase antimicrobial resistance in commensal *E. coli*. The study established that tetracycline, aminoglycosides and quinolones had a strong effect on the development of antimicrobial resistance. This is also in line with another study done by Tadesse *et al*, (2012) in which a significant upward trend in resistance was observed for ampicillin, sulfonamide and tetracycline.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. ESBL producing *E. coli* are present at the University Teaching Hospital.
2. The ESBL producing *E.coli* isolated from the UTH specimens have genes which confer antibiotic resistance and include *bla*TEM, *bla*CTXM, *bla*SHV, INT and CS2.
3. These ESBL producing E.coli have multi drug resistant phenotypes.

6.2 Recommendations

1. There is need for continued antimicrobial resistance surveillance to monitor resistance patterns in Zambian hospitals and clinics.
2. Detection of the CTX-M ESBLs should be part of routine susceptibility testing by the Microbiology laboratory at UTH in UTI specimens
3. There should be prudent management of UTIs caused by ESBL producing *E. coli* and to prevent emergency of new antibiotic resistance patterns.
4. Reinforcement of infection control measures, especially hand washing in healthcare settings and antibiotic stewardship, is critical to reduce the spread of ESBL-producing *E. coli.*
5. Policies restricting sale and use of antibiotics in Zambia should be strengthened and enforced based on these findings.

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APPENDICES

Appendix 1: Information sheet

THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

This information sheet is for men, women and children who are in-patients or out patients at the University Teaching hospital who have urinary tract infections and are willing to participate in the research on urinary tract infections caused by *E. coli*. The title of the research is "Extended Spectrum Beta Lactamase productivity and class I integrons in *E. coli* from Urinary tract infections at UTH".

Introduction

My name is Chirwa Emmanuel a post graduate a Masters' Degree student at the University of Zambia, School of Medicine. I am carrying out a research on urinary tract infections caused by *E. coli*, which is very common in this country. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me or any member of staff at UTH.

Purpose of the research

UTIs are one of the most common and serious diseases in Zambia. The drugs that are currently used to treat people with UTIs are limited and even after treatment there is always a possibility of the UTI recurring. This research sought to get more information on the bacteria that causes UTIs so as to contribute to better preventive measures of these infections.

Type of Research Intervention

This research requests you to donate a sample of your urine only once.

Participant selection

All adults and children with UTIs who come for treatment at UTH are invited to participate in the research.

Voluntary participation

Your participation in this research is entirely voluntary. Whether you choose to participate or not, all other services you receive at UTH will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this hospital for UTIs. You may change your mind later and stop participating even if you agreed earlier.

Duration

The research will take about 30 days in total. There will be no need for you to provide a second sample during this period.

Side effects and Risks

There are no side effects or risks in this study. Any information you have provided is strictly confidential and will not be used for anything else outside this study

Benefits

There may not be any direct benefit to you but your participation may help us find the answer to the research questions. There may not be any benefit to the society at this stage of the research, but future generations may benefit from the research findings.

Reimbursements

You will not be given any money or gifts to take part in this research.

Confidentiality

The information that is collected from this research project will be kept confidentially. Information about you that will be collected during the research will only bear a number for identity and will be kept in secure storage and no-one but the researchers will have access to it. This information will not be shared with or given to anyone.

Sharing of results

The knowledge that we get from this research will be shared with you through health care givers in your community before it is made available to the public. Results will be published for the purpose of sharing it with other interested people that may wish to learn from this research.

Right to Refuse or withdraw

You do not have to take part in this research if you do not wish to do so. It is your choice and all of your rights will -still be respected.

Who to contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following: The Chairperson University of Zambia Biomedical Research Ethics Committee (P.O BOX, 50110 Lusaka, +2601256067). Dr Chirwa Emmanuel (0979205130, echirwa07@gmail.com, Dr Gina Mulundu 0977788804, gmulundu@zamtel.zm, Prof B. Han'gombe (0977326288, mudenda68@yahoo.com).

Appendix 2: Consent form

I have read the information in the information sheet, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this research.

Name of Participant_____

Signature of Participant _____

Date _____

Day/month/year

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____ AND Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done.

1. The participant shall donate a sample of their urine
2. The participant shall not be given any money
3. The participant's information shall be kept confidential

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this information sheet has been provided to the participant.

Chirwa Emmanuel……

Signature…….

Date _____

Day/month/year

Appendix 3: Assent form

Project Title: Determination of Extended Spectrum Beta Lactamase Producers and Class I intergrons in *E. coli* isolates from UTI patients at UTH from November 2016 to January 2017

Main Investigator: Chirwa Emmanuel

We are doing a research study about germ called *E. coli*. A research study is a way to learn more about things. If you decide that your child should be part of this study, your child will be asked to donate a sample of thir urine**.**

No benefit will be given to your child or anyone who takes part in this study.

When we are finished with this study we will write a report about what was found and learned. This report will not include your child's name or that your child was in the study.

Your child does not have to be in this study if you decide to stop after we begin, that's okay too.

If you decide you want your child to be in this study, please sign your name on behalf of your child.

I, _____, want my child to be in this research study.

_____ _____

(Sign your name here) (Date day/month/year)

Appendix 4: Translated information sheet

THE UNIVERSITY OF ZAMBIA
RIDGEWAY CAMPUS
SCHOOL OF MEDICINE

Aya amashiwi ayali mumabuula aya, yabaume, abanakshi elyo na abaana abalwele, abateekwa nabashateekwa pa cipataala ca University Teaching Hospital (UTH) abakwata amalwele ya Urinary Tract kabili abali abapimpa ukuibimbamo mukfwalisha pa malwele Urinary Tract ayaletwa na *E. coli*. Umutwe wakufwailisha uku ni "Extended Spectrum Beta Lactamase productivity and class I intergrons in *E. coli* from Urinary tract infections at UTH".

Ifya ku tampilapo

Ishina lyandi nine Chirwa Emmanuel (kafwailisha mukalamba). Ndi musambi mukalamba pa University of Zambia, Ridgeway Campus, School of medicine. Ndefwailishako pa malwele ya Urinary Tract ayaletetwa na *E. coli*, ayaseeka muno caalo. Nalamipeela amshiwi yamo na ukumilalika kukuibimbamo mukufwailisha uku. Tamulefwaikwa ukusala leelo nga kuti mwasendamo ulubali nangu iyo. Ilyo tamulasala, kuti mwalanda nabantu abali bonse pali uku ukufwailisha.

Kuti kwaba amashiwi ayo mushingomfwikisha. Kuti mwanjeba naimininako panoono ilyo tulepita mumashiwi aya kabili ndi na ukumilondolwela. Nga muli na ukukwata ameepusho, kuti mwanjipusha nangu abali bonse ababonfi aba pa UTH.

Chinshi tule fwaya

Amalwele ya Urinary Tract yaba pamalwele yabiipisha muno caalo cesu ica Zambia. Imiti iilebomfiwa mukwafwilisha abantu abakwata aya amalwele yaba iyapelebela pantu na panuma yakundapishiwa, aya amalwele kuti yabweela. Umulandu wakufwailisha uku, kufwaya ukusenda amashiwi pakashishi akalenga amalwele ya Urinary Tract pakuti twingasanga inshila ishisuma ishakucingililamo amalwele aya.

Ubupusana ubwa ku fwaya

Mukufwailisha uku, mwalalombwa ukupeelako imisu shenu umukufye umo.

Abalimo aba ku sala

Bonse abakalamba eloyo nabaice abakwata amalwele ya urinary tract abeesa mukundapwa ku UTH nabeetwa mukuibimbamo mukufwalisha uku.

Aba kui pelesha

Ukusendamo ulubali muli uku ukfwailisha kwakuipeeleshafye. Busalo bwenu ukusendamo ulubali nangu iyo. Nga cakuti mwasala ukusendamo ulubali nangu mwakana, bonse ubwafwilisho musenda ku UTH buli nokukonknyapo ukwabula ifintu ukwaluka. Nga mwasala ukukaansendamo ulubali mukufwailisha uku, muli na ukupelwa ubwafwilisho buntu mupeelwa lyonse ubwamalwele ya urinary tract. Kabili, kuti mwaleka ukusendamo ulubali pa nshita iili yonse nangu mwasumina nomba ukuibimbamo pali ino inshita.

Inshita

Ukufwailisha kuli na kusenda inshiku amakumi yatatu (30). Tachakafwaikwe ukuti mukapeele imisu nakabili muli iyi inshita.

Ifibi ifinga chitika

Tamuli ifyakutinya nangu ifibi ifkatumbuka mumasambililo aya. Amashiwi yonse ayo muli naukupeelwa yali na ukusungwa munkaama kabili tayakabonfiwe kufintu fintu ukucila pamasambililo aya.

Ubusuma ubwa bamo

Teti kube ifyabukumu ifyo mwinganonkelamo mukufwailsha uku lelo lelo ukusendamo ulubali kwenu kuli na ukutwafilisha ukusanga amaasuko yakufwailisha kwesu. Teti kube ukunonkelamo kebakala caalo pali ino nshita lelo abenganonkelamo bamu nkulo shakuntanshi.

ilambo

Tamuli nokupeelwa icilmbu icili conse pakusendamo ulubali mukufwailisha uku.

Ifya nkama

Amashiwi yonse ayasendwa mukufwailisha uku yali na ukusungwa munkaama.

Amashwi yonse ayasendwa pali imwe panshiat yakufwailisha uku yali na ukubikwapo impendwa nga akeshilbilo kabili yali nokubikwa mucifulo icafisha ukwingeshafye bakafwailisha. Kabili amashiwi aya tayakapeelwe ku muntu uuli onse nakalya.

Ukusabankanya ifyafumamo

Amaano ayo tuli na ukusanga ukufuma mukufwailisha uku, yali nowakanishiwa kuli imwe ukupitila mubabomba kufyabuumi mu mishi yenu ilyo tayalasalnganishiwa ku cintu bwingi. Ifikatunmbuka mukufwailisha uku, fili na ukusabankanishiwa pamulandu wakkwakana amano nabo bonse abengafwaya.

Insambu isha ku kana nangula uku kana

Tamufwile ukusendamo ulubali nga tamulefwaya. Busalo bwenu kabili insambu shenu isho ishili nokucindikwa.

Abaku tumina

Nga muli na ameepusho, kuti mwaipusha nombalie nangu inshita imbi nangu nipanuma yakufwailisha nakutendeka. Nga kuti mwafwaya ukwipusha ifipusha ifipusho inshita imbi kuti mwatumina ba: The Chairperson Ethics Committee- UNZA Ridgeway campus, Chirwa Emmanuel (0966 205130, 0979205130, echirwa07@gmail.com), Dr Gina Mulundu (0977788804, gmulundu@zamtel.zm) Prof B. Han'gombe (0977326288, mudenda68@yahoo.com).

Appendix 5: Translated assent form

Umutwe wakufwailisha: "Determination of Extended Spectrum Beta Lactamase Producers and Class I intergrons in *E. coli* isolates from UTI patients at UTH from November 2016 to January 2017."

Kafwailisha mukalamba: Chirwa Emmanuel

Tulefwailisha pakashishi akeetwa *E. coli*. Ukufwailisha enshila imo iyo tusambililamo pafintu. Nga mwasumina ukusendamo ulubali, mwalipushiwa ukupeelako imisu. Takuli ubukumu ubwalapeelwa kuli bonse abalasendamo ulubali. Ubukumu, nico icawama nganshi kuli imwe.

Nga twapwisha ukufwailisha, tuli na ukulemba insebo yafyo tuli na ukusambililamo. Insebo iyi tayakamilumbulemo ishina nangu ukutiila emo mwalimukufwailisha uku.

Tamufwile ukusendamo ulubali ngatamulefwaya. Nga mwasala ukulekela pakati kanshila, kuti mwaleka. Abafyashi benu naabeshiba pamasambililo aya.

Nga mwasala ukubamo mumasambililo, kuti mwasaina.

Ine, _____, ndefwaya ukusendamo ulubali mukusambilila uku.

_____ _____

(Sign your name here) (Date day/month/year)

Appendix 6: Translated consent form

Nimbelenga amashiwi yonse, nangu nabambelengelako. Nacikwata inshiat yakwipusha ameepusho kabili nameepusho yandi yonse naayasukwa bwino, naasumaina mukuipelesha ukuibimbamo mukufwailisha uku.

Ishina lyaulesendamo ulubali: _____

Signature iya wauleisendamo ulubali: _____

Date _____

ubushiku/umweshi/umwaka

Aba sha ishiba uku belenga

Naali kambone uko balebelengela uwasumine ukusendamo ulubali kabili wene aalikwete inshita yakwipusha ameepusho. Nashininkisha ukuti uyu uulesendamo uubali naasumina ukuibimbamo.

Ishina lyakwa kambone_____ **NA Apakufwatika kambone**

Signature yakwa kambone _____

Date _____

Day/month/year

Statement by the researcher/person taking consent

Nimbelenga amashiwi yose kuulesendamo ulubali kabili naceesha namaka yonse ukulenga ukuti omfwikishe ukuti ifili peesamba fili nokucitwa.

1. Uulesendamo ulubali ali na ukupeela imisu.
2. Uulesendamo ulubali taapeelwe ulupiya.
3. Fyonse ifya twalaishiba paulesendamo ulubali fyalasungwa munkaaama.

Nashininkisha ukuti uwaibimbilemo aalipeelwe inshiata yakwipusha ameepusho kabili naameepusho yonse ayaipushiwe yaliasukwa bwino nganshi umwabeela amaka yandi. Nashininkisha ukuti uwasendeemo ulubai taapatikishiwe ukuibimbamo lelo aaliipeleshe.

A copy of this information sheet has been provided to the participant.

Chirwa Emmanuel……

Signature…….

Date _____

Day/month/year

Appendix 7: Questionnaire

This is your medical history form to be completed prior to screening. All information will be kept confidential. This information will be used for analysis and evaluation of the risk factore assocaiated with UTIs caused by *E. coli*. Please take time and complete it carefully and thoroughly and then review it to be certain you have not left out anything.

If you have any concerns, we will help you with clarifications. We realise that some parts of the form may be not familiar to you. All your questions will be thoroughly adressed.

Participant Name: Name of number??

Assigned # :

Signature :

Tick where appropriate

1. Marital Status

   Single ☐ Married ☐ Divorced ☐ Widowed

2. Gender:

   Male ☐ Female ☐

3. Age in years

   4-16 ☐ 16-20 ☐ 20-40 ☐ Above 40 ☐

   Less than 20 too wide considering you:re starting at 6! Why not less than 10, then 10-20

4. Education

   Grade school ☐ Junior high ☐ High school ☐

   Diploma ☐ Degree ☐

5. Hospitalization status

   Currently hospitalized ☐ Less than 3 months ago ☐

   More than 3 months ago ☐ Never ☐

6. If you are currently hospitalised, how long have you been admitted

   Less than 1 week ☐ 2-3 weeks ☐ More than 3 weeks ☐

7. Urinary tract catheter

   Currently catherterised ☐ Less than 3 months ago ☐

   More than 3 months ago ☐ Never catheterized ☐

8. Have you been treated for a Urinary tract infection before?

   No ☐ Yes ☐

9. Do you have any known underlying urinary system infection?

   No ☐ Yes ☐

10. Do you have any known history of surgery?

    No ☐ Yes ☐

11. Antibiotic use in past 3 months

    Yes ☐ No ☐

12. Hand washing after using the toilet

With soap ☐ Without soap ☐ Never ☐

Thank you very much for your participation