RISK FACTORS AND ALLELES OF EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING *Escherichia coli* AT THE UNIVERSITY TEACHING HOSPITAL, ZAMBIA

BY

SHAWA MISHECK

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School of Veterinary Medicine

DECLARATION

I, **Shawa Misheck**, do solemnly declare that this dissertation is my own original work. It has been presented in accordance with the guidelines for Msc 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.

Signed _____ Date _____

CERTIFICATE OF APPROVAL

The University of Zambia approves the dissertation submitted by **SHAWA MISHECK**, as fulfilling the partial requirements for the award of the Master of Science Degree in One Health Analytical Epidemiology by the University of Zambia.

Prof. Bernard M. Hang'ombe

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Supervisor	Signature	Date
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Chairperson (Board of Examiner)	Signature	Date

ABSTRACT

The limitation of antibacterial treatment options imposed by the emergence of bacterial resistant organisms calls for the correct identification of the genes involved in mediating resistance. In this study, antimicrobial resistance was determined in terms of the presence of extended-spectrum beta-lactamases (ESBL) among *Escherichia coli* isolates obtained from patients admitted at the University Teaching Hospital (UTH) in Zambia. A total of 400 rectal swabs were obtained and subjected to initial screening using MacConkey agar supplemented with cefotaxime. *bla*TEM, *bla*SHV and *bla* CTX-M genes in ESBL producing *E. coli* were detected using Polymerase Chain Reaction (PCR). All CTX-M positive isolates were subjected to sensitivity patterns using 11 different antibiotics: 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 prevalence of ESBL producing *E. coli* was 19% (76/400). Among the ESBL producing *E. coli*, 25 out of 76 (32.9%) were positive for CTX-M genes. Of the 25 CTX-M positive isolates, 9 (36%) isolates were positive for SHV genes and 9 (36%) were positive for TEM genes. The highest resistance was found to be to nalidixic acid (96%), followed by sulfamethoxazole-trimethoprim, tetracycline and ceftazidime (92% each). The least resistance was to ampicillin (60%). Male gender (p=0.014, 95% CI=1.198-4.813) and history of surgery (p=0.01, 95% CI=1.196-3.740) were found to be significant risk factors for ESBL presence. The findings emphasize that ESBL-producing bacteria are present among patients at the UTH, and that these organisms exhibit co-resistance to several classes of antibiotics.

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DEDICATION

To God almighty for the abundant favor. My dedication also extends to my mother Pastor Grace Nkani for the moral support, prayers and encouragement throughout my studies.

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LIST OF ABBREVIATIONS AND ACRONYMS

AMP	Ampicillin
AmpC	Ampicillin class C
CAZ	Ceftazidime
CHL	Chloramphenicol
CI	Confidence Interval
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CTX-M	Cefotaxime-hydrolyzing, first isolated in Munich
DNA	Deoxyribonucleic acid
E. coli	Escherichia coli
ESBL	Extended Spectrum Beta Lactamase
GEN	Gentamicin
IMViC	Indole, Methyl red, Voges-Proskauer and Citrate
MIC	Minimum Inhibitory Concentration
n	Number of isolates or samples
NAG	N-acetyl glucosamine
NOR	Norfloxacin
NPHL	National Public Health Laboratory
OXA	Oxacillin-hydrolyzing beta-lactamase
PBP	Penicillin Binding Protein
PEN	Penicillin
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
RAPD	Random Amplified Polymerase DNA
SHV	Sulphydryl variable
SPSS	Statistical Package for Social Sciences
STR	Streptomycin
SXT	Sulphamethoxazole/Trimethoprim
TE Buffer	Tris –Ethylenediaminetetraacetic acic buffer
TEM	Temoniera type beta-lactamase
TET	Tetracycline
UTH	University Teaching Hospital
UTI	Urinary Tract Infection

INTRODUCTION

Extended spectrum beta lactamases (ESBL) are beta lactamases capable of conferring bacterial resistance to antibiotics including penicillins, first, second, and third generation cephalosporins, and aztreonam (but not cephamycins or carbapenems) by hydrolysis (Bush *et al*., 1995; Paterson and Bonomo, 2005). They are inhibited by β-lactamase inhibitors such as clavulanic acid. They are a group of plasmid-mediated or chromosomally encoded, miscellaneous, intricate and evolving enzymes that have led to major therapeutic challenges today in the management of hospitalized and community-based patients (Rawat and Nair, 2010).

The ESBL producing organisms have been found to be associated with a wide range of infections that vary in severity from uncomplicated infections to life threatening sepsis (Rawat and Nair, 2010). Both gram negative and gram positive bacteria can produce ESBLs. The true prevalence of ESBLs is unknown, but it is obviously increasing (Angel *et al*., 2009; Ender *et al*., 2009), and studies in many parts of the world report that 10-40 % of strains of *Escherichia coli* and *Klebsiella pneumoniae* are ESBL-producers (Rupp and Fey, 2003), while some studies in Africa suggest that more than 50% of strains of *K. pneumoniae* express ESBLs (Mshana *et al*., 2009).

ESBL-producing organisms have a worldwide distribution as evidenced by the large number of outbreaks of infection reported on every populated continent of the globe (Rupp and Fey, 2003). Most outbreaks have occurred in critically ill patients in intensive care units (De Champs *et al*., 1989; Laurent *et al*., 2008). However, infections due to ESBLs are ubiquitous in hospitals. A study carried out at Muhimbili National Hospital in Tanzania showed that more than 80% of isolates exhibited resistance to ampicillin and 25% of *E. coli* isolates demonstrated resistance to third generation cephalosporins (Mshana *et al.*, 2009), while high carriage rates of ESBL strains were observed among post-delivery women and neonates at Bugando Medical Centre in Tanzania (Nelson *et al*., 2014). On the other hand, community acquired infections are not uncommon (Paterson, 2006; Livermore *et al*., 2007), and outbreaks have been reported in nursing homes and rehabilitation units (Rooney *et al*., 2009). In

a study conducted in Nigeria, 35% of the overall ESBL isolates had a community origin while the remaining 65% had a hospital origin, and these organisms exhibited co-resistance to other antibiotics including gentamycin, tetracycline, ceftriaxone, cefuroxime, ciprofloxacin, and Augmentin (amoxicillin and clavulanic acid combination) (Shaikh *et al*., 2014). In another study conducted at the National Public Health Laboratory (NPHL), Kathmandu, Nepal, it was reported that 31.6% of the *E. coli* isolates produced ESBL that were resistant to several classes of antibiotics (Shaikh *et al*., 2014). Thus, very broad antibiotic resistance extending to multiple antibiotic classes is a feature frequently seen among ESBL-producing isolates, making it difficult to treat infections by such organisms and also leading to an increase in health care costs. Patients infected with such organisms are more likely to require more complicated treatment associated with increased morbidity and mortality (Kayange *et al*., 2010). This increased morbidity and mortality may also be due to a holdup in correct antimicrobial treatment (Gupta *et al*., 2003).

Studies done in Europe, North America, Latin America, Asia and Australia suggest that ESBL-producing organisms are distributed worldwide and that their prevalence varies markedly even in closely related regions (Rupp and Fey, 2003). Various research data show that the prevalence of ESBLs ranges from 6-88% in various hospitals (Ahmed *et al*., 2013). Although infections due to different clones of ESBL-producing organisms have been reported in some African countries, data on such clones and alleles are scarce in Zambia as there is no national surveillance of these strains. This study aims at quantifying the burden, identifying the risk factors, and determining alleles associated with ESBL in Zambia.

1.1 STATEMENT OF THE PROBLEM

In many hospitals, ESBL-producing organisms are already prevalent, and outbreaks of infections with such organisms have been reported worldwide including some African countries (Paterson and Bonono, 2005; Ben-Ami *et al*., 2006). Several pathogens of public health importance, including *E. coli, Salmonella, Shigella* and *Vibrio cholera,* are ESBL producers (Rawat and Nair, 2010). The situation today is very worrying because multidrug-resistant bacteria including ESBL-producing bacteria causing community acquired urinary tract infections are readily encountered in clinics and outpatient settings (Mahesh *et al*., 2010). Clinicians, clinical microbiologists and

infection control professionals face serious challenges when managing infections due to ESBL-producing organisms (Serefhanoglu *et al*., 2009).

Patients infected with ESBL producers experience greater likelihood of poor treatment outcome with mortality rates ranging from 42-100% (Rupp and Fey, 2003). Although the actual proportion of infections due to ESBL in Zambia is not known, it is clear that many human bacterial diseases are increasingly becoming more difficult to treat because of antimicrobial resistant organisms, leading to an increase in healthcare costs as patients infected with such organisms are likely to have longer treatment period and may require more expensive treatment.

1.2 JUSTIFICATION OF THE STUDY

Despite a number of outbreaks of infection with ESBL producers having been reported in some African countries, very limited ongoing efforts are directed at addressing this emerging problem. Very limited antimicrobial surveillance figures have been published, and such data are scarce in Zambia. The production of beta-lactamases among hospital-acquired bacterial strains differs between countries, units and patients, but resistance rates are generally known to be more in third world countries such as those in Africa (Rao, 2012), where carriage rates can be as high as 66% (Kluytmans-van den Bergh and Kluytmans, 2012). Thus it is necessary to investigate the burden, risk factors and alleles of ESBLs in Zambia. Such a study would significantly increase effectiveness of prevention, treatment and control measures through appropriate antibacterial choice which is usually complicated by multi-drug resistance. A study conducted at the University Teaching Hospital (UTH), Zambia, revealed that 100% of *K. pnuemoniae* isolates obtained from neonates in the neonatal intensive care unit produced ESBLs and were multi-drug resistant (Mumbula *et al*., 2015). However, no information regarding ESBL producing *E. coli* in Zambia has been availed, hence the significance of this study.

1.3 RESEARCH QUESTION

What is the prevalence, alleles and associated risk factors of ESBL infections among patients admitted at the UTH in Zambia?

1.4 OBJECTIVES

1.4.1 General objective

To determine the prevalence, risk factors and alleles of ESBLs present at the UTH.

1.4.2 Specific objectives

(a) To determine the prevalence of ESBL producing *E. coli* among patients in general medical and surgical wards of the UTH.

(b) To determine the risk factors associated with ESBL producing *E. coli.*

(c) To determine the susceptibility pattern to beta lactam and non-beta lactam antibiotics among ESBL producers

(d) To determine the common ESBL alleles at the UTH.

CHAPTER TWO

LITERATURE REVIEW

2.1 Gram-positive and Gram-negative bacteria

Bacteria are minute prokaryotic organisms that are a few micrometers in length. Being prokaryotes, bacterial cells lack a nucleus and rarely contain membrane-bound organelles (Aderiye and Oluwole, 2014). The majority of bacteria in the human body are harmless and some are even valuable. However, there are several types of bacteria that cause disease in the human host. Examples of such bacterial infectious diseases include cholera, syphilis and tuberculosis (Stamm and Mudrak, 2013).

Bacteria can be broadly classified as Gram-positive or Gram-negative depending on whether or not they retain the crystal violet stain used in the Gram-staining method (Beveridge, 2001). Gram-positive bacteria retain the crystal violet stain while Gram-negative bacteria do not retain the stain but appear pink. Gram-positive and Gram-negative bacteria differ in structure and function (Wada *et al*., 2012). Gram-negative cells consist of a cell membrane, a thin peptidoglycan layer, an outer membrane (containing lipopolysaccharides and phospholipids), porins in the outer membrane, and a periplasmic space between the outer membrane and the cytoplasmic membrane (Brooks, 2007). The backbone of peptidoglycan is made of units of N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) which are arranged in an alternating and repeating fashion (Brooks, 2007). A tetrapeptide side chain consisting of L-lysine, D-glutamic acid, diaminopimelic acid and d-alanine is attached to NAM (Rao, 2012). The bacterial enzyme d-d-transpeptidase catalyses the cross-linking of diaminopimelic acid in the side chain of one backbone to d-alanine in the side chain of another backbone in Gram-negative cells. This cross-linking is very imperative for bacterial growth and multiplication as it results in the constant remodeling of the cell wall by degrading and synthesizing portions of cell wall (Rao, 2012). Gram-negative bacteria of medical importance include *Neisseria meningitidis, N. gonorrhoeae, Hemophillus influenzae, K. pneumoniae, Pseudomonas aeruginosa, E. coli,* and *Salmonella typhi* (Kayser *et al.,* 2005). The family *Enterobacteriaceae* is a broad group of Gram-negative non-spore-forming bacteria belonging to Phylum

Proteobacteria, Class Gammaproteobacteria and Order Enterobacteriales. Examples include *E.coli, Enterobacter spp., Citrobacter spp. and Klebsiella spp.*

Gram-positive cells consist of a cytoplasmic lipid membrane, a thick peptidoglycan layer, teichoic acids and lipoids (forming lipoteichoic acids), and a much smaller periplasm relative to that of Gram-negative bacteria. Medically important Gram-positive bacteria include *Streptococcus, Staphylococcus, corynebacterium, Lysteria,* and *Clostridium* (Kayser *et al.,* 2005).

Bacteria store their genetic information in chromosomes and in plasmids. A bacterial chromosome is located within a nucleoid region, not bounded by a nuclear envelope, and contains genes that are crucial for cellular function (Michod *et al*., 2008). Each cell contains only one chromosome. Plasmids are small DNAs which are located in the cytosol of bacterial cells. They rarely play any role in the growth and metabolism of bacteria but they may be relevant for the survival of bacteria as they may carry resistance genes and virulence factors (Srivastava and Srivastava, 2003).

Some bacteria are able to transfer genetic material among themselves using one of three mechanisms; transformation, transduction or conjugation (Trevors, 1999). In transformation, DNA from the environment is incorporated into bacteria. In transduction foreign DNA is incorporated into the bacterial chromosome through a bacteriophage, while in conjugation DNA is transferred from one cell to another by direct contact. The rapid multiplication of bacteria increases the likelihood of mutations, resulting in easy adaptation to the ever-evolving environmental conditions (Denamur and Matic, 2006).

2.2 Antibiotics and their action

An antibiotic is a drug produced by a microorganism that kills or inhibits the growth of other microorganisms (Makut and Owolewa, 2011). Therefore, following this strict definition, antimicrobial agents that are synthetic (e.g. sulfonamides and quinolones) and those that are semisynthetic (e.g. amoxicillin) should not be considered to be antibiotics. Antibiotics that kill bacteria are said to be bactericidal while those that slow the growth of bacteria are bacteriostatic (Bernatova *et al*., 2013). Bactericidal drugs include aminoglycosides, beta-lactams, vancomycin, quinolones, rifampicin and

metronidazole, while bacteriostatic drugs include chloramphenicol, erythromycin, clindamycin, sulfonamides, trimethoprim and tetracyclines (Pankey and Sabath, 2004). There are five main ways in which antibiotics act (Shaikh *et al.*, 2014). These are summarized in Table 1 below:

Table 1: Action of antibiotics.

Action	Mode
a) Inhibition of cell wall synthesis	Beta-lactam antibiotics block the action of the enzyme d-d transpeptidase which is responsible for the formation of peptidoglycan layer.
b) Inhibition of protein synthesis	Tetracyclines and aminoglycosides interfere with protein synthesis by binding to the 30S ribosomal subunit while chloramphenicol and macrolides do so by binding to the 50S ribosomal unit.
c) Interference with nucleic acid synthesis	Rifampicin exerts its bactericidal action by interfering with a DNA-directed RNA polymerase while quinolones act by interfering with type II topoisomerase, DNA gyrase and type IV topoisomerase.
d) Inhibition of a metabolic pathway	Sulphonamides and trimethoprim act by blocking the key steps involved in the synthesis of folate, and therefore compromising the biosynthesis of nucleotides.
e) Disorganizing of the cell membrane	Polymyxins are thought to act by increasing the permeability of bacterial membrane, causing bacterial cell contents to seep out of the cells.

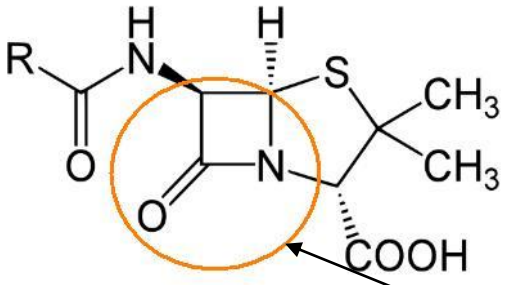
2.3 Beta-lactam antibiotics

A beta-lactam ring is a four-membered cyclic amide which is made up of three carbon atoms and one nitrogen atom, and antibiotics that exhibit these structures are called beta-lactam antibiotics (Rao, 2012). The beta-lactam ring is the functional group of beta-lactam antibiotics.

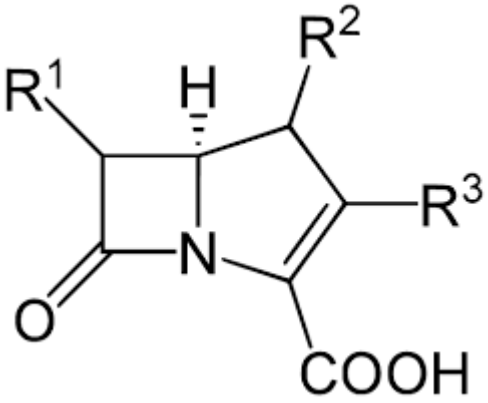
These antibiotics are classified based on the structure of the component fused to the beta-lactam ring (Table 2) as:

a) Beta-lactams merged with saturated five-membered rings (e.g. penicillins).
b) Beta-lactams merged with unsaturated five-membered rings (e.g. carbapenems).
c) Beta-lactams merged with unsaturated six-membered rings (e.g. cephalosporins).
d) Beta-lactams not merged with any other ring (monobactams).

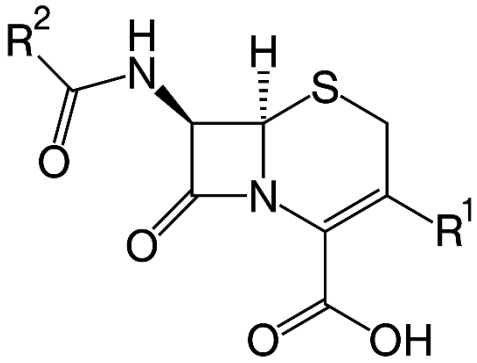
Table 2: Examples of beta-lactam antibiotics core structures.



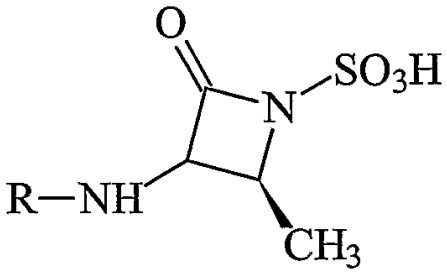
(a) Penicillin beta-lactam ring



(b) Carbapenems



(c) Cephalosporins



(d) Monobactams

Types of beta-lactam antibiotics include penicillins, cephalosporins, cephamycins, monobactams and carbapenems. The penicillins may be narrow spectrum (e.g. benzylpenicillin, benzathine benzylpenicillin, procaine benzylpenicillin and cloxacillin) or broad spectrum (e.g. amoxicillin and ampicillin) (Rao, 2012). Cephalosporins may be first-generation (e.g. cefalexin), second-generation (e.g. cefuroxime), third-generation (e.g. ceftriaxone, cefotaxime and cefpodoxime), fourth-generation (e.g. cefepime) or fifth-generation (e.g. ceftobiprole) (Laudano, 2011). Cephamycins include cefoxitin and cefotetan, while monobactams include aztreonam and tabtoxin (Rao, 2012). Carbapenems include imipenem, meropenem and doripenem (Sakyo *et al*., 2006).

Beta-lactam antibiotics target and bind to penicillin-binding proteins (PBPs), rendering them unable to perform their normal function in cell wall synthesis, leading to death of the bacterial cell due to osmotic instability and autolysis (Rao, 2012).

2.4 Antibiotic resistance mechanism

"Antibiotic resistance is the reduction in effectiveness of a drug such as an antimicrobial or an antineoplastic in curing a disease or condition" (Shaikh *et al*., 2014). The mechanisms of resistance to beta-lactams seen among bacteria can be grouped into three categories. These are: (1) Inactivation or modification of the beta-lactam antibiotic by the production of beta-lactamases, (2) alteration of the target site by production of newer penicillin binding proteins with less affinity for the antibiotic, and (3) reduced drug accumulation due to reduced permeability to the antibiotic through the cell envelope due to modified porin channels, and active efflux pumps that pump the drug out of the cell (Rao, 2012). Penicillin binding protein modification and bypassing are the drivers of resistance in Gram-positive cocci, whereas Gram-negative species predominantly utilize beta-lactamases (Livermore, 1998).

2.5 Beta-lactamases

Beta-lactamases are a family of enzymes produced by many Gram-positive and Gram-negative bacteria that inactivate beta-lactam antibiotics by cleaving the beta-lactam ring (Lakshmi *et al*., 2014). They do so by opening the amide bond of the beta-lactam ring and thus the beta-lactam antibiotics become harmless to the bacteria. Many beta lactamases possess serine in their active site but a few employ a zinc ion to

attack the beta-lactam ring (Livermore, 1998). Many studies show that the preponderance of ESBLs is higher in *K. pneumoniae* and this has been attributed to the organism's ability to withstand harsh environmental conditions such as desiccation (Paterson and Bonono, 2005; Rao, 2012). Relative to other enteric bacteria, *Klebsiellae* survive longer on hands and environmental surfaces, therefore making infection transmission possible within hospitals (Paterson and Bonono, 2005). A number of factors are known to play a role in enhancing the ability of beta-lactamases to cause resistance. The capacity of beta-lactamases to cause resistance is facilitated by rapid turnover of substrate, high affinity for the substrate, large amount of enzyme, and, in the case of Gram-negative bacteria, the low permeability of the outer membrane (Livermore, 1998). Beta-lactamases include ESBLs, Ampicillin class C (AmpC), sulphydral variable (SHV), Temoniera type (TEM), oxacillin-hydrolyzing (OXA), Pseudomonas Extended Resistance (PER) type, and Guyana Extended-Spectrum β-lactamase (GES) (Rawat and Nair, 2010). Since bacteria multiply at a high rate, the probability of random mutations in their genes is high. Point mutations in the beta-lactamase gene occurring by chance may result in changes in the primary structure of the enzyme and this can in turn lead to modified substrate specificity (Rao, 2012).

The native Temoniera type 1 (TEM-1) beta lactamase is associated with resistance to ampicillin, penicillin and first generation cephalosporins (Rupp and Fey, 2003). It was the first plasmid-mediated beta lactamase to be described after it was obtained from a urine specimen of a woman named Temoniera from Greece in 1965 (Rawat and Nair, 2010; Rao, 2012). The first variant described differed from TEM-1 through the substitution of a lysine for a glutamine at position 39, was found in a *P. aeruginosa* isolate in Great Britain in 1969, and was termed TEM-2 (Rup and Fey, 2003; Rao, 2012). The native sulphydryl variable type 1 (SHV-1) beta lactamase, which is associated with resistance to penicillins and first generation cephalosporins, was described in 1972 in a *K. pneumoniae* isolate (Rup and Fey, 2003; Rao, 2012). It was the second plasmid-mediated beta lactamase to be described. Both TEM-1 and SHV-1 lack broad spectrum activity and are unable to hydrolyze oxyimino-cephalosporins. They are therefore narrow spectrum beta-lactamases. It must be noted that TEM-1, TEM-2 and SHV-1 are not ESBLs. However, mutations in the beta-lactamase genes

may broaden the hydrolysis potential of TEM-1 and SHV-1 to extended-spectrum cephalosporins and monobactams (Rupp and Fey, 2003).

2.6 Classification of beta-lactamases

Classification of beta-lactamases has evolved over time and can be traced back to as early as the late 1960s (Bush *et al*., 1995). Early scientists classified beta-lactamases based on a variety of parameters such as hydrolytic range, hydrolysis rates, vulnerability to inhibitors, and whether they were chromosomal or plasmid mediated (Rao, 2012). Currently, beta-lactamases are classified based on amino acid similarity as well as on functional similarity of the enzymes. Two classification systems are commonly used; these are the Ambler Molecular Classification which divides beta lactamases into four major classes (A to D) based on amino acid sequence (Ambler, 1980), and the Bush-Jacoby-Medeiros functional classification which groups beta lactamases based on functional properties, i.e. the substrate and inhibitor profiles (Rawat and Nair, 2010; Shaikh *et al*., 2014). The Bush-Jacoby-Medeiros functional classification is of much more practical application to the clinician or microbiologist in a diagnostic laboratory because it considers beta-lactamase inhibitors and beta-lactam substrates that have relevant implications on the management of patients (Paterson and Bonono, 2005).

The Bush-Jacoby-Medeiros functional classification groups beta-lactamases into 3 groups and several subgroups.

Group 1 comprises cephalosporinases that are inactivated by cloxacillin and aztreonam but not clavulanic acid.

Group 2 is made up of a variety of enzymes that hydrolyze penicillins, cephalosporins, broad-spectrum beta-lactams and serine carbapenems, and are inhibited by clavulanic acid.

Subgroup 2a beta-lactamases are penicillinases that readily hydrolyze benzylpenicillin and are inhibited by clavulanic acid and tazobactam.

Subgroup 2b enzymes, just like those in subgroup 2a, are inhibited by clavulanic acid and tazobactam. However, these enzymes easily hydrolyze penicillins and

cephalosporins whereas subgroup 2a enzymes hydrolyze cephalosporins at rates not more than 10% those for benzylpenicillin or ampicillin. TEM-1, TEM-2 and SHV-1 enzymes belong to this group, along with several other TEM and SHV enzymes (Bush and Jacoby, 2010).

Subgroup 2be is a subset of subgroup 2b. Members from this group have properties of group 2b but, in addition, these enzymes hydrolyze one or more oxyimino-beta-lactams (ceftazidime, cefotaxime and aztreonam) at a rate more than 10% that of benzylpenicillin (Rao, 2012). They are extended spectrum beta-lactamases which are mostly mutants of TEM-1, TEM-2 and SHV-1. The group also includes several other ESBLs not closely related to TEM-1 or SHV-1 (Bush *et al*., 1995).

Subgroup 2br enzymes are said to be inhibitor resistant as they are resistant to inhibition by beta-lactamase inhibitors.

Subgroup 2ber enzymes have a substrate profile similar to subgroup 2be. However, these enzymes are resistant to inhibition by clavulanic acid (Rao, 2012).

Subgroup 2c enzymes are penicillinases that preferentially hydrolyze carbenicillin or ticarcillin and poorly hydrolyze cloxacillin or oxacillin and are inhibited by clavulanic acid or tazobactam (Thirapanmethee, 2012).

Subgroup 2d beta-lactamases preferentially hydrolyze cloxacillin or oxacillin and hence they are also called OXA enzymes (Bush and Jacoby, 2010).

Subgroup 2de consists of OXA enzymes with a broader substrate profile that includes oxyimino-beta-lactams but not carbapenems.

Subgroup 2df and subgroup 2f beta-lactamases are serine carbapenemases. Subgroup 2df enzymes have the ability to hydrolyze oxacillin and carbapenems, while subgroup 2f enzymes are capable of hydrolyzing oxyimino-cephalosporins, carbapenems and cephamycins (Rao, 2012).

Subgroup 2e enzymes poorly hydrolyze penicillins but are able to hydrolyze extended-spectrum cephalosporins.

Group 3 enzymes include metallo-beta-lactamases that have need of either one or two zinc atoms in their active site (Rao, 2012).
Subgroup 3a enzymes require two zinc atoms for their hydrolytic activity while subgroup 3b enzymes require only one zinc atom in their active sites.

The Ambler's molecular classification, introduced by RP Ambler in 1980, considers the primary structure of the beta-lactamases (Ambler, 1980). The system initially classified beta-lactamases into two classes (A and B) but currently four classes (A to D) are identified (Rawat and Nair, 2010).

Class A enzymes have serine in their active sites, can be either chromosomal or plasmid-mediated, and may confer resistance to penicillins, cephalosporins and carbapenems.

Class B enzymes are metallo-beta-lactamases which contain the zinc (2+) ion in their active sites. They confer resistance to penicillins, cephalosporins and carbapenems, and are not inhibited by common beta-lactamase inhibitors (Rao, 2012).

Class C beta-lactamases are serine-containing cephalosporinases which confer resistance to aminopenicillins, oxyimino-cephalosporins, cephamycins and beta-lactamase inhibitors (Thirapanmethee, 2012).

Class D beta-lactamases are the OXA-type beta-lactamases, named so because of their ability to hydrolyze oxacillin and cloxacillin at high rates (Danel *et al*., 1998).

The simplicity of the Ambler's molecular classification makes it easier to understand and probably has more universal acceptance than the Bush-Jacoby-Medeiros function classification.

2.7 Description of ESBLs

Extended spectrum beta-lactamases (ESBLs) are mutant enzymes with a broad spectrum of activity compared to their parent molecules (Lakshmi *et al*., 2014). More than 150 different ESBLs have been documented worldwide (Rupp and Fey, 2003). ESBLs have led to serious reductions in the efficacy of beta-lactam antibiotics as evidenced by the loss of effectiveness of these previously potent antibacterial agents with resultant treatment failure (Bush, 2010). Because of their ability to confer resistance to the extended spectrum cephalosporins, these beta-lactamases were termed extended spectrum beta lactamases. ESBL-mediated resistance is believed to be spreading throughout the community and not primarily within healthcare-related institutions (Leistner *et al*., 2013).

ESBLs are usually plasmid-mediated and these plasmids may carry genes that lead to resistance to other antibiotics (Rupp and Fey, 2003; Paterson and Bonomo, 2005). This explains why ESBL-producers are resistant to several other antibiotics including aminoglycosides, trimethoprim, suphonamides, tetracycline and chloramphenicol. A study conducted in India showed that multidrug resistance was higher in ESBL-producers than in non-producers (Chatterjee *et al*., 2012).

Most ESBLs are derivatives of TEM-1, TEM-2, and SHV-1, with generally not more than 6 amino-acid sequence substitutions (Livermore and Paterson, 2006; Rao, 2012). Amino acid substitutions at different amino acid positions, acting independently or in unison with other structural gene mutations, have been demonstrated in over 90 TEM-1 or TEM-2-derived ESBLs (Rup and Fey, 2003). The resultant change in substrate profile is due to alteration and modification of the enzyme's active site, making it able to accommodate the side chain of a cephalosporin and explains the transformation of a beta-lactamase enzyme to an extended spectrum beta-lactamase after amino acid substitutions in the parent enzyme. For example, substituting lysine and arginine with glycine at position 240 in TEM and SHV ESBLs decreases activity against ceftazidime because the electrostatic interaction between the positively charged residues (Lys and Arg) of the enzyme and the negatively charged carboxyl group from ceftazidime is lost as the substitute (glycine) carries no charge (Rao, 2012). Mutants of TEM and SHV beta-lactamases make up the largest group of ESBLS. Differentiation between these enzymes requires PCR amplification of the *bla*TEM

and *bla*SHV genes with oligonucleotide primers, followed by amino acid sequencing (Shaikh *et al*., 2014).

Non-TEM and non-SHV ESBLs are not closely related to TEM-1 or SHV-1-derived enzymes. These include OXA-type, CTX-M-type, VEB-type, BES-type and PER-type beta lactamases (Paterson and Bonomo, 2005; Jacoby and Munoz-Price, 2005). ESBL-producing organisms exhibit co-resistance even to non-beta-lactam antibiotics, resulting in limitation of therapeutic options (Rawat and Nair, 2010; Mshana *et al*., 2013).

The CTX-M-type is the second largest group of ESBLs with 5 subgroups and around 40 members, classified based on their amino acid sequence similarities (Brin˜as *et al*., 2005; Rawat and Nair, 2010). The 5 groups are CTX-M-1, -2, -8, -9, and -25. The CTX-M-type enzymes are classically known to exhibit more resistance to cefotaxime than to ceftazidime (Brin˜as *et al*., 2005; Livermore *et al*., 2007) but some CTX-M enzymes, such as CTX-M-15 and -19, also hydrolyze ceftazidime efficiently, thus making their phenotypic identification difficult. This higher affinity for cefotaxime is demonstrated by the higher mean inhibitory concentrations (MICs) observed for cefotaxime than for ceftazidime in organisms producing CTX-M-type beta-lactamases. In addition to the high resistance against cefotaxime, another unusual feature of CTX-M beta-lactamases is that they are more likely to be inhibited by tazobactam than by sulbactam and clavulanate (Shaikh *et al*., 2014). The CTX-component of the name of this group of enzymes comes from “cefotaxime”, pointing out that these enzymes preferentially hydrolyze cefotaxime relative to ceftazidime. The –M component comes from “Munich”, as the first clinical isolate was observed in Munich, Germany (Rao, 2012). It is thought that the CTX-M-type ESBLs, especially CTX-M-15, are now the most prevalent ESBL type worldwide and are still progressing to become a global pandemic (Bonnet, 2004; Lewis *et al*., 2007; Woerther *et al*., 2013). Unlike TEM and SHV derived ESBLs which are more frequently observed among hospital-acquired strains, CTX-M enzymes are more predominant in community strains (Rao, 2012). The origin of the CTX-M enzymes is considered to be different from that of TEM and SHV ESBLs in that while SHV-ESBLs and TEM-ESBLs were products of amino acid substitutions in their parent

enzymes, CTX-M ESBLs are believed to have been acquired by the horizontal gene transfer from other bacteria (Shaikh *et al*., 2014).

2.8 Geographic distribution of ESBLs

There has been a realization that the epidemiology of ESBL-producing bacteria is still very difficult to understand because of its complexity and increasing unclear boundaries between hospitals and the community (Shaikh *et al*., 2014). As noted earlier, ESBL-producing organisms have a global distribution (Babini and Livermore, 2000; Rup and Fey, 2003). Different beta-lactamase types are distributed differently across the globe as certain types are preponderant in certain geographical areas (Casellas, 1999). For example, TEM-type beta-lactamases were a major concern in France while PER-1 class A cephalosporinase is a problem in *Salmonella* and *P. aeruginosa* from Turkey (Rao, 2012).

There are over 1.1 billion ESBL carriers in the community populations of Southeast Asia, while the Western Pacific and Eastern Mediterranean have 280 and 180 million carriers, respectively, ahead of Africa, which has approximately 110 million carriers (Woerther *et al*., 2013). The main mechanism which leads to the spread of antibiotic resistance is horizontal transfer of antibiotic resistance genes by conjugation, transformation or transduction (Shaikh *et al*., 2014).

The first description of ESBLs was in England and Germany in 1983 (Rup and Fey, 2003). This was in *K. pneumoniae* and *Serratia marcescens* isolates (Gupta *et al*., 2003). Later, studies confirmed the presence of ESBLs in the Netherlands, France and Italy. In the United States, the presence of ESBL-producing organisms was first documented in 1988, and the rates of ESBL acquisition have been noted to vary from 0 to 25% with an estimated national average of 3% (Rup and Fey, 2003; Paterson and Bonomo, 2005), while in Latin America, the SENTRY antimicrobial surveillance programme concluded that 45% of *K. pneumoniae* and 8.5% of *E. coli* were ESBL-producers (Rup and Fey, 2003). In Asia, the presence of ESBL-producing organisms has been noted in Korea, Indonesia, China, Thailand, Taiwan, Singapore, Japan, and the Philippines, and in Australia such outbreaks have a wide distribution (Paterson and Bonomo, 2005).

Despite the limited surveillance data in most African countries, outbreaks of infection due to ESBL-producing organisms have been noted in some African countries, including South Africa, Tanzania, Rwanda, Ethiopia, Kenya, Tunisia, Egypt, Nigeria and Algeria (Mshana *et al*., 2009; Nelson *et al*., 2014; Storberg, 2014). Research on ESBLs has been done locally in a number of African countries but there are no generalized results describing the situation in the continent as a whole. ESBLs have been reported in different regions of the continent with varying prevalence (Storberg, 2014). Heavy antibiotic use and poor hygiene standards in Africa may be responsible for the transmission of ESBLs in the continent (Woerther *et al*., 2013). In Northern Africa, the prevalence of ESBLs ranges from 16.4-31.4% in Algerian hospitals, 11-42.9% in Egyptian hospitals and communities, and 11.7-77.8% in Tunisian hospitals (Storberg, 2014). In Eastern Africa, ESBLs exist in Ethiopia and Kenya at carriage rates of 62.8% and 37.4%, respectively, while in Rwanda the prevalence is 38.3% in hospital urine samples and 5.9% in community urine samples (Storberg, 2014). Studies in Tanzania show that ESBL producing organisms are common and pose a challenge to antibiotic therapy as they exhibit resistance to most antibiotics used in hospitals. The antibiotics include gentamycin, tetracycline, and sulphamethoxazole/trimethoprim (Mshana *et al*., 2009). In Central African Republic, 11.3% of community urine samples contained ESBL producers, while in West Africa, ESBL existence has been documented in Ghana, Mali, Nigeria and Senegal, with prevalence ranging from 10-96% (Storberg, 2014). In Southern Africa, ESBLs have been reported in South Africa at a prevalence of 36.1% among *K. pneumoniae* isolates collected during a study in 1998 and 1999 (Paterson and Bonomo, 2005). Very limited studies of this kind have been conducted in Zambia.

These geographical discrepancies and some retrospective studies of patients with ESBL-producers led to the development of the hypothesis that history of travel to countries with higher occurrences of ESBL-producing *Enterobateriaceae* could be a risk factor for the acquisition of ESBLs (Tham *et al*., 2012), and this hypothesis was proved in a study that showed a strong association ($P<0.001$) between travel to India and ESBL acquisition (Kuster *et al*., 2010; Ta¨ngde´n *et al*., 2010). Another study confirmed this hypothesis when travel to Africa, Southeast Asia, India, Thailand and the Middle East was found to be associated with high ESBL acquisition rates (Woerther *et al*., 2012).

2.9 Risk factors

Numerous studies aimed at assessing risk factors for colonization and infections with ESBL-producing organisms have been conducted (Mahesh *et al*., 2010; Ta¨ngde´n *et al*., 2010; Woerther *et al*., 2012). Analysis of the results of these studies has given rise to a broad array of contradictory results, possibly due to the variations among different study populations, methods employed in selection of cases and controls, and sample size (Paterson and Bonono, 2005). Regardless of these disparities in findings, some generalizations have been made.

Infections with ESBL producers can be community-acquired or healthcare associated (Rup and Fey, 2003). Studies show that ESBL carriage differs between patients, health care workers, and healthy subjects. A study conducted by Moubareck and colleagues (2003), which involved analysis of fecal samples, showed an ESBL carriage of 16% among patients, 3% among health workers, and 2% among healthy subjects, while other researchers in Lebanon observed that there was a preponderance of ESBL-producing isolates among inpatients (15.4%) relative to outpatients (4.5%) (Shaikh *et al*., 2014). A study done in Basel, Switzerland showed that nosocomial transmission of ESBL-producing bacteria from non-isolated index patients to room-mates is astoundingly low (1.5%) when standard infection prevention measures are in place (Kluytmans-van den Bergh and Kluytmans, 2012), suggesting that low levels of hygiene may be a significant risk factor. Established risk factors associated with acquisition of ESBL-producing organisms include prolonged hospital stay, invasive medical devices (e.g. nasogastric tubes, gastrostomy and jejunostomy tubes, or arterial lines), administration of total parenteral nutrition, recent surgery, hemodialysis, decubitus ulcers, diabetes, low birth weight in preterm infants, mechanical ventilation, and poor nutritional status (Gupta *et al*., 2003; Rupp and Fey, 2003; Pasricha *et al*., 2013).

Male gender has also been found to be a significant risk factor of urinary tract infection (UTI) by an ESBL-producing bacterium (Mahesh *et al*., 2010). One would expect to find a higher incidence of urinary tract-related ESBL-producing bacteria in women since females are generally more prone to UTI than men due to the shorter course of the urethra compared to men. However, the opposite is true. The longer course of the urethra and the bacteriostatic properties of the prostate in men are

protective factors against UTI. The usual microorganisms cannot overcome this barrier, but ESBL-positive organisms are able to resist these obstacles, leading to a higher incidence in males (Mahesh *et al*., 2010).

Patients with diabetes mellitus have a compromised immune system with lower number of white blood cells in the urinary tract, which is an essential factor in protecting the host (Mahesh *et al*., 2010). The reduced local immunity significantly increases the risk of UTI by multidrug resistant microorganisms.

Recently, there has been a lot of discussion linking the food chain to human colonization with ESBL-positive organisms (Leistner *et al*., 2013). Indirect evidence of transmission of ESBL-producing bacteria from food-producing animals or food to humans is provided by data about clonally related ESBL-producing *E. coli* and significant similarities between plasmids in animals and humans (Overdevest *et al*., 2011; Hammerum *et al*., 2014). However, no experimental study has been conducted to authenticate the possibility of colonization through the consumption of contaminated food, although a study conducted in Germany suggested that frequent consumption of pork was associated with the acquisition of ESBL-positive *E. coli* in the community (Leistner *et al*., 2013).

Low socioeconomic status may be a risk factor for ESBL carriage. A study conducted in Madagascar in which 10% of outpatients carried ESBLs also showed that low socioeconomic status was significantly associated with ESBL carriage (Herindrainy *et al*., 2011). The high carriage rates of ESBL-producing bacteria in parts of Asia and the Indian subcontinent has been attributed to unsafe sewage routines and unavailability of adequately treated drinking water, as well as lack of strict policies promoting antimicrobial stewardship (Shaikh *et al*., 2014).

Other risk factors include heavy antibiotic use (Bisson *et al*., 2002; Peña *et al*., 2006), old age (above 65years), and dementia (Rawat and Nair, 2010). Countries with fewer restrictions on antibiotic use are expected to have higher rates of ESBL carriage while those with strict policies on antibiotic use are expected to have relatively fewer cases of ESBLs. It is therefore surprising that the ESBL colonization rates in Switzerland are as high as 15% in pigs and 63% in chickens (Woerther *et al*., 2013) despite the

prudent use of antibiotics in that country. One possible explanation for this observation could be that some farmers could be illegally using antibiotics for growth promotion, leading to selection for resistant strains.

Placement in close proximity with patients infected or colonized with ESBL-producing organisms is by far the greatest risk factor for nosocomial acquisition of an ESBL-producing organism (Rawat and Nair, 2010), although this risk was found to be insignificant in the ICU setting (Harris *et al*., 2007). In the same way, expectant women who are carriers of ESBL-producing organisms in the gastrointestinal tract may pass these microbes on to their neonates (during intrapartum and postpartum periods), to other patients in the same ward, and to health care providers (Nelson *et al*., 2014). It is therefore important that special precautions are taken during hospital stay to stop these microorganisms from spreading to other patients. Some of the precautions may include having the patient remain in a private room, placing a sign on the door of the room to alert every one of the precautions needed, and practicing frequent hand washing (Rupp and Fey, 2003).

2.10 Clinical features

Clinical features in patients infected with ESBL-producing organisms vary depending on the infecting organism, duration of infection, organ-system involved, and whether appropriate therapy has been instituted or not. There is need to distinguish between infection and colonization. Colonization refers to the presence of an organism in the body without clinical disease while infection is the presence of an organism accompanied by clinical disease (Casadevall and Pirofski, 2000). The presenting sign in patients infected with ESBL-producing organisms is treatment failure, and the mortality rate in such patients can range from 42-100% (Rupp and Fey, 2003). Common community acquired ESBL- infections are urinary tract infections (UTIs) with CTX-M–producing *E. coli* (Rawat and Nair, 2010). These organisms may be resistant to most or all antimicrobial agents commonly used to treat UTIs, such as ciprofloxacin, trimethoprim-sulfamethoxazole, gentamycin, nitrofurantoin and ceftriaxone. Patients with such an infection may present with frequency, urgency, chills, fever, nausea, and bilateral back pain. Physical examination may be unremarkable or there may be mild abdominal or loin tenderness. Urinalysis may show high numbers of leukocytes per high-powered microscopic field and urine

culture may grow over 100,000,000 colony-forming units/L of *E. coli*, resistant to trimethoprim/sulfamethoxazole and ciprofloxacin (Longmore *et al*., 2005).

Catheterization is generally associated with urinary tract infection and may be a risk factor for acquisition of ESBL-bacteria (Mahesh *et al*., 2010). It has been suggested that the increased risk of infection may be mainly due to lapses in following standard sterile procedures during the insertion of the catheter, or due to introduction of infection into the collecting system via the lumen of the catheter, and that the risk may be reduced by applying better sterile techniques and by using silicone catheter in high-risk patients (Mahesh *et al*., 2010). Orienting clinicians and infection control specialists on the risk factors associated with ESBL positive urinary tract infections is invaluable for determining the empiric antibiotic therapy of high-risk cases (Mahesh *et al*., 2010).

Several pathogenic organisms of public health importance are known to be ESBL-producers, including *Salmonella*, *Shigella* and *Vibrio cholera* (Paterson and Bonomo, 2005). *Salmonella serovar typhi* is the causative agent of typhoid fever, a systemic *Salmonella* infection which is spread by the fecal-oral route and is usually very severe with high rates of morbidity and mortality, especially in third world countries (Naiemi *et al*., 2008). *Salmonella* infections are usually treated with common antimicrobial agents, including fluoroquinolones, chloramphenicol, cefotaxime, azithromycin, and amoxicillin, but recent studies show that resistance is a problem (Longmore *et al*., 2005; Naiemi *et al*., 2008).

Typhoid fever begins one to two weeks after ingestion of the organism. In the early stages of the disease the fever is low grade, but as the illness progresses the fever becomes high grade (39° to 40°C), and may be associated with relative bradycardia (Ostergaard *et al*., 1996). It is characterized by a rising temperature which is worse during the course of each day and subsides by the subsequent morning. The temperature chart usually shows successively taller peaks as the illness progresses. Over the course of the first week of illness, patients usually develop abdominal symptoms and signs. These include abdominal pain and tenderness which is usually not localized, but in some cases patients may develop localized pain in the right upper quadrant due to gall bladder or liver involvement (Bhandari *et al*., 2009). Patients may

also present with constipation but diarrhea is also common especially in children and HIV-infected young adults (Parry *et al*., 2002). The individual may then develop a dry cough, dull frontal headache, epistaxis, rose spots, anorexia, nausea, confusion, and an increasingly stuporous malaise (Parry *et al*., 2002). *Salmonella* spp may be isolated from blood and fecal samples of the patients, which may be resistant to extended spectrum cephalosporins (Uma *et al.*, 2010).

Gastrointestinal perforation and hemorrhage are among the most feared complications of typhoid fever, and are often fatal. Other complications include septicaemia, diffuse peritonitis, encephalitis, metastatic abscesses, cholecystitis, endocarditis, osteitis, psychotic states, meningitis, impairment of coordination, bronchitis, pneumonia (*Salmonella enterica* serotype *typhi, Streptococcus pneumoniae*), anemia, disseminated intravascular coagulation, pharyngitis, miscarriage, relapse, and chronic carriage (Parry *et al*., 2002; Baylis *et al*., 2011)

2.11 Methods for ESBL detection

The laboratory has an extremely important role to play in the control of the spread of ESBLs as it is in the laboratory where the diagnosis of ESBLs is made. Ignorance about ESBLs and their detection may lead to high incidence of treatment failure among patients treated with inappropriate antibiotics (Pitout *et al*., 2005). It is therefore necessary for laboratory personnel to be well oriented in the methods used to detect ESBLs. However, many laboratories in Zambian hospitals are both underequipped and understaffed, restricting their operations only to basic laboratory tests and procedures.

ESBL detection involves both phenotypic and genotypic detection. Based on guidelines proposed by the Clinical Laboratory Standards Institute (CLSI) of the United States of America in 1999, the occurrence of an ESBL is suspected if bacterial growth is supported despite a concentration of 1µg/ml of at least one of three extended spectrum cephalosporins (ceftazidime, ceftriaxone or cefotaxime) or aztreonam, or growth is observed despite a concentration of 4µg/ml of cefpodoxime (Paterson and Bonomo, 2005).

Phenotypic confirmatory tests may employ comparison of minimum inhibitory concentration (MIC) for ceftazidime or cefotaxime in combination with clavulanic acid versus its MIC when tested alone, where a decrease of at least 3 serial dilution concentrations is regarded as a positive phenotypic test for an ESBL. If disk diffusion is used, zone diameter for either cefotaxime or ceftazidime tested alone is compared to its zone diameter when tested with clavulanic acid. Increase of at least 5mm is considered a positive phenotypic test. The principle on which these guidelines are based is that most ESBLs confer resistance to third-generation cephalosporins although they are susceptible to clavulanate (Shaikh *et al*., 2014). Other methods used to phenotypically detect ESBLs include Double Disk Approximation Test, Three-Dimensional Test, Epsilometer test (E-Test), and Vitek (Rupp and Fey, 2003).

Various molecular approaches exist for the screening of ESBL-positive organisms. PCR amplification of the *blaTEM* and *blaSHV* genes with oligonucleotide primers, with subsequent sequencing, can be used to differentiate between the non-ESBL parent enzymes (e.g. TEM1, TEM2, or SHV1) and diverse variants of TEM or SHV ESBLs (e.g TEM3, SHV2, etc), while PCR amplification of CTX-M specific products with no sequencing, in an isolate that is known to harbor an ESBL, is generally suggestive of the presence of *blaCTX-M* gene (Shaikh *et al*., 2014).

2.12 Treatment of infections due to ESBL-producing organisms

Beta-lactam antimicrobial agents are the most commonly used form of treatment for bacterial infections and continue to be the antibiotic class that is most affected by resistance conferred by Gram-negative bacteria worldwide (Shaikh *et al*., 2014). Treatment of infections due to ESBL producers can be very challenging as these bacteria exhibit resistance to many other types of antibacterial drugs making antibiotic choice for treatment of such infections markedly limited. Knowing the resistance patterns of bacterial strains in an institution or community can significantly increase the effectiveness of treatment of bacterial infections through appropriate antibiotic use.

Beta-lactam/beta-lactamase inhibitor combinations have been deemed to be a possible form of treatment for infections due to ESBL-producing organisms because *in vitro* and *in vivo* models indicate a possibility of successful treatment outcomes following

use of these combinations (Rupp and Fey, 2003). These combinations are usually effective if the isolate produces only a single ESBL (Paterson and Bonono, 2005). However, the simultaneous occurrence of multiple beta-lactamases as well as porin deficient mutants in ESBL-producing organisms limits the use of these agents as first line therapy (Rupp and Fey, 2003). Currently, the treatment of choice for grave infections with ESBL-producing *E. coli* and *K. pneumoniae* are the carbapenems (imipenem, meropenem, ertapenem, doripenem) as studies show that more than 98% of the ESBL-producing *E. coli, K. pneumoniae and P. mirabilis* are still susceptible to these antimicrobials (Shaikh *et al*., 2014). Imipenem and meropenem have been shown to be resistant to extended spectrum TEM and SHV derivatives, and to exhibit a high level of penetration to the target site. Besides these properties, the carbapenems also have a high affinity for penicillin binding proteins and are not susceptible to attack by most beta-lactamases (Horii *et al*., 1998; Rao, 2012). Carbapenems are resistant to hydrolysis brought about by ESBLs and have been found to be effective against strains of *Enterobacteriaceae* expressing ESBLs (Rupp and Fey, 2003). Use of imipenem/cilastatin combination has been shown to reduce mortality (Rao, 2012).

2.13 Hospital infections due to ESBLs

Most outbreaks of infection due to ESBL-producing organisms occur in hospitalized patients, especially those in Intensive Care Units (Rodriguez-Ban˜o and Paterson, 2006; Razazi *et al*., 2012), those with history of undergoing solid organ transplant, burn patients, cancer patients, and neonates (Rupp and Fey, 2003). Reservoirs of ESBL-producing organisms may include health care workers' hands and clothing, medical equipment such as thermometers, oxygen probes and stethoscopes, contaminated ultrasonography gel, liquid soap, and vectors such as cockroaches (Rupp and Fey, 2003). In addition, ESBLs may be found in the environment (e.g. water and soil) and in wild and domestic animals (Meyer *et al*., 2012; Shaikh *et al*., 2014), but the gastrointestinal tract is the main reservoir of both community and hospital acquired enterobacteria (Woerther *et al*., 2013). There is some evidence to implicate cockroaches in the transmission of infection (Rupp and Fey, 2003). In hospitals cockroaches may mechanically transmit pathogens to patients. These insects may be attracted to secretions and excretions from patients including vomits, blood, pus, sweat, tears, urine, mucosal secretions, serum seepage, sputum and feces (Al-

bayati *et al*., 2001). These may in turn contaminate food and surfaces, thus facilitating transmission of bacterial infections.

Evidence suggests that ESBL-producing organisms can be transferred from one patient to another owing to temporal carriage on the hands of health care workers, while ESBL-producing organisms have also been found in patients' soap, sink basins, and babies' baths, but the extent to which this kind of contamination increases the chance of infection has not been determined (Paterson and Bonono, 2005).

2.14 Control of infections due to ESBL producers

Most infection control programs in institutions mainly focus on reducing transmission of nosocomial bacterial infections (Paterson and Bonomo, 2005). The approach towards control of ESBL-associated infections depends on whether ESBLs are already endemic or not. Recognizing the reservoir of infection in an outbreak setting can be invaluable for infection control practitioners to curtail the outbreak. For example, contaminated ultrasonography gel can be replaced quickly, while thermometers, stethoscopes or bronchoscopes colonized with ESBLs can be disinfected and maintained properly to avoid transmission. In some cases temporarily closing a ward or unit may be necessary in order to control an outbreak (Macrae *et al*., 2001).

ESBL associated infections may occur as new cases occurring at a higher frequency than expected, suggesting a significant imbalance between the agent and host with the agent having an upper hand, or may become established with regular occurrence. It is important to be able to distinguish between nosocomial infections that are caused by the same clone of organisms (monoclonal or oligoclonal outbreaks) from those that are caused by different clones of organisms because this may give an idea about the dynamics of transmission of the infection. If the same strain of organisms is responsible for infections in an outbreak then it could mean that there is horizontal transfer of organisms from patient to patient (Paterson and Bonomo, 2005). This information is indispensable as it determines the action to be taken following the outbreak. For example, stringent infection control measures may be taken if the infections are caused by the same clone of organisms to avoid horizontal transfer of pathogens. On the other hand, nosocomial infections with organisms of the same

species which are not of the same strain (polyclonal outbreaks) could be due to heavy antibiotic use with resultant selection for resistant strains (Paterson and Bonomo, 2005).

Infection control can also be accomplished by studying the epidemiology of ESBL-producing bacteria (Rodriguez-Ban˜o and Paterson, 2005). The methods involved in these studies may include plasmid profiles, pulsed-field gel electrophoresis (PFGE), ribotyping, arbitrarily primed polymerase chain reaction (PCR) and random amplified polymerase DNA (RAPD) (Rupp and Fey, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical consideration

Ethical clearance was obtained from Excellence in Research Ethics and Science (ERES) converge and a written informed consent was obtained from each patient or caregiver.

3.2 Sample size

Since the prevalence (p) of ESBLs in Zambia was not known at the time of the study, we set p=0.5 to yield the maximum value of the sample size (n). Assuming that we require the estimate to be within 5% of the true value in either direction then, at 95% CI, the sample size, $n=z^2.p(1-p)/d^2$,where z is the value of the standard normal distribution corresponding to a significance level of α (1.96 for α=0.05), p=0.5, and d=0.05. This gives n=384. This was the minimum number of patients to be recruited.

3.3 Data collection

Data collection on variables of interest was done by administering a structured questionnaire to 400 patients admitted at the UTH (Questionnaire attached as Appendix 1).

3.4 Sampling

Between 1st and 30th of May 2015, a total of 400 rectal swabs were collected from patients admitted in general medical and surgical wards of the UTH.

The Inclusion criteria included patients in the general medical and surgical wards aged 18 years and above, and willing to participate in the study. The specimens were collected over a period of 4 weeks. All laboratory work was done from the Hokkaido Centre for Zoonosis Control in Zambia, University of Zambia.

3.5 Initial screening for ESBL production

Initial screening was done by dilution susceptibility test as proposed by CLSI. Each sample was inoculated on MacConkey agar (Oxoid, UK) supplemented with cefotaxime at a screening concentration of 1µg/ml and incubated at 37ºC for 24 hours. Growth at this screening antibiotic concentration was suggestive of ESBL production (Paterson and Bonomo, 2005).

3.6 Phenotypic confirmatory test for ESBL production

This was done with a cefotaxime disk (30 µg) alone and in combination with clavulanic acid (30 µg /10 µg). Increase in zone diameter of at least 5mm for the cephalosporin and its cephalosporin/clavulanate disk was considered to be a positive phenotypic confirmatory test.

3.7 Identification of *E coli*

Colonies that appeared pink to red on MacConkey agar were suspected to be *E. coli* based on the fact that *E. coli* (and *Enterobacter aerogenes*) ferments the lactose producing acid and forms colonies that are pink to red. Lactose fermentation-positive colonies were subjected to a battery of biochemical tests known as IMViC (Indole, Methyl red, Voges-Proskauer and Citrate). Any isolate demonstrating an IMViC pattern of positive-positive-negative-negative was considered to be *E. coli* (Zahera *et al*., 2011).

3.8 Genetic detection and characterization

3.8.1 Preparation of bacterial DNA

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 × *g* for 5 minutes. After centrifuging, the supernatant was discarded. The remaining cell pellet was washed with 500µl of normal saline, centrifuged at 13000 × *g* for 5 minutes and supernatant was discarded. After washing, 500µl of TE buffer (pH 8.0) was added to the cell pellet and boiled at 100ºC, then immediately transferred to ice for 10 minutes. Cell debris was removed after

centrifuging at 13000 × *g* for 5 minutes, while the supernatant was transferred into a new microfuge tube and maintained at -20ºC until use.

3.8.2 PCR procedure

3.8.2.1 Preparation of Master Mix

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. The primers used are shown in table 3.

The reaction tubes were covered with special standard sealing cover (highly heat resistant) so that there was no evaporation while the thermal cycler machine (PCR machine) was running. The reaction tubes were placed into the holes of the thermal cycler and the door of the machine was closed.

Table 3: Primers used in this study

Gene	Primer	Sequence	Expected Amplicon
bla_{TEM}	bla_{TEM} -F	TCGGGGAAATGTGCG	1074
	bla_{TEM} -R	TGCTTAATCAGTGAGGCACC	
bla_{SHV}	bla_{SHV} -F	GCCGGGTTATTCTTATTTGTCGC	1016
	bla_{SHV} -R	ATGCCGCCGCCAGTCA	
bla_{CTX-M}	bla_{CTX-M} -F	CGATGTGCAGTACCAGTAA	585
	bla_{CTX-M} -R	TAAGTGACCAGAATCAGCGG	

3.8.2.2 PCR Conditions

Conditions during reaction 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.

3.8.2.3 Preparation of 1.5% Agarose Gel

A 1.5% Agarose Gel was prepared by adding 1.5g of Agarose gel powder to 100ml of TAE Buffer and heating the mixture to dissolve completely in the microwave oven. The mixture was then cooled to about 45 to 50℃. 10 microliters ethidium bromide was added to the molten gel and swirled to mix completely. 20 milliliters of this mixture was poured on the plate in the trough and a comb was placed in the molten gel on the plate. The mixture was allowed to cool at room temperature for about 30 minutes, and after it had solidified a small amount of TAE buffer was added. The comb was removed from the solidified gel.

3.8.2.4 Running Gel Electrophoresis

Enough TAE buffer was applied in the electrophoretic tank. The solidified gel was placed on the base (holder) of the tank and was completely immersed in the TAE buffer.

3.8.2.5 Loading of samples on gel

Gene ruler (marker) was applied in the first well of the gel in the electrophoresis operating machine. 5 microliters of 6X loading dye was applied on a piece of para-film. To the 5 µl of the 6X loading dye on para-film, 5 µl of PCR product was added and the two were mixed completely by pipetting. The mixture was transferred into the well of gel. This was done repeatedly for all the samples in question, ensuring that each well only had one sample. Electrophoresis was run for 40 minutes, after which the gel was viewed on a trans-illuminator machine (Fig. 8 and Fig. 9 in results section).

3.9 Antimicrobial sensitivity test

The CTX-M positive isolates were subjected to antimicrobial sensitivity test using the Kirby-Bauer agar disk diffusion method (Changkaew *et al*., 2015). Antimicrobial discs were placed on growth plates containing Muellar-Hinton agar (BD Difco,

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.

3.10 Data analysis

Data was analyzed using SPSS version 20. The dependence of the categorical response variable (ESBL result) on the hypothesized explanatory variables (which were either continuous or categorical) was modeled using logistic regression. Statistical significance was set at $P \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Demographic data

4.1.1 Gender distribution

A total of 400 patients were recruited in this study. There were more males (244) than females (176) recruited in the study as shown in figure 1 below.

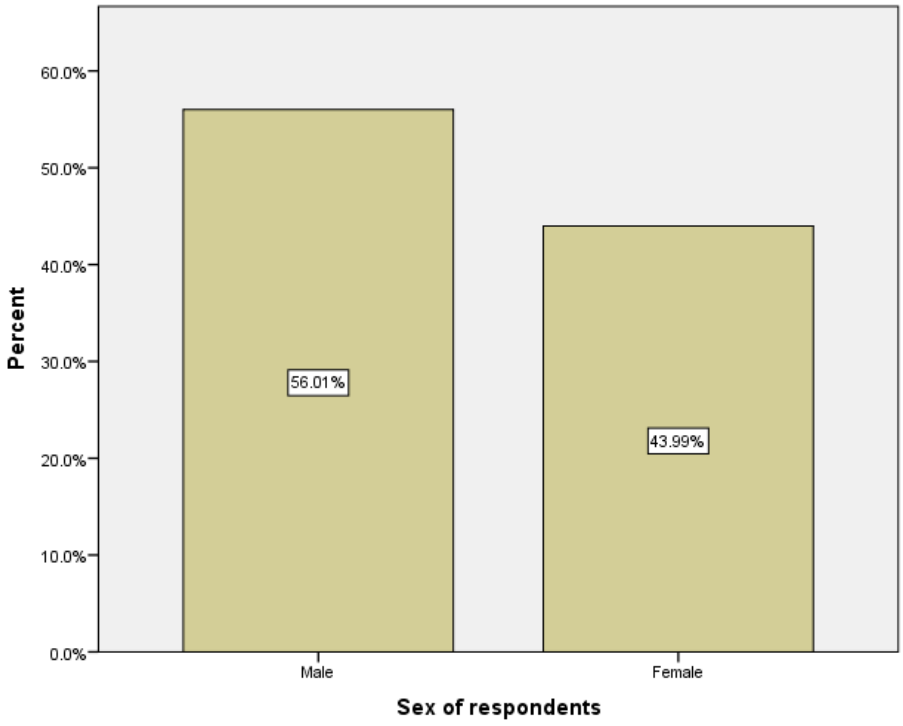


Figure 1: Gender distribution on the sampled population

4.1.2 Age distribution

The most frequent age range (in years) was the “30-39” category (27.8%), followed by the “over 50” category (24.9%), then “40-49” (24.4%), “20-29” (20.8%) and “15-19” (2.3%) (Figure 2).

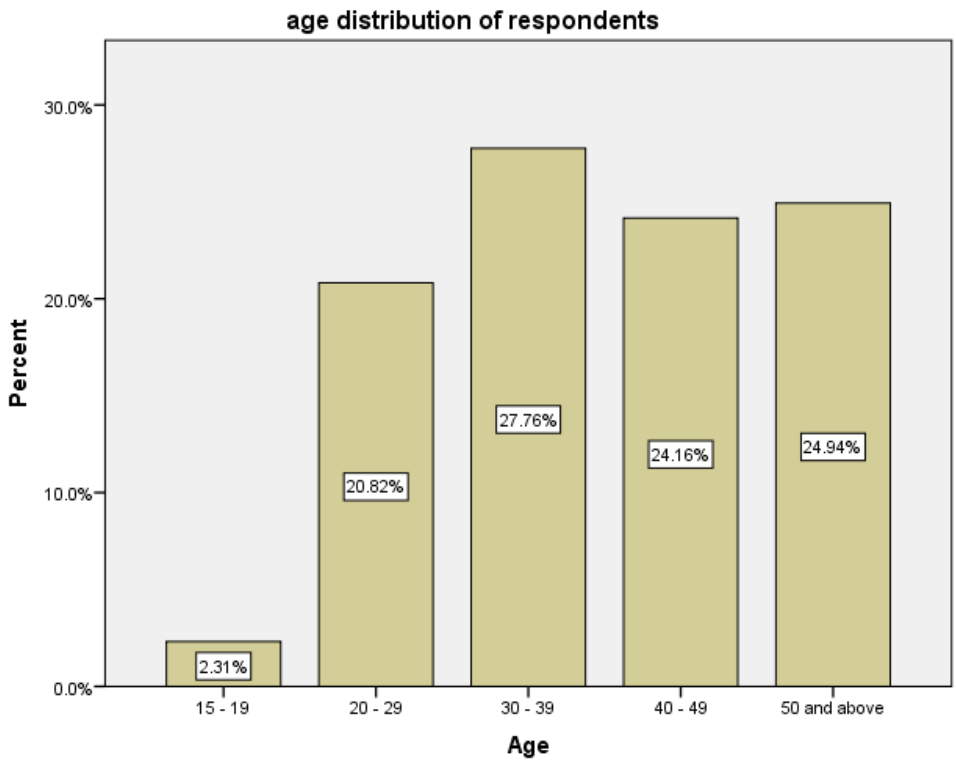


Figure 2: Age distribution of the sampled population

4.1.3 Education status of respondents

In this study, 10% of the respondents had not attained any education while 29% had only attained primary education. Twenty seven per cent (27%) had attained junior secondary, 26% went up to high school, 3% up to certificate level, and 4% had gone up to diploma level. Only 1% of the respondents had a university degree. Therefore more than 90% of the respondents had not attained tertiary education despite the majority (97.7%) being above the age of 20 years (Figure 3).

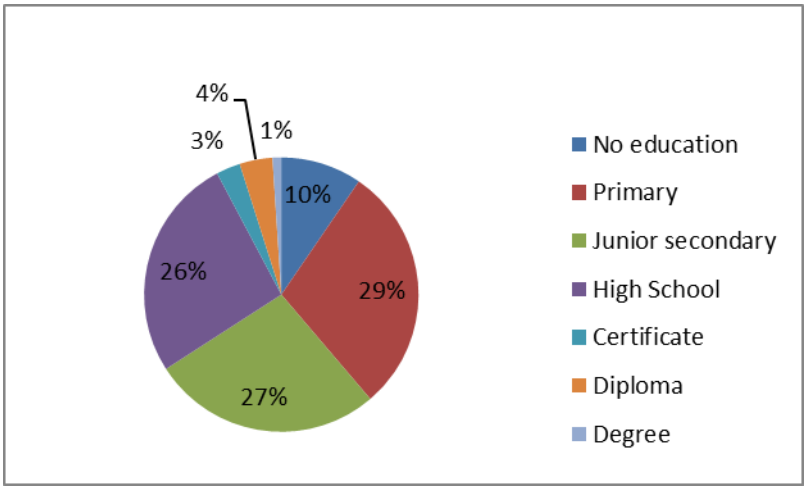


Figure 3: Education status of the study population

4.1.4 Occupation of respondents

In terms of employment status, 28.6% of the respondents were unemployed while 26.9% were daily wage earners, 22.5% were farmers, 6.6% were self-employed, 1.8% were students and 1.5% were retired. Only 12% of the respondents were formally employed (Figure 4)

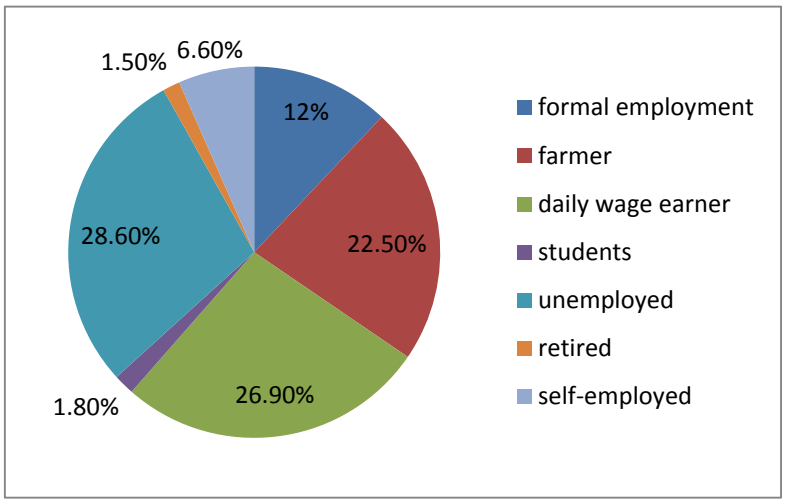


Figure 4: Occupation of respondents

4.1.5 Monthly income of respondents

The study furthermore revealed that 33% of the respondents had a monthly income of less than K500, 32.7% earned between K500 and K1000 per month. Only 33.5% of the respondents earned more than K1000 in a month (Figure 5).

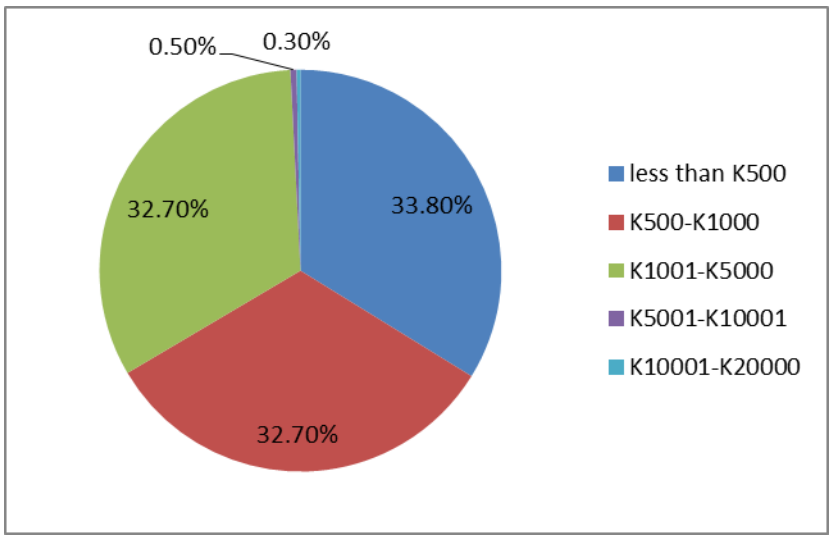


Figure 5: Monthly income of respondents

4.2 Antibiotic treatment

In terms of treatment, 235 out of 400 (58.8%) patients in the study were on antibiotic treatment (figure 6).

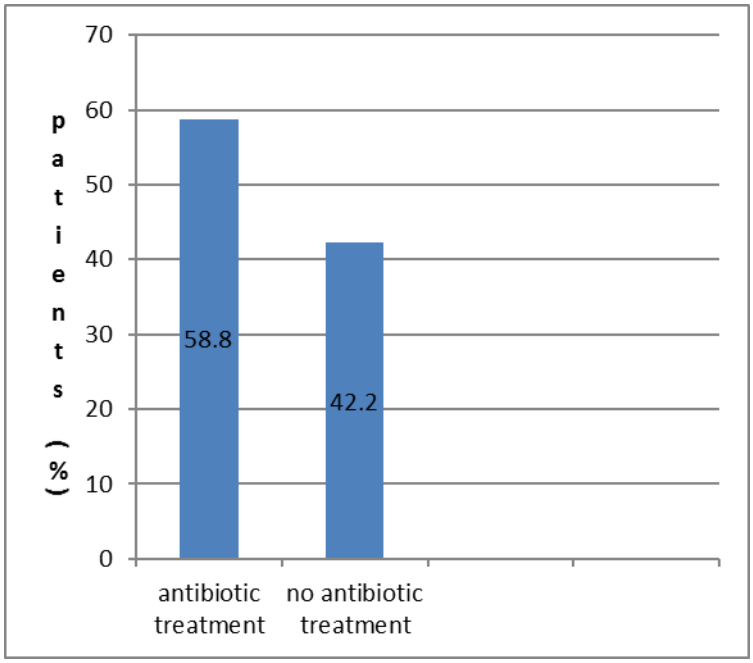


Figure 6: Antibiotic treatment of study participants

4.3 Types of antibiotics

The penicillins (38%) and cephalosporins (41%) (both beta-lactam antibiotics) accounted for 79% of antibiotic use among the patients (figure 7).

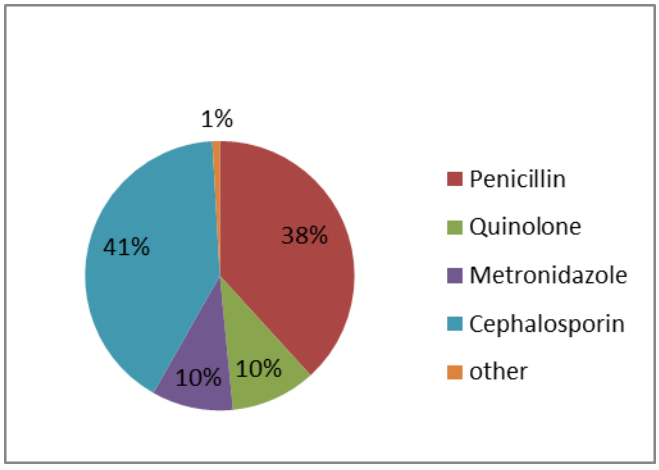


Figure 7: Antibiotic use by class

4.4 ESBL results

A total of 76 *E. coli* isolates were confirmed to be ESBL producers on cefotaxine growth on the media, representing 19% (76/400) of all isolates collected during the study. Among the ESBL producing *E.coli*, 25 (32.9%) out of 76 were positive for CTX-M genes. Of the 25 CTX-M positive isolates, 9 (36%) isolates were positive for

SHV genes and 9 (36%) were positive for TEM genes. Seven (28%) out of the 25 CTX-M positive isolates were positive for both TEM and SHV genes, simultaneously (Figure 10).

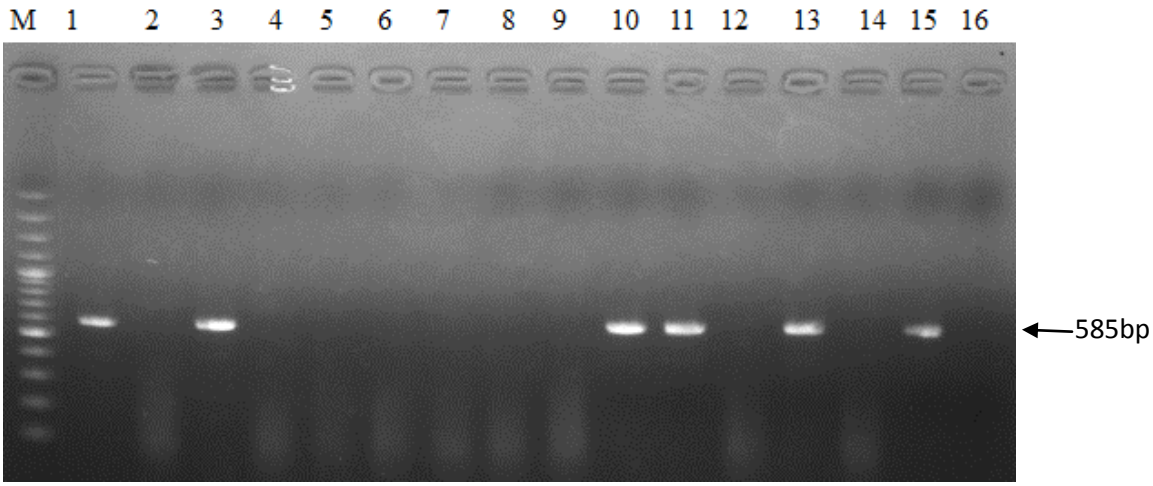


Figure 8: PCR results for *bla*CTX-M gene. M; DNA ladder, Lane 1 to 14 are samples, while Lane 15 being the positive control and Lane 16 negative control.

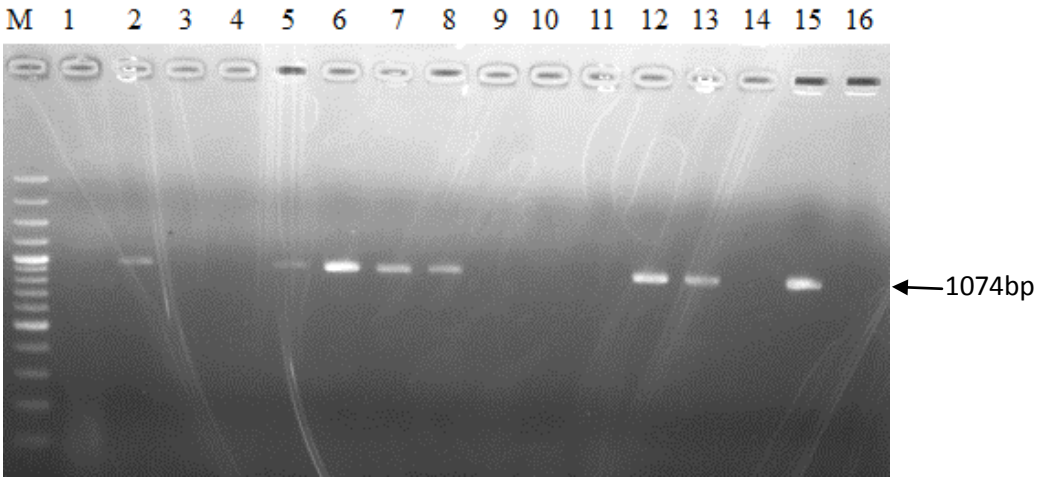


Figure 9: PCR results for *bla*TEM. M; DNA ladder, Lane 1 to 14 are sample, while Lane 15 being the positive control and Lane 16 negative control

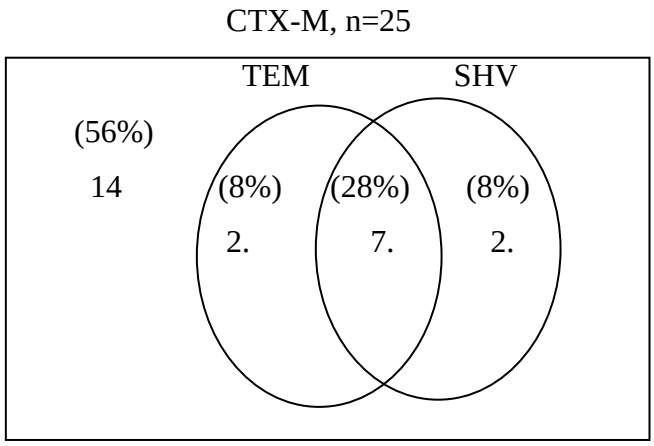


Figure 10: TEM and SHV genes in CTX-M positive isolates

4.5 Antimicrobial susceptibility test results

All the 25 CTX-M positive isolates were subjected to antimicrobial susceptibility tests. All the tested isolates had significant drug-resistant phenotypes. All the isolates were resistant to cefotaxime. Eight out of 25 (32%) CTX-M positive isolates were resistant to all the 11 antibiotics, while 23 out of 25 (92%) CTX-M positive isolates were resistant to at least 6 antibiotics. The highest resistance among non-beta-lactam antibiotics was found to be to nalidixic acid (96%), followed by sulfamethoxazole-trimethoprim, tetracycline and ceftazidime (92% each), then gentamycin and norfloxacin (80% each), streptomycin (76%), chloramphenicol (72%), ciprofloxacin (68%), and ampicillin (60%) (Table 4).

Table 4: Pattern of antimicrobial susceptibility in CTX-M producing *E. coli* isolates (n=25)

Sensitivity	Sensitive		Resistant	
Name of antibiotic disc	Number of isolates	percentage	Number of isolates	percentage
Ampicillin (AMP)	10	40	15	60
Sulfamethoxazole-trimethoprim (SXT)	2	8	23	92
Streptomycin (STR)	6	24	19	76
Tetracycline (TET)	2	8	23	92
Gentamycin (GEN)	5	20	20	80
nalidixic acid (NAL)	2	8	23	92
Ceftazidime (CAZ)	2	8	23	92
Chloramphenicol (CHL)	7	28	18	72
Norfloxacin (NOR)	5	20	20	80
Ciprofloxacin (CIP)	7	28	18	72
Cefotaxime (CTX)	0	0	25	100

4.6 SPSS output

The SPSS output showed that male gender (p=0.014) and a history of surgery (p=0.01) were the only significant risk factors for the presence of ESBLs (Table 5).

The logistic regression model can be summarized as follows:

$$\ln (p/1-p)=\alpha+(\beta 1 \times Sex)+(\beta 2 \times SGY),$$

where "ln" is the natural logarithm (log to base e), p/1-p is the odds of occurrence of ESBL, and SGY represents a history of surgery. Sex is coded as 0-female, 1-male, and SGY is coded as 0-no history of surgery, 1-history of surgery. From the output, $\alpha=0.590$, $\beta 1=0.876$, $\beta 2=0.749$. The model is:

$\ln (p/1-p)=0.590+(0.876 \times Sex)+(0.749 \times SGY)$

Or equivalently,

estimated probability of ESBL carriage

$$= \frac{\exp(0.590 + 0.876 \text{ x Sex} + 0.749 \text{ x SGY})}{1 + \exp(0.590 + 0.876 \text{ x Sex} + 0.749 \text{ x SGY})}$$

The coefficient of sex, β1, is estimated as 0.876. This means that for patients with the same surgical history, the log odds (base e) of a male is 0.876 units higher than that of a female. In simpler terms, exp (β1) is the amount by which the odds of ESBL carriage are multiplied for a male compared to a female with the same surgical history. In this case, exp (β1) =2.401. Therefore, the odds of ESBL carriage in males are 2.401 times the odds of ESBL carriage in a female with the same surgical history. In the same way, the odds of ESBL carriage in a patient with history of surgery are 2.115 times the odds of ESBL carriage in a patient with no history of surgery, assuming they are of the same sex.

The Hosmer and Lemeshow test gave a non-significant result (P=0.929) indicating that the model fits adequately (Table 6).

Table 6: Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	3.078	8	.929

CHAPTER FIVE

DISCUSSION

There has been an alarming rise in the rate of resistance in Gram-negative pathogens (Bush, 2010) and the problem of ESBLs is becoming a global concern. Studies conducted in different countries (including African countries) show that ESBLs are widespread and that their prevalence is increasing (Rupp and Fey, 2003). Increased use, misuse, overuse, and lack of control over prescription and sales of antibiotics can cause microbes to become resistant to antibiotics. Beta-lactams are the most used antibiotics in the world, accounting for over 50% of global antibiotic consumption (Livermore, 1998; Shaikh *et al*., 2014). This heavy usage is the major drive in the development of bacterial resistance as it exerts considerable selection for resistance.

The rate at which resistance develops and spreads is a complex process that depends on a broad array of factors such as selection pressure and use of infection control measures (Rupp and Fey, 2003). The correct identification of ESBLs requires competent laboratory personnel and adequately equipped laboratories. This is probably the reason why most developing countries do not screen for ESBLs. This current study was conducted at the University Teaching Hospital in Zambia, and as far as we are concerned it is the first of its kind in Zambia to concentrate on ESBL producing *E. coli*. Documented and published work conducted in Zambia on this subject matter is limited to ESBL producing *K. pneumoniae* (Mumbula *et al*., 2015).

5.1 Prevalence of ESBL producing *E. coli*

The present study was conducted on 76 ESBL producing *E.coli* isolates that were obtained after screening 400 clinical samples isolated from patients admitted to a tertiary care hospital. Thus the prevalence of ESBL producing *E. coli* was determined to be 19% (76/400). This was not so different from figures reported in similar studies conducted in Africa. A study conducted in Tanzania reported a 24.4% prevalence of ESBL producing *E.coli (*Mshana *et al*., 2009), while a similar study at a tertiary hospital in Ghana reported a 37.2% prevalence of ESBL producing *E. coli* (Obeng-Nkrumah *et al*., 2013). Poverty could be a major player in contributing to the observed pattern in these resource-constrained countries as it may lead to poor quality of drinking water, poor sewage services, inadequate diagnostics promoting

inappropriate antibiotic use, overcrowding and lack of surveillance of antimicrobial resistance.

5.2 ESBL alleles

In this study, genes coding for CTX-M, SHV and TEM ESBLs were identified. However, sequencing was not done (due to lack of specific primers at the time of the study) to discriminate between the non-ESBL parent enzymes (TEM-1, TEM-2, and SHV-1) and ESBL producing variants of TEM and SHV. A non-systemic literature review of research published between 2008 and 2012 showed that CTX-M and TEM genes are common among *E. coli* isolates in Tanzania and South Africa (Storberg, 2014).

5.3 Antimicrobial susceptibility pattern

The results show that antibiotic usage is high at the UTH (more than 50%) (Fig. 6) and that beta-lactam antibiotics are the most used (79%) (Fig.7). It is therefore worrisome to have organisms that may not be susceptible to the main treatment options at the institution. In fact, this apparent problem was made clear by the findings of the antibiotic sensitivity pattern (Table 4). Eight (8) out of 25 (32%) CTX-M positive isolates were resistant to all the 11 tested antibiotics, while 23 out of 25 (92%) CTX-M positive isolates were resistant to at least 6 antibiotics. This clearly shows the limitations in treatment options brought about by ESBL mediated resistance. The resistance patterns were extended even to non-beta-lactam antibiotics (92% each for sulfamethoxazole/trimethoprim, nalidixic acid and tetracycline, 80% each for gentamycin and norfloxacin, 76% for streptomycin, and 72% each for ciprofloxacin and chloramphenicol). These results show that apart from being resistant to beta-lactam antibiotics, ESBL producing isolates are resistant to several other classes of antibiotics. This observation could be explained by the fact that large plasmids that harbor ESBL genes also harbor genes for resistance to other antimicrobials (fluoroquinolones, aminoglycosides, cotrimoxazole), leading to multidrug resistance (Patterson, 2006; Rao, 2012).

The high resistance rates observed for cefotaxime (100%) and ceftazidime (92%) rule out the use of third generation cephalosporins as a treatment option for ESBL producing isolates. The study results also show that cephalosporins (mostly third

generation) are the most used antibiotics at the UTH (41%). The very high resistance rate observed for cefotaxime could be attributed to the fact that cefotaxime was used in the initial screening of isolates. Therefore, this figure could exaggerate the true resistance rate to this drug. The observed resistance rate in ceftazidime (92%) seems to have been higher than expected as CTX-M-type genes preferentially hydrolyze cefotaxime. However, in some cases CTX-M-type beta-lactamases hydrolyze ceftazidime and may cause significant resistance to this cephalosporin (Paterson and Bonono, 2005). Besides, if these isolates simultaneously carry PER-type ESBLs then resistance rates to ceftazidime may be high (Rupp and Fey, 2003). Unfortunately, the presence of PER-type ESBLs was not explored in this study.

The observed multidrug resistance in ESBL producing isolates implies limited treatment options. Beta-lactam/beta-lactamase inhibitor combinations may seem to be a solution to this problem (Harris *et al*., 2015; Nguyen *et al*., 2014). These combinations are usually effective if the isolate produces only a single ESBL (Paterson and Bonono, 2005). However, in this study, 11 out of 25 (44%) CTX-M positive ESBL isolates also produced either TEM or SHV genes, or both, which may be ESBLs (figure 10). Besides, when non-ESBL-producing beta-lactamases are hyperproduced by an isolate there may be resistance to beta-lactamase inhibitors (Paterson and Bonono, 2005). Resistance to beta-lactam/beta-lactamase inhibitor combinations in *E. coli* may be due to hyperproduction of penicillinase enzyme brought about by *bla*TEM-1 gene, or due to hyperproduction of constitutive AmpC cephalosporinase and Class D beta-lactamases, or due to inhibitor resistant TEM beta-lactamases (Rao, 2012).

Quinolones have been used to treat complicated urinary tract infections associated with ESBL-producing organisms when there is no *in vitro* resistance to quinolones (Paterson and Bonono, 2005). However, recent studies associate ESBL production with fluoroquinolone resistance (Rupp and Fey, 2003), and in this study, resistance was high for ciprofloxacin (72%), norfloxacin (80%) and nalidixic acid (92%). Thus, quinolones cannot be recommended as treatment of choice for ESBL producing organisms at the UTH.

Since cephamycins are not susceptible to hydrolysis by ESBLs, drugs such as cefoxitin and cefotetan have been suggested as possible treatment for ESBL-producing organisms (Rao, 2012). However, studies show that there is a tendency of selecting for resistant organisms (through mutation in genes coding for porins) during the course of therapy, resulting in significant cephamycin resistance and infection resurgence (Paterson and Bonono, 2005). This makes the use of cephamycins controversial. Therefore, these drugs cannot be recommended as first line treatment for ESBL infections. Carbapenems are recommended as first line treatment for severe infections with ESBL-producing organisms (Paterson and Bonono, 2005; Endimiani and Paterson, 2007) as these drugs are stable to hydrolysis by most ESBLs (Rao, 2012). However, emergence of carbapenem-resistance has been reported in some studies (Paterson and Bonono, 2005; Shaikh et al., 2014). One challenge associated with the use of carbapenems is difficulty in administration as they can only be administered parenterally. This entails hospitalization of patients who would normally be treated with an oral antibiotic in the community. Another challenge is that these drugs are not readily available in developing countries despite ESBL carriage being even higher in these countries.

One of the limitations of this study was the failure to include cephamycins and carbapenems to the list of antibiotics assessed during susceptibility testing as these antibiotics were not available at the time of the study. A study conducted at the UTH, Zambia, on isolates collected in 2013 showed that all the 45 ESBL-producing isolates tested were susceptible to amikacin and imipenem, making them possible treatment options (Mumbula *et al*., 2015)

5.4 Risk factors

In this study, male gender was found to be a risk factor for ESBL presence (p=0.014, 95% CI=1.198-4.813) (Table 5). It is not clear as to why males were found to be at a higher risk of ESBL presence than females. Mahesh *et al.,* (2010) reported male gender to be a risk factor for acquiring ESBL-positive urinary tract infection but Ena *et al.,* (2006) found no such association. The explanation provided for the higher risk in males with regard to urinary tract infection by an ESBL producing organism was that the longer urethra in males prevents ordinary organisms from causing infection but ESBL-producing organisms (extraordinary organisms) overcome this barrier and

thus lead to a higher incidence of ESBL-related UTI in males (Mahesh *et al*., 2010). In this study, patients were recruited with no regard to the diagnosis and yet male gender was still found to be a risk factor. More research is needed to explain the observed association.

A history of surgery was found to be a risk factor for ESBL presence (p=0.01, 95% CI=1.196-3.740) (Table 5). The findings agree with those from other studies (Paterson and Bonono, 2005). Surgery is usually followed by increase in interleukin 10 (IL-10), decrease in T lymphocytes and depressed antigen presentation by macrophages (Scholl *et al.,* 2012). These immune responses to surgery are responsible for the increase in susceptibility to infection. These infections are usually bacterial infections which may or may not be ESBL-producers. In a study conducted at Bugando Medical Centre in Tanzania, it was shown that there was high prevalence of ESBL and methicillin resistant *Staphylococcus aureus* (MRSA) (Moremi *et al*., 2014).

Age, hospital stay, family size, monthly income, diabetes, smoking, alcohol intake and pressures were not significantly associated with ESBL presence in this study (p>0.05).

It seems ESBLs are already endemic at the UTH. There was no baseline data to which our study findings could be compared. Therefore it was difficult to determine whether the observed ESBL frequency was low (hypoendemic), moderate (mesoendemic) or high (hyperendemic). Another challenge faced in this study was the inability to sequence TEM, SHV and CTX-M enzymes. However, PCR amplification of CTX-M-specific products was regarded as proof that a *bla*CTX-M gene was responsible for the observed phenotype (Shaikh *et al*., 2014). However, the case was different for TEM and SHV enzymes because there was need for sequencing in order to distinguish between the non-ESBL parent enzymes and different variants of TEM and SHV ESBLs. This was not done due to lack of specific primers.

There was no association between ESBL carriage and gastrointestinal disease. Thus most patients in our study population were colonized with ESBL-producing organisms but they were not infected. A possible explanation could be that most of

the isolates analyzed were commensal strains belonging to phylogenetic groups A and B1 of *E. coli*. Phylogenetic analyses of the isolates may be necessary to understand and explain the observed results. Since ESBLs can be transmitted horizontally among different organisms, it is necessary to consider putting in measures to prevent or minimize this spread as transfer of these genes to pathogenic strains may lead to increased rates of treatment failure for many infections. This can in turn lead to increased morbidity and mortality. To control the spread of ESBL-producing pathogens, effective infection control strategies should be put in place for all patients who are infected or colonized with ESBL-producing bacteria. Infectious disease control specialists need to take a multidisciplinary approach in the fight against ESBL-producing infections (Knudsen and Andersen, 2014). Treating patients alone is not sufficient as the reservoir of these organisms may be vast, including the environment (e.g. water and soil), wildlife and pets. Therefore experts from various disciplines should play a role in controlling ESBL-related infections. These may include physicians, public health officers, pharmacists, microbiologists, and veterinarians.

Recommendations on the interventions required for effective control of ESBL infections vary in different geographic locations. Implementation of surveillance of antimicrobial resistance is key to rapid diagnosis and control of ESBLs as it can aid in identification of individuals with ESBL infection as well as those colonized with ESBLs (Mshana *et al*., 2013). Identification of these individuals is pertinent to effective control of ESBL transmission as it can alert infection control specialists to take measures necessary to correct the situation. These interventions include identification of patients, isolation measures, and more prudent antibiotic use. Improvement of water and sewage services as well as proper disposal of hospital waste may reduce the circulation of these bacteria in the environment. Although controversial, other infection control measures that can be applied to curb outbreaks of ESBL-producing bacteria include gut decontamination with appropriate antibacterial agents, decontamination of the nasopharynx with povidone-iodine nasasl spray, and staff cohorting and reorganization (Rupp and Fey, 2003). Polymyxin, neomycin, and nalidixic acid, colistin and tobramycin, or norfloxacin to selectively decontaminate the digestive tract in patients with gastrointestinal colonization with ESBL-producing organism, while nasal spray with povidone-iodine is used in patients

with nasotracheal colonization (Paterson and Bonono, 2005; Huttner *et al*., 2013). This decolonization leads to reduced horizontal transmission of ESBL-producing organisms and may be invaluable in outbreak settings.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. ESBL-producing *E. coli* are present among patients admitted to the UTH, Zambia, at a prevalence of 19%.
2. These organisms are multiply resistant to most antimicrobials available at the UTH.
3. CTX-M, SHV and TEM genes are common among ESBL-producing *E. coli*.
4. Male gender and a history of surgery are significant risk factors for ESBL presence ($p<0.05$).

6.2 Recommendations

1. There is need for establishment of an antimicrobial resistance surveillance network in Zambia for ESBL-producing *Enterobacteriaceae* to monitor the resistance patterns emerging in hospitals.
2. It is necessary to equip laboratories in general and district hospitals with the capacity to detect ESBLs.
3. There is need to strengthen policies restricting unnecessary sale and use of antibiotics.
4. Good hygiene practices and standard infection control measures at the UTH may reduce transmission and spread of ESBLs.

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APPENDICES

8.1 Appendix 1: Information sheet

Information sheet for study participants for the study entitled "Risk Factors And Alleles Of Extended Spectrum Beta–Lactamase (Esbl) Producing *Escherichia Coli* At The University Teaching Hospital"

NOTE: This information should be translated into local language for participants who may not read or understand English.
Good morning/afternoon. My name is
I am a health worker/student from _____ . We are conducting a study entitled "Risk Factors and Alleles of Extended Spectrum Beta – Lactamases (Esbl) at the University Teaching Hospital". I wish to invite you to participate in this study.

What is this study about?

This is a research project being conducted by Dr Shawa Misheck and supervised by Professor Benard Hang'ombe. The aim of the study is to determine the presence of certain germs that are difficult to treat using the currently available medicines. We also wish to identify the factors that increase the likelihood of infection by such organisms, as well as the common type of these organisms in our setting. Such a study can significantly increase effectiveness of treatment through appropriate treatment choice.

What will I be asked to do if I agree to participate?

If you agree to participate in this study you will answer questions regarding yourself, your current and past illnesses, as well as your social life. It will also involve obtaining a sample from you which will be sent to the laboratory. The sample will be a wound swab. Completing this questionnaire will take between 10 and 15 minutes while collection of the sample will take about 5 minutes.
Samples will be collected by swabbing the rectum with sterile cotton wool.

What will happen to the specimens afterwards?

After the specimens are worked on in the laboratory, they will be decontaminated prior to disposal. Decontamination of these specimens will result in the removal of microorganisms to a lower level, so that they don't pose a danger to the environment. After treatment the waste will then be disposed of in the regular waste stream.

Will my participation in this study be kept confidential?

We will do our best to keep your personal information confidential. To help protect your identity, your name will not appear with the information pertaining to results of our tests. Only authorized staff will have access to information bearing your name.

What are the risks of this research?

There may be slight discomfort when obtaining rectal swabs from you. However, this is a normal routine process used in hospitals. We will do everything possible to minimize discomfort.

What are the benefits of this research?

The results obtained in this research may be used to modify your treatment to a more appropriate one. On a larger scale, the study will help in

(a) Establishment of a better understanding of these resistant organisms and their characteristics in Zambia,

(b) Revision of standard treatment protocols of bacterial infections in Zambia,

(c) Identification of factors associated with transmission of these organisms in Zambia.

Do I have a choice to refuse to participate and am I free to withdraw from the study in the long run? What are the consequences if I decide to withdraw?

Your participation in this study is completely voluntary. Refusing to participate in this study is without any consequences and you will still be treated with the best care

available. You may also choose not to answer questions that you consider personal or that you are not comfortable answering. You are also free to withdraw from the study at any time without giving reasons. Withdrawing from the study is also without any consequences and you will still receive the best available care.

What if I have questions?

Should you have any questions regarding this study, please contact:

Dr Shawa Misheck
C/O Department of paraclinical studies
School of Veterinary Medicine
University of Zambia
P.O. BOX 32379
LUSAKA
Mobile: 0968-467086
Or
Professor Benard Hang'ombe
Department of Paraclinical Studies
School of Veterinary Medicine
P.O. BOX 32379
LUSAKA
Mobile: 0977-326288
Or
ERES Converge IRB
33 Joseph Mwila Road
Rhodes Park
LUSAKA
Mobile: +260955 155 633/4

8.2 Appendix 2: Consent form

TITLE OF STUDY: EXTENDED SPECTRUM BETA – LACTAMASES (ESBL) AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

NAME OF RESEARCHER: MISHECK SHAWA
POSITION OF RESEARCHER: STUDENT MSC. ONE HEALTH ANALYTICAL EPIDEMIOLOGY
CONTACT ADDRESS OF RESEARCHER: UNIVERSITY OF ZAMBIA, P.O.BOX 32379, LUSAKA, ZAMBIA, PHONE No; 0968467086
ADRESS OF RESEARCH ETHICS COMMITTEE:
ERES Converge IRB, 33 Joseph Mwila Road, Rhodes Park, LUSAKA, +260955155633
NOTE: This information should be translated into local language for participants who may not read or understand English.

1. I confirm that I have read and understood the information sheet for the above study and have had an opportunity to ask questions.

2. I understand that my participation is purely voluntary and that I am free to withdraw at any time, without giving reasons.

3. I agree to take part in the above study

4. I do not agree to take part in the study

Enrollment number	Date	Signature or thumb print
Name of interviewer	Date	Signature

8.3 Appendix 3: Questionnaire used in Data Collection

QUESTIONNAIRE FOR ESBL STUDY

ENROLLMENT ID……

WARD……..

PATIENT FILE NUMBER……..

BED NUMBER……....

SECTION A : SOCIO AND DEMOGRAPHIC

(1). Sex

a) Male
b) Female

(2). Age in years

a) 0 – 4
b) 5 – 14
c) 15 -19
d) 20 -29
e) 30 – 39
f) 40 – 49
g) 50 and above

(3). Length of Hospital stay

a) 0 – 3 days
b) 4 – 7 days
c) 8 – 14 days
d) over 14 days

(4). Place of Residence

……
……

(5). Religion

a) Christian
b) Muslim
c) Other,
   specify……
   …….

(6). Education Status

a) No education
b) Primary
c) Junior Secondary
d) High school
e) Certificate
f) Diploma
g) Degree
h) Other,
specify……
…..

(7). Occupation

a) Formally employed
b) Farmer
c) Daily wage earner
d) Student
e) No particular job
f) Other,
specify……
……

(8). Marital status

a) Married
b) Single
c) Separated / divorced
d) Widowed

(9). Family Size

a) Less than 4
b) 4 to 7
c) More than 7

(10). Approximately how much income in cash do you earn in a month?

a) Less than K500
b) K501 – K1000
c) K1001 – K5000
d) K5001 – K10, 000
e) K10, 001 – K20, 000

f) Other,
specify……

SECTION B: PAST AND CURRENT MEDICAL AND SURGICAL HISTORY

(11). Diagnosis (System involved)

a) Respiratory System (RS)
b) Gastrointestinal Tract (GIT)
c) Genitourinary System (GUS)
d) Cardiovascular System (CVS)
e) Central nervous system (CNS)
f) Musculoskeletal System (MSS)
g) Other, specify

(12). Have you ever undergone surgery?

a) Yes
b) No

(13). If yes, which type?

a) Abdominal
b) ENT
c) Obstetric
d) Orthopedic
e) Other,
specify……..

(14). If yes, how long ago?

a) Less than 3 months ago
b) 3 to 6 months ago
c) 6 months to 12 months ago
d) More than 12 months ago

(15). In your current illness/ admission, has any of the following procedures/devices been done/inserted on you?

a) Naso-gastric tube (NGT) YES/ NO
b) Gastrostomy/Jejunostomy/Ileostomy YES/NO
c) Haemodislysis YES/NO

(16). Do you have any pressure sores

a) Yes
b) No

(17). Which antibiotics have you taken during this current illness? (Review drug chart)

a) None
b) Penicillin
c) Quinolone
d) Metronidazole
e) Cephalosporin

(18). Are you diabetic?

a) Yes
b) No

SECTION C: SOCIAL

(19). Do you drink alcohol?

a) Yes
b) No

(20). If yes, please quantity

a) Regularly
b) Moderate
c) Occasionally/rarely

(21) Do you smoke?

a) Yes
b) No

(22). If yes, please quantity

a) Non – Smoker
b) Ex – Smoker
c) Light smoker (less than 5 cigarettes a day)
d) Heavy smoker (more than 5 cigarettes a day).

THANK YOU FOR YOUR TIME!