ANTIBIOTIC SUSCEPTIBILITY OF *E. COLI* ISOLATED FROM DRY FISH SOLD IN LOCAL MARKETS IN LUSAKA, ZAMBIA

BY

NYIMBILI LILLIAN

A RESEARCH REPORT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH FOOD SAFETY.

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VET MED

DECLARATION

I, **NYIMBILI LILLIAN**, do hereby declare that this Research Report represents my own original work. It has been presented in accordance with the guidelines of the University of Zambia. It has not been submitted before for the award of any degree or examination in any other University.

Signed: _____Date: _____

CERTIFICATE OF APPROVAL

The University of Zambia approves the research report submitted by **NYIMBILI LILLIAN**, as fulfilling the partial requirements for the award of the Master of One Health Food Safety by the University of Zambia.

Prof. Bernard M. Hang'ombe ……. ……...

Supervisor Signature Date

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Examiner Signature Date

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Examiner Signature Date

ABSTRACT

Escherichia coli has been known as one of the most common bacteria found in the intestinal tract of human and warm blooded animals. It is the major causative agent of serious infections and a mediator of drug resistance through the production of Extended Spectrum Beta-Lactamase enzymes(ESBL) that hydrolyse the beta-lactam ring on most of the beta-lactam antibiotics including of penicillins, cephalosporins, and the monobactam aztreonam. These antimicrobial resistant strains have become a global public health challenge affecting both humans and domestic livestock. In Lusaka and worldwide Fish is considered as a universal protein source consumed by a larger population of people. Some people also favour consuming raw dry fish. The objective of this study was to determine the antibiotic susceptibility and presence of resistant genes of *E. coli* isolated from dry fish sold in open markets of Lusaka district.

A total of 120 fish samples were collected between July 2018 and August 2018. The fish samples were subjected to bacteriological analysis. Of the 120 samples of fish analysed for *E. Coli* 69 percent were positive for *E. coli* and 31 percent were negative. The determination of *E. coli* as an ESBL producing organism was determined by growing the bacteria on MacConkey agar containing 2 mg/L of cefotaxime. Following culturing of *E. coli* on MacConkey agar containing 2 mg/L of cefotaxime 46 isolates 55.4 percent were detected as ESBL-producing. The *E. coli* isolates presumably identified to be ESBL producing following culture on MacCkonkey agar supplemented with cefotaxime were subjected to PCR. A total of 35 out of 46 isolates were tested for the presence of the *bla*CTXM gene and out of these 21 were positive for the *bla*CTXM gene. Of the samples subjected to antimicrobial sensitivity test suggested that ESBL producing *E. coli* isolates had conferred resistance to beta-lactum antibiotics and other common antimicrobial agents. The results obtained indicate the need for surveillance on the emergence of antimicrobial resistance in fish sold in open markets and improve the food safety and hygiene of this important source of protein.

DEDICATION

This study is dedicated to my father, Mr. James Henry Nyimbili, my mother Mrs. Tyness Kondowe Nyimbili, my Husband Chakwiya Bornface, and children Kondwani and Temwani. Their encouragement and continuous prayers have necessitated the successful completion of this study. To them I say, thank you very much and may God richly bless you. Above all I acknowledge the grace and the eternal love of the Almighty God for the gift of life.

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LIST OF ABBREVIATIONS

AMC	Amoxycllin
AMP	Ampicillin
CTX-M	Cefotaxime – Munich
CAZ	Ceftazidime
C	Chloramphenicol
CIP	Cipofloxacin
CLSI	Clinical and Laboratory Standards Institute
DEC	diarrheagenic *Escherichia coli*
DAEC	Diffusely adhering *Escherichia coli*
DNA	Deoxyribonucleic acid
DO	Doxycline
ERES	Excellence in research Ethics and Science
E. coli	*Escherichia coli*
EIEC	*Enteroagregative Escherichia coli*
ESBL	Extended Spectrum Beta Lactamase
EPEC	Enteropathogenic *Escherichia coli*
E	Erythromycin
ETEC	Enterotoigenic *Escherichia coli*
EXPEC	Extraintestinal pathogens
GEN	Gentamicin GPS Geographical Position System
≥	Greater or equal to
IMViC	Indole Methyl red, Voges-Proskauer and Citrate
IRB	Institutional research board
MAEC	NeonataL Meningitis
MFNP	Ministry of finance and national planning
n	Number of isolates or samples
NA	Nalidixic acid
N	Neomycin
NX	Norfloxacin
PEN	Penicillin
PCR	Polymerase Chain Reaction
SHV	Sulphydryl variable
Spp	species

STEC	Shiga toxigenic producing *Escherichia coli*
S	Streptomycin
TEM	Temoniera
TE	Tetracycline
TSI	Triple Sugar Iron
UTI	Urinary tract infection
VTEC	Verocytotoxin producing *Escherichia coli*
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1. Background information

Fish is an important food source that is consumed by the majority of the population in Zambia. It is rich in vitamins and minerals. Despite all the benefits, fish is easily susceptible to common bacterial diseases making it a major concern in public health and food safety. This is due to an increased incidence of gastro-intestinal disease further aggravated by the presence of flies that serve as vectors for a variety of pathogenic microorganisms. Flies that besets on fresh fish sold in Zambian markets carry genes of Extended-spectrum beta-lactamases (ESBL) that are resistant to some commonly used antibiotics (Mwansa *et al*., 2017). Particularly in urban areas near human dwellings with a high human population density, flies have conducive conditions to grow and reproduce year-round and can play an important role in cross-contamination between dirty contaminated environments and food sources as they fly from one place to another. Fish as a food which easily degrades is usually plagued by house flies, which may transfer pathogens as described above. Poor sanitary conditions that may prevail in food markets create a potential for higher housefly populations. Additionally, simple pit latrines commonly used in most of the Zambian markets may provide increased access to human excreta for flies, further increasing the potential for fly-borne transmission of disease-causing microorganisms from foods being sold in markets such as fish (Butaye, 2006).

The contamination of food products with enteropathogenic *Escherichia coli* generates serious health and economic consequences. *Escherichia coli*, a commensal bacterium of humans and animals, is a significant cause of gastro-intestinal disease, ranging from simple diarrhea to dysentery-like conditions (Ryan, 2004). *Escherichia coli* has also been shown to carry drug resistant genes that may complicate management of diarrheal diseases (Nakajavani, 2013). *Escherichia coli* is sometimes used as a sentinel for monitoring antimicrobial drug resistance in fecal bacteria because it is found more frequently in a wide range of hosts and acquires resistance easily (Brenner *et al.*, 2007). Surveillance data show that resistance in *Escherichia coli* is consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine (Holt and Moore, 2007). Researchers and policy makers in Africa lack up-to-date information on potential disease-causing microorganisms that carry resistance genes to commonly used antibiotics and their impact on human health. Therefore,

there is need to carry out a study that will isolate *Escherichia coli* from dry fish sold from open markets in Zambia, and subsequently determine if these bacteria carry resistance genes to commonly used antibiotics, which would indicate problems in eradicating these pathogens.

1.2. Statement of the Problem

Outbreaks of infections due to ESBL-producing organisms has been noted in some African Countries (Rupp and Fey, 2003). Prevalence of ESBLs as high as 50 percent have been observed among *Klebsiella pneumoniae* from in-patients in Zambia, Mozambique, Democratic Republic of Congo and Tanzania (Mshana, 2013). Furthermore, Extended-spectrum β-lactamase-producing *Enterobacteria* (ESBL-E) have become one of the main challenges for antibiotic treatment largely because of the current cefotaxime (CTX-M) resistant organisms. Generally speaking, individuals with infections caused by bacteria carrying ESBLs have a higher mortality rate and require longer hospitalization (Kayange *et al*., 2010; Bloomberg *et al*., 2005). Zambia is one of the sub-Saharan African countries most affected by bacterial infectious diseases. These organisms are frequently resistant to many other antimicrobial agents usually recommended for the treatment of infections caused by *E. coli*, such as gentamicin, fluoroquinolones, and trimethoprim-sulfamethoxazole (Rodriguez, 2008). Fish has become an increasingly important source of protein, and other elements necessary for the maintenance of a healthy body, and constitute an important food component for a large section of the world population. Consumption of fish may cause diseases due to infection or intoxication; some of these diseases have been specifically associated with pathogens, which are resistant to antibiotics such as *E. coli* (Adebayo-Tayo *et al*., 2012). Several studies have demonstrated a number of bacterial species encountered in different fish, which are potentially pathogenic under certain conditions as reported for *Pseudomonas angulluseptica* and *Streptococcus spp*. (Emikpe *et al*., 2011). In view of the above, in order to have safe, wholesome and acceptable fish to avoid diseases there is need to undertake a study to isolate *E. coli* from dry fish sold in open markets in Lusaka and asses its susceptibility to antibiotics. This provided the necessary evidence as to whether *Escherichia coli* isolated from dry fish is one of the carriers of ESBL producing organisms.

1.3. Justification of Study

The paucity of updated data on ESBLs in the communities of Zambia makes the understanding of the extent and complexity of the problem with regards to circumstances leading to bacterial infectious diseases as well as the populations at risk very challenging. This cripples the prospect of putting up appropriate preventive and control intervention

measures, which has negative consequences on the already pressured health service delivery system of Zambia. The study endeavored to reduce the knowledge gap about the existence of ESBLs producing *E. coli* in dry fish that carry resistance genes to commonly used antibiotics in Zambia. Knowledge on the ESBL producing bacteria carriage will be useful in the determination of appropriate antibiotic drugs for the current strains of ESBLs as one of the intervention directed at reducing mortality due to this scourge. This study would also be important in stimulating further research on this important public health problem.

1.4. Research Question

What is the antimicrobial susceptibility of *E. coli* isolated from dry fish sold in Local markets in Lusaka District, Zambia?

1.5. Study Hypothesis

E. coli isolated from dry fish sold in local markets in Lusaka District, Zambia carry genes that are resistant to commonly used antibiotics.

1.6. Objectives

1.6.1. General Objectives

The objective of this study was to determine the antibiotic susceptibility and presence of resistant genes of *E. coli* isolated from dry fish sold in open markets of Lusaka district.

1.6.2. Specific objectives

1. To isolate *E. coli* from dry fish sold in open markets in Lusaka, Zambia.
2. To assess the antibiotic susceptibility of *E. coli* isolated from dry fish.
3. To determine the presence of ESBL producing *E. coli* in dry fish.

CHAPTER TWO

LITERATURE REVIEW

2.1. Description of Fish in line with food safety

Fresh fish contains up to 80 percent of water. It is highly perishable material and having a short storage life (Bala and Mondol, 2001). Drying of fish is mainly carried out traditionally under open sun. Sun drying represents a low cost processing technique to preserve fish. Natural sun drying has been used since time immemorial for agricultural products. Open sun drying has limitation to control the drying process and other for example, weather uncertainties, high labour costs, require large drying area, insect infestation, mixing with dust and other foreign materials and so on. However, open sun drying is widely practiced in tropical and subtropical countries to preserve agricultural products, where solar radiation is convenient (Szulmayer, 2001). It is abundant inexhaustible and environmental friendly (Basunia and Abe, 2001).

2.2. Role of Fish in Food Security

The issue of food security is a complex one in both developed and developing countries, where proteins source from animals such as meat and meat products, fish and fish products are generally regarded as high risk and unwholesome commodities with respect to pathogen contents, availability of natural toxins and other possible contaminants and also the use of adulterants (Clarence *et al*., 2009). Food borne infections and illnesses have become a major international health problem with consequent reduction in economic growth. It is also identified as a major cause of illness and death worldwide (Adak *et al*., 2005). Recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety (Adak *et al*., 2005).

In the developing world, food borne infection leads to the death of many people, as well as resulting in diarrheal disease which can have long-term effects on children's growth as well as on their physical and development a well being (Clarence *et al*., 2009). Food borne infections also heavily affects the healthcare systems (Adak *et al*., 2005). According to Clarence *et al*., (2009), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the

individuals to the toxin (Clarence *et al*., 2009). Lengthy food supply procedures, mass catering complex associated with increased international movement, changes in eating habits and poor hygiene practices are major contributing factors (Hedberg *et al*., 1992).

Contaminated raw meat and fish is one of the main sources of food-borne illness (Bhandare *et al*., 2007). It has been known that most food contaminations are caused by food-borne pathogens such as bacteria, fungi, mold and others. Clarence *et al*., (2009) reported that gram negative bacteria accounts for approximately 69 percent of the cases of bacterial food borne diseases. Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income generating countries that have developed surveillance and control programs (Ternhag *et al*., 2008). The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained while in case of fish, the organisms may come from the water and the fish handlers. Other potential sources of microbial contaminations are the equipment used for each operation that is performed until the final product is consumed, the clothing and hands of personnel and the physical facilities themselves are all implicated (Rombouts and Nouts, 1994).

2.3. Strains of *E coli*

The species *E. coli* is serologically divided in serogroups and serotypes on the basis of its antigenic composition (somatic or O antigens for serogroups and flagellar or H antigens for serotypes). Many strains express a third class of antigens (capsular or K antigens) that although important in pathogenesis only occasionally are used in serotyping. The species comprise intestinal and extra intestinal pathogens. The intestinal pathogens are also known as Diarrheagenic *E. coli* (DEC) of which six categories have been characterized: enteropathogenic *E. coli* (EPEC), shiga toxin-producing *E. coli* (STEC) or verocytotoxin-producing *E. coli* (VTEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adhering *E. coli* (DAEC) (Smith *et al*., 2007; Kaper *et al*., 2004; Nataro and Kaper, 1998). The extraintestinal pathogens (EXPEC) are more prevalent strains that include those associated with urinary tract infections (UPEC), neonatal meningitis (MAEC), and bacteremia (Kaper, 1998).

Systematic O serotyping of *E. coli* began in the early 1930s (Nataro and Kaper, 1998), and many studies showed that the O serotype of *E. coli* are generally associated with pathogenesis (Wang *et al*., 2011; Yayue *et al*., 2006). Other researchers reviewed that O serotyping

became important tools to classify *E. coli* in clinical settings. It has been shown repeatedly that antigenic typing of *E. coli* is extremely useful in epidemiological studies (Vu-Khac *et al*., 2007; Orskov and Orskov, 1992).

2.4. Definition of Extended Spectrum Beta – Lactamases (ESBL)

There is no consensus regarding the precise definition of ESBLs. A commonly used working definition is that ESBLs are chromosomal or plasmid- mediated β-lactamases (enzymes that cleave the β-lactam ring) which have mutated from pre-existing broad-spectrum β-lactamases (TEM-1, TEM-2, and SHV-1). This results from a consequence of widespread use of third generation Cephalosporins as well as Aztreonam (Giriyapur *et al.,* 2012; Shukla *et al.,* 2004). The ESBL producing bacteria can cause serious infections that involve urinary tract, septic conditions, wound, meningitis and respiratory tract (Paterson, 2005).

2.4.1. Distribution of ESBL

Enterobacteriaceae producing β-lactamases are a worldwide problem and are now found in a significant percentage of *Escherichia coli* and *Klebsiella pneumoniae* strains in certain countries (Ben-Ami, 2006). Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars (Gonzalez, 1999). Recently *E. coli* strains have emerged as a common cause of community-acquired infections. Community-acquired infections caused by ESBL-producing *E. coli* have been reported worldwide including the United States and continue to increase, although there are regional differences in rates (Rodriguez, 2008).

Extended Spectrum Beta Lactamase producing *Enterobacteriaceae* in hospitalized patients and in communities varies largely between different geographic regions and countries but is common in Africa. The ESBL-producing *Enterobacteriaceae* in hospital and community settings in Africa is common. For example, data from a review published showed that less than 10 percent of *Enterobacteriaceae* isolates expressed ESBLs in Australia, Sweden, Japan, Korea and Singapore, compared to rates higher than 30 percent in Portugal, Italy, Turkey and most Latin American countries (Hawser *et al*., 2009).

In general, the spread of infections due to ESBL producers has been greater in countries with lower economic resources. This is very clear when comparing prevalence data from Sweden (3 percent) with those from Greece, Turkey, Portugal (>25 percent) (Casellas, 1999) or South America (>30 percent) (Sader, 2006). Several reasons have been attributed to account for this

disparity and these include poorer social and economic conditions, crowded hospitals frequently with high patient/nurse ratios, self-prescription of antibiotics that are sold over the counter, deficient hospital hygiene resulting in high rates of colonisation and infection with *Klebsiella* species. This last factor is very important because *Klebsiella* species have a particular ability to acquire plasmids determining ESBL production (Villegas *et al*., 2007).

2.4.2. Classification of ESBLs.

There are various types of ESBLs. These are classified accordingly as stated below:

2.4.2.1. TEM-type

Most TEM ESBLs are derived from TEM-1, an extremely common enzyme known since 1965. The first plasmid-mediated beta lactamase in gram-negative bacteria was discovered in Greece in the 1960s. It was named TEM after the patient named Temoniera from whom the bacteria were isolated. Subsequently, a closely related enzyme was discovered and named TEM-2. It was identical in biochemical properties to the more common TEM-1 but differed by a single amino acid with a resulting change in the isoelectric point of the enzyme (Public Health England, 2013).

The TEM-type enzymes are grouped according to different combinations. These combinations are based upon changes of TEM-type enzymes. There are 140 TEM-type enzymes that have been described. The TEM-10, TEM-12, and TEM-26 are among the most common in the United States and many European Countries (Villegas *et al*., 2004). TEM-1 is the most commonly encountered beta-lactamase in Gram-negative bacteria. Up to 90 percent of ampicillin resistance in *E. coli* is due to the production of TEM-1. This is also responsible for the ampicillin and penicillin resistance that is seen in *H. influenza* and *N. gonorrhoea* (Paterson, 2003). There have been reports of CTX -M-12 ESBL in *Klebsiella pneumonia* in Kenya, TEM-53, TEM-63, SHV-2, SHV-5, SHV-19, SHV-20, SHV-21 and SHV-22 in *K. pneumonia* in South Africa, CTX-M 15 and SHV-12 in Tanzania, SHV-12 in *Salmonella enterica* serotype and TEM-3 in *S. typhimurium* in Morocco (Ndugulile *et al*., 2005). Although TEM-type beta-lactamases are most often found in *E. coli* and *K. pneumoniae*, they are also found in other species of Gram-negative bacteria with increasing frequency. The amino acid substitutions responsible for the ESBL phenotype cluster around the active site of the enzyme and change its configuration, allowing access to oxyimino-beta-lactam substrates (Bradford, 2001).

2.4.2.2. SHV-type

Sulfhydryl variable (SHV)-type ESBLs are all derived from SHV-1 by point mutations, with more than 90 SHV ESBL variants so far described (Paterson, 2003). This family of ESBLs currently predominates and today, SHV-5 and SHV-12 are among the most common members of this family. The SHV types of ESBLs have been detected in a wide range of *Enterobacteriaceae* and outbreaks of SHV–producing *Pseudomonas species* and *Acinetobacter species* have been reported (Mshana, 2011).

2.4.2.3. CTX-M-type

The CTX-M-type enzymes are a group of class A extended-spectrum beta-lactamases (ESBLs) that are rapidly spreading among *Enterobacteriaceae* worldwide. More than 50 allotypes are known, clustered into six sub-lineages. The CTX-M-encoding genes have been captured from the chromosome of *Kluyvera* spp. on conjugative plasmids that mediate their dissemination among pathogenic enterobacteria. The CTX-M-type ESBLs exhibit powerful activity against cefotaxime and ceftriaxone but generally not against ceftazidime, which has important implications for laboratory detection. However, several CTX-M variants with enhanced ceftazidimase activity have been detected. The rapid and massive spread of CTX-M-type ESBLs is rapidly changing the ESBL epidemiology and, in some geographical areas, these enzymes are now the most prevalent ESBLs in *Enterobacteriaceae* (Rossolini *et al*., 2008).

2.4.3. Reservoirs of ESBL infection

The faecal flora of children in the community can represent a reservoir for ESBLs genes which are located on highly transmissible plasmids because intestinal carriage is a key factor in the epidemiology of ESBL-producing *Enterobacteriaceae.* The study on the prevalence of these resistant bacteria and risk factors in young children is of particular interest (Birgy *et al*., 2012). The cause of this sudden upsurge in community-acquired infections with ESBL-producing organisms is not yet clear, but associations with foodstuffs, animal consumption of antibiotics, and frequent patient contact with health care facilities need to be explored (Paterson and Bonomo, 2005). Animals may also be the reservoir of the resistant faecal flora. Knowledge concerning colonisation of the human gut is still incomplete but it is well-known that endogenous faecal flora of animal origin can spread via the food chain and transiently colonise the human gut. Resistant gut colonisers (principally, *E. coli*) may be subsequent agents of urinary infection in vulnerable patients. (Carattoli, 2008). The frequent administering of antibiotics in the treatment of poultry may contribute to emergence of

antimicrobial resistant strains. The antimicrobial sensitivity test in the study which was conducted in Zambia revealed that 85.7 percent of ESBL producing *E. coli* isolates conferred resistance to Beta-lactam and antimicrobial agents. These results indicated that poultry is a potential reservoir for ESBL-producing *E. coli* which could spread into the food chain (Chishimba *et al*., 2016). The study conducted in Zambia on the detection of *E. coli* in flies that besets on fresh fish sold in market places in Zambia indicated that fish selling business is affected negatively by high fly numbers. About 56 ESBL-producing *E. coli* were obtained from flies in Lusaka out of these 42 isolates were subjected to antimicrobial susceptibility tests where drug resistant phenotypes were observed, this indicated that flies are reservoirs for ESBL producing *E. coli* (Mwansa *et al*., 2017).

2.5. Antibiotic Resistance to *E. coli*

Escherichia coli has been known as one of the most common bacteria found in the intestinal tract of human and warm blooded animals (Levine, 1987). Their ability to survive outside the body for longer periods of time makes them an ideal indicator organism to test food and environmental samples for fecal contamination (Lihan *et al*., 1999; Levine 1987). Though people generally understand *E. coli* as harmless intestinal flora, they are opportunistic and some of the strains have been identified as the serious causal agents of various illnesses (Levine, 1987). In our local setting, the health hazards associated with *E. coli* have become complicated by the fact that some of the causal agents have over the years, developed resistance against commonly used antibiotics (Wan *et al*., 2003; Son *et al*., 2001; Son *et al*., 1999; Son *et al*., 1998; Son *et al*., 1997).

The ESBL producing strains of *Enterobacteriaceae* have emerged as a major problem in hospitalized as well as community based patients (Rodriguez *et al.*, 2004). Organisms producing ESBLs are clinically relevant and remain an important cause of failure of therapy with cephalosporins and these are primarily produced by the *Enterobacteriaceae* family, in particular *Klebsiella pneumoniae* and *Escherichia coli* (Bradford, 2001). Furthermore, bacteria harbouring ESBLs may also acquire and most often exhibit additional resistances to other antimicrobial classes such as the quinolones, tetracyclines, cotrimoxazole, trimethoprim, and aminoglycosides, which further limits therapeutic options and thus pose a therapeutic dilemma (Chopra *et al*., 2008).

Emergence of ESBL-producing isolates has important clinical and therapeutic implications. In most bacterial isolates, resistance determinants for ESBL production are carried on

plasmids that can easily be spread from organism to organism and the spread of resistance toward extended-spectrum cephalosporins further limits the utility of the ß-lactam class and may lead to increased prescription of more broad-spectrum and expensive drugs such as imipenem. In addition, these resistant isolates may escape detection with routine susceptibility testing performed by a clinical microbiology laboratory, which can result in adverse therapeutic outcomes (Tenover, 1999)

A heavy use of antibiotics has been reported to be a risk factor for acquisition of ESBL producing organisms (Mshana *et al*., 2009). Production of ESBL is the most common amongst the mechanisms of resistance to third generation cephalosporins like cefotaxine, cefdinir, and cefpodoxime in Gram-negative bacilli. Most of the ESBL- producing isolates are multi-drug resistant making available therapeutic choices limited (Black *et al*., 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area.

The study was conducted in Lusaka, the Capital City of Zambia. Lusaka covers an estimated area of 360 $km^2$and is located at 15-30^{o}latitude south and 28-17^{o}longitude east. The city lies on a plateau 1280m above sea level (MFNP 2006 to 2011).

3.2. Sample size estimation

The sample size was estimated by using the following formula for sample estimation at 95 percent level of confidence.

$$\mathbf{n = z^2p\ (1\text{-}p)/d^2}$$

Where n = required sample size

p = estimated prevalence for fresh fish 50 percent

p = estimated prevalence for dry fish 50 percent

d = precision 5 percent

Z = value of the standard normal distribution corresponding to a significance level of a 1.96 for a 2-sided test at the 0.05 level.

3.3. Isolation of *E. coli* from Fish

Fish samples were randomly collected from three local markets (Table 3.1) in Lusaka District. The fish samples were packaged in sterilised plastic bags and labeled appropriately. Furthemore the collection of the dry fish was aseptically done.

Table 3.3. Information on sample size per market of fish

District	**Markets**	**Samples**	**No: of Samples**
LUSAKA	Soweto	Dry fish	80
	Chawama	Dry fish	20
	Mutendere	Dry fish	20
	TOTAL		**120**

At the laboratory, 1 gram of the fish samples was suspended into the sterilised normal saline and then a 100 µl was inoculated on MacConkey agar (Himedia, Mumbai, India) to isolate *E. coli*. The suspected *E. coli* was identified as lactose fermenters. Identification of *E. coli* lactose-fermenting positive colonies was further done using phenotypic characteristics and confirmed by the Triple Sugar Iron (TSI) and IMViC tests as described by other workers (Rayamajhi *et al.,* 2008; Batchelor *et al.,* 2005).

3.4. Determination of Extended Spectrum Beta-Lactamase (ESBL) Producing *E. coli*

The detected *E. coli* from the dry fish was inoculated on MacConkey agar containing 2 mg/L of cefotaxime for preliminary screening of ESBL-producing bacteria (Reich and Klein, 2016). The plates were incubated at 37 ^{o}C for 24 hrs and the colonies that grew on MacConkey agar were subjected to genetic determination and confirmation as ESBL producing. The *E. coli* growing on MacConkey agar containing 2 mg/L of cefotaxime was cultured on brain-heart-infusion broth (Himedia, Mumbai, India) followed by DNA extraction using the heat treatment method. The *E. coli* isolates were then subjected to PCR for confirmation of resistance gene CTX-M (Cefotaxime–Munich) using primers previously used by other workers (Kuroda, 2014). The primer sequences were CTX-M-uni-F CGATGTGCAGTACCAGTAA and CTX-M-uni-R TAAGTGACCAGAATCAGCGG. The PCR (FinnzymesOy, Espoo, Finland) was performed in a total reaction volume of 10 µL consisting of 5µL Phusion master mix, 2µL sterile distilled water, 2µ L primers (forward and reverse) and 1µL bacterial DNA template. The PCR was performed using the rapid cycle DNA amplification method comprising of an initial denaturation step at 98^{o}C for 30 s, followed by 35 cycles of template denaturation at 98^{o}C for 1 s, primer annealing at 60^{o}C for 5 s and 72^{o}C for 1 s with final extension at 72^{o}C for 10 s. The PCR products was later viewed with ethidium bromide after electrophoresis through 1.5 percent agarose gel.

3.5. Antibiotic Susceptibility Test

The antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar based on the Clinical Laboratory Standard Institute (CLSI) guidelines (Stern and Badley, 2001). The antibiotic discs which was used includes ampicillin (10µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), streptomycin (300 µg), ciprofloxacin (5µg), tetracycline (30µg), gentamicin (10µg), nalidixic acid (30 µg), chloramphenicol (30 µg), ceftazidime (30 µg), norfloxacin (10µg) and cefotaxime (30 µg). The phenotypic confirmation of ESBL isolates was done by the combination of disc approximation method using either ceftazidime (30µg) or cefotaxime (30 µg) alone followed

by over-night incubation at 37^{o}C for 18 to 24 hrs. Interpretation of susceptibility patterns on other anti-microbial discs was done using guidelines laid down in the CLSI, which provides break points corresponding to zones of inhibition diameter. Quality control standard laboratory procedures was strictly followed to avoid contamination. *Escherichia coli* ATCC 25922 was used as a quality control organism.

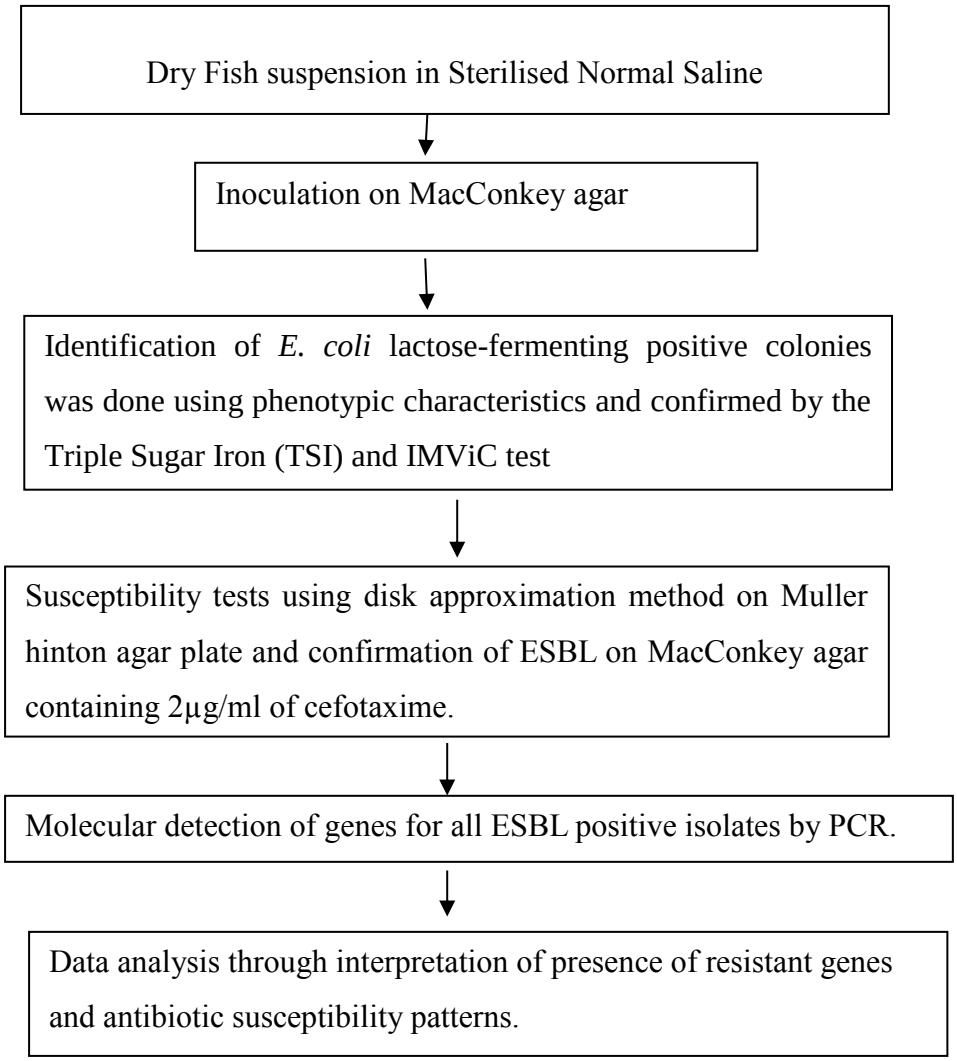

Figure 3.1: Summary of the laboratory work conducted in this study

3.6. Data Analysis

The data collected was entered into Microsoft excel and thereafter, exported to STATA 13 for processing and analysis using the presence of drug resistant genes and antibiotic sensitivity patterns. Presentation of results for the study were done in form of charts, graphs and tables.

3.6 Ethical Considerations

Before proceeding to collect research data from the community, permission was obtained from the Lusaka City Council and approval from the Excellence in Research Ethics and Science (ERES) institutional research board (IRB). The researcher ensured that the information collected were treated with the strictest confidence.

CHAPTER FOUR

RESULTS

4.1. Isolation of *E. coli*

A total of 120 samples of fish were analysed for *E. coli*. Of which, 83 (69 percent, 83/120) were positive for *E. coli* and 37 were negative. (Figure 4.1). The breakdown of the *E. coli* isolated from the various sampled markets was 57 (69 percent) from Fish Sampled from Soweto market, 10 (12 percent) from Chawama market and 16 (19 percent) from Mtendere market (Table 4.1).

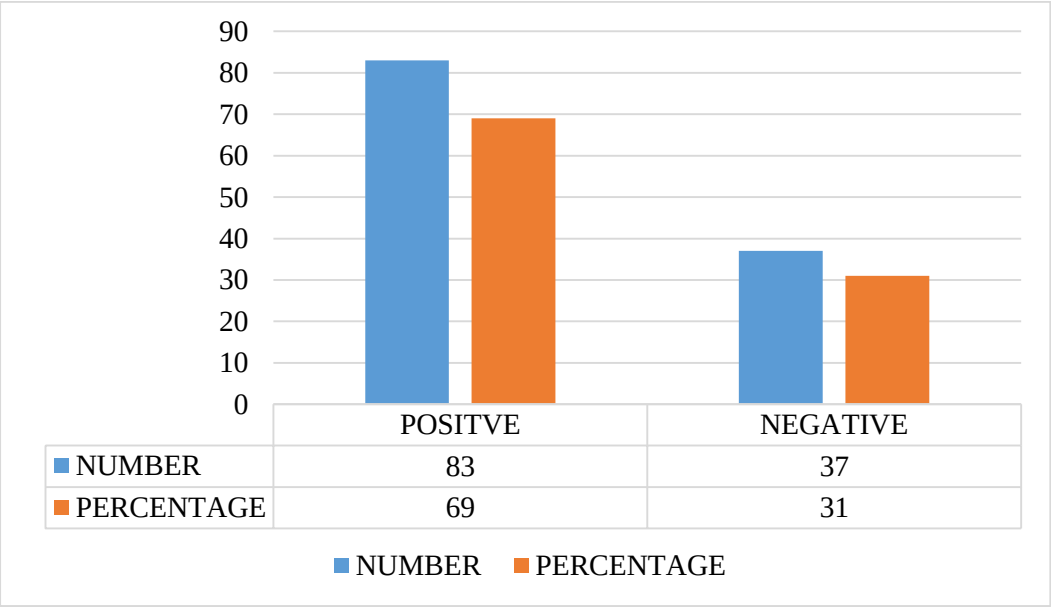

Figure 4.1: *E. coli* isolation from dry fish

Table 4.1. *E. coli* isolated from dry fish at various markets sampled

	SOWETO		CHAWAMA		MTENDERE		Total	
	Fish Sampled	*E. coli* (+ve)	Fish Sampled	*E. coli* (+ve)	Fish Sampled	*E. coli* (+ve)		
NUMBER	80	57	20	10	20	16	120	83
PERCENT		71		50		80		69

4.2. Determination of ESBL Producing *E. coli* in Dry Fish

The determination of *E. coli* as an ESBL producing organism was determined by growing the bacteria on MacConkey agar containing 2 mg/L of cefotaxime. Following culturing of *E. coli* on MacConkey agar containing 2 mg/L of cefotaxime a total of 46 (55.4 percent) were

suspected to be ESBL-producing *E. coli* isolates while 37 (44.6 percent) did not grow. (Figure 4.2). Furthermore it was observed that 73.9 percent of *E. coli* detected as ESBL producing was from Soweto market, 6.5 percent from Chawama market and 19.5 percent from Mtendere market (Figure 4.3).

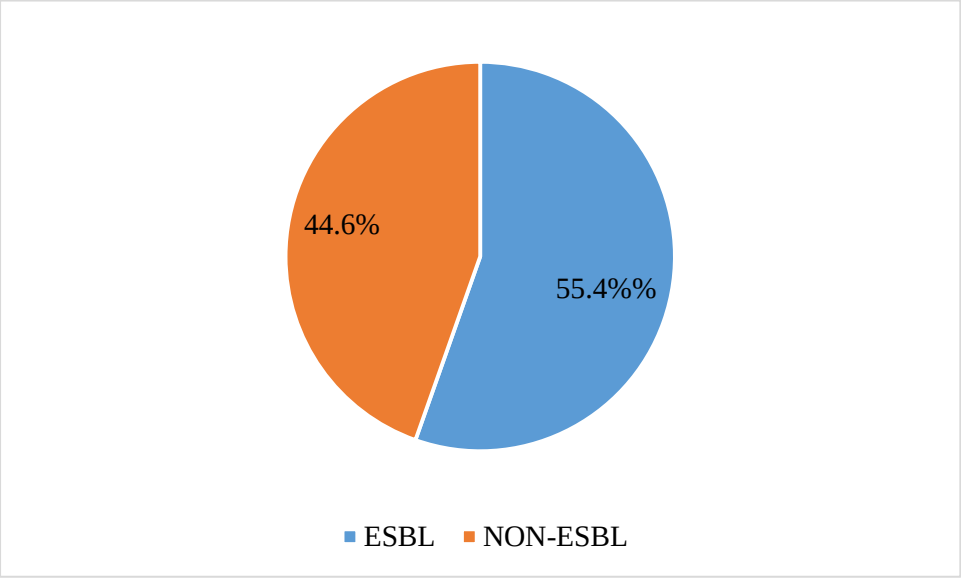

Figure 4.2: Determination of ESBL producing *E. coli* isolated from dry fish

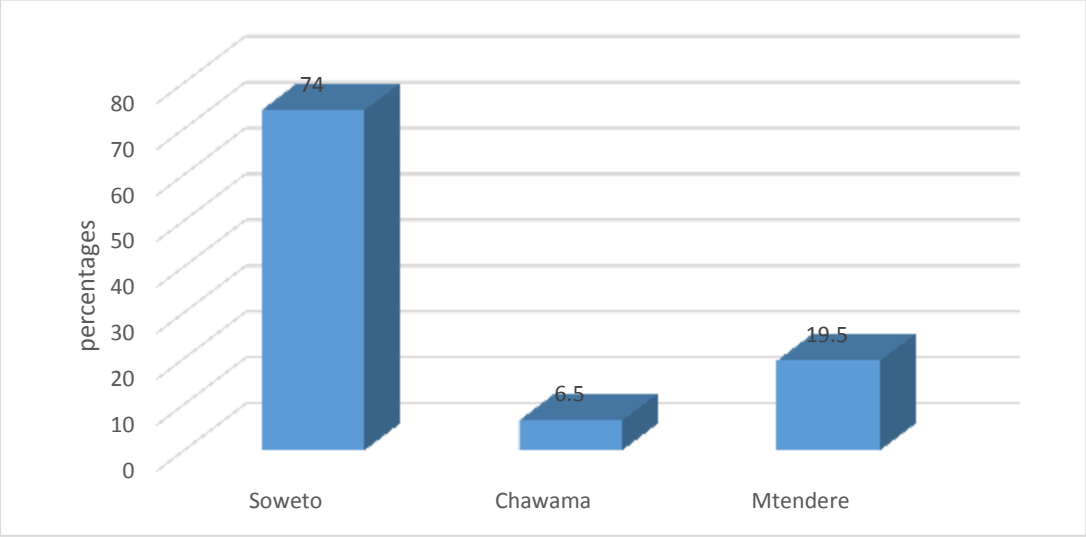

Figure 4.3: Determination of ESBL producing *E. coli* isolated from dry fish in various markets

The *E. coli* isolated from various markets segregated as ESBL producers or non ESBL producers as shown in Figure 4.4.

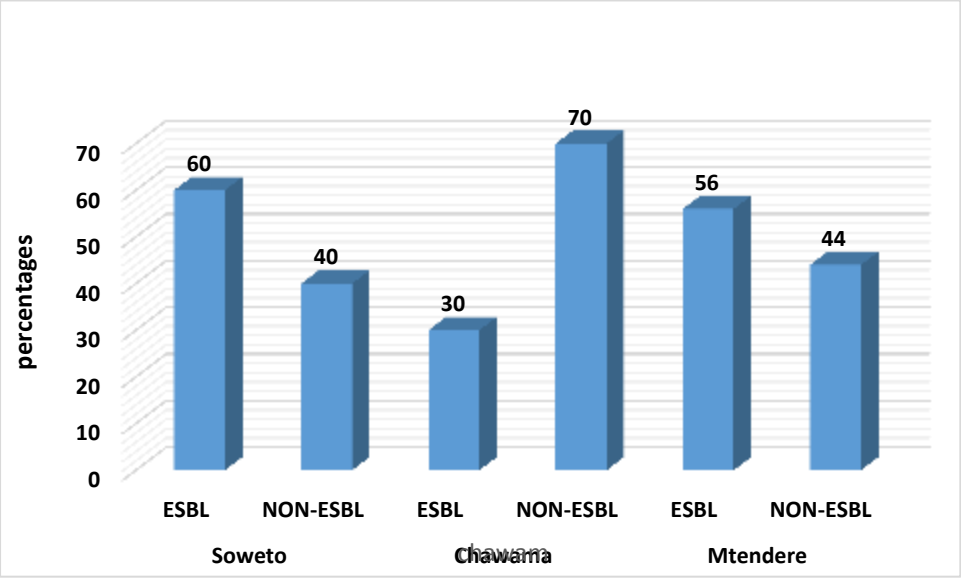

Figure 4.4: *E. coli* isolates segregated as non ESBL producers and ESBL producers.

4.3. Molecular confirmation of ESBL producers

The *E. coli* isolates presumably confirmed to be ESBL producing following culture on MacConkey agar supplemented with cefotaxime were subjected to PCR. A total of 35 isolates were tested for the presence of the *bla*CTXM gene and out of these 21 were positive through the amplification of the 589bp fragment (Figure 4.5).

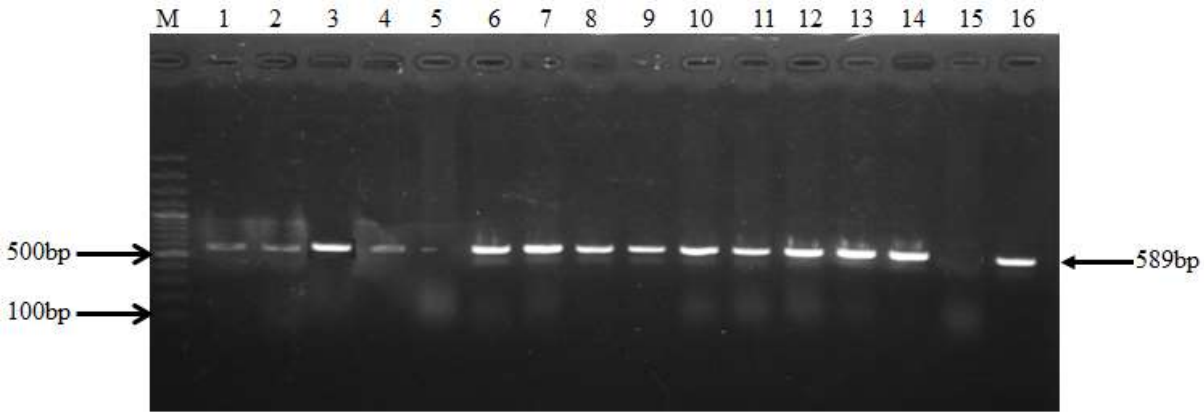

Figure 4.5: PCR results for *bla*CTX-M gene. M; DNA ladder, Lane 1 to 14 are samples, while Lane 15 and 16 as negative and positive controls respectively.

4.4. Antimicrobial susceptibility Pattern

Of the 46 *E. coli* isolates that grew on MacConkey agar supplemented with cefotaxime, the resistant were observed in all the drugs which included, erthromycin, Chlorampenicol, Ampicillin, Ceftazidime, doxycycline, tetracycline, norfloxacin, naldixic, ciprofloxacin, gentamicin, strptomycin, neomycin and Amoxycllin. The highest intermidiate was observed

with Chloramphenicol and the lowest with ampicillin, ceftazidime and doxycycline. On susceptibility the highest was with norfloxacin (48 percent) and some were not susceptible to some antibiotics that included tetracycline, doxycycline and erythromycin (Figure 4.6). For *E. coli* which did not grow on cefotaxime media showed resistance to all antibiotics (Figure 4.7).

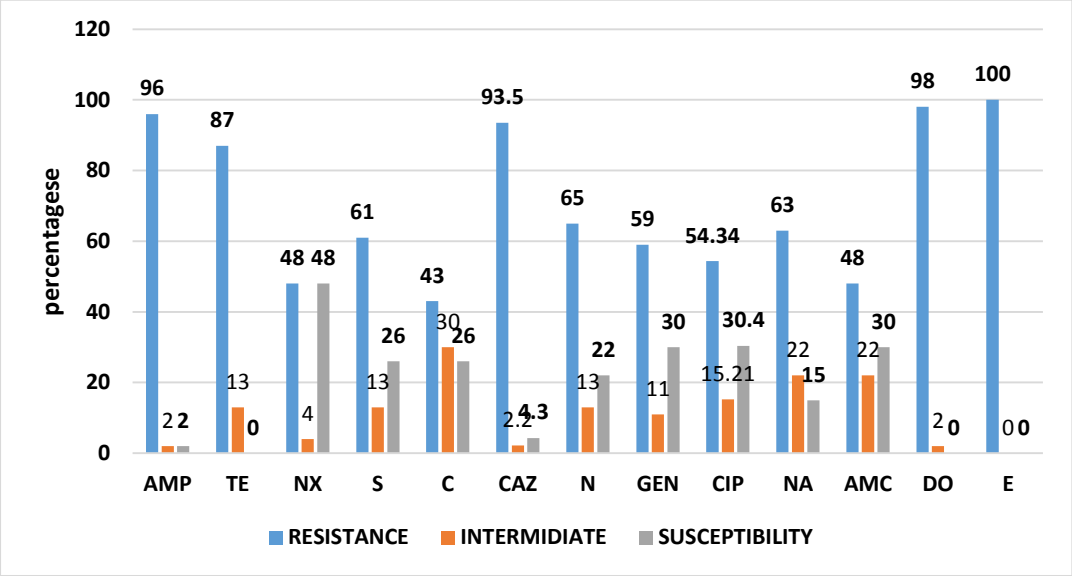

Figure 4.6: Antimicrobial susceptibility pattern of *E. Coli* that grew on MacConkey agar supplemented with cefatoxime media

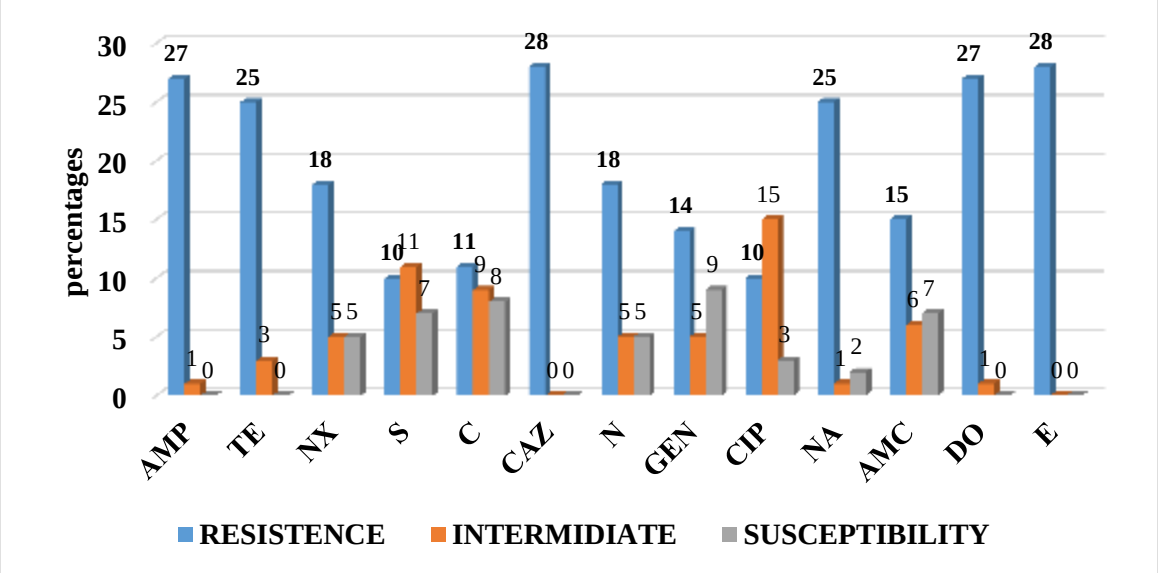

Figure 4.7: Antimicrobial susceptibility pattern of *E. coli* that did not grow on MacConkey agar supplemented with cefatoxime media. Key for antibiotics: Ampicillin – AMP; Tetracycline – TE; Norfloxacin – NX; Streptomycin – S; Chloramphenicol – C; Ceftazidime – CAZ; Neomycin – N; Gentamicin – GEN; Cipofloxacin – CIP; Nalidixic acid – NA; Amoxycllin – AMC; Doxycline – DO; Erythromycin - E

The diversity of the antibiotic resistance, intermidiate and susceptible *E. coli* isolates that were found to be ESBL producing and non ESBL producing *E. coli* are presented in Figure 4.7. A total of 74 isolates were subjected to antibiograms which consisted 46 ESBL producers and 28 non ESBL producers.

CHAPTER FIVE

DISCUSSION

Fish is an important food commodity in the international food trade but they deteriorate rapidily especially when storage facilities are lacking. It has also been widely accepted as a good source of protein and other elements necessary for the maintance of a healthy body. In order to preserve this prestigous protein source, drying of fish is very common. In the study, dry fish (tilapia) was sampled from three markets in Zambia and examined for the presence of *E. coli*. A total of 83 *E. coli* isolates were isolated from 120 dry fish samples. The isolation rate of *E. coli* was high (69 percent). The isolation of *E. coli* is of high importance because this bacteria plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as a spoilage organism. This is in accordance with what was previously reported by Koutsoumaris and Nychas (2000). From the results of the study, it was observed that dry fish sold in open markets in Lusaka has high contamination of *E. Coli*. This may be as a result of poor hygienic practices of fish handlers. The contamination could be due to water taken in by the fishes which may contain faecal matter in their ecosystem. This is in agreement with an earlier report by Agbu *et al.,* (1998) in kastina found high viable counts of coliform density in the ecosystem.

Thampuran *et al.,* (2005) reported that the microbial quality of the tilapia indicated that all tissue samples were contaminated with fecal coliform and *E. coli* were the most common contaminant encountered. The presence of contamination was attributed to cross contamination from the environment, source of fish (water) and handling by the sellers (Byan, 1988; Byan *et al.*, 1981). In Lusaka the possible contamination routes includes poor supply of safe water in the markets, improper disposal of fecal matter and poor sanitation. This is in support with Mwansa, (2017) who reported that poor sanitary conditions and lack of formal refuse collection facilitates are breeding grounds for disease-causing organisms in the markets further stated that flies are associated with spreading of diseases because of their intimate relationship with decaying matter, garbage, and faeces.

55.4% isolates were confirmed to be ESBL producing *E. coli* while 44.6% were found to be non-ESBL producing following growth on MacConkey agar containing 2 mg/L of

cefotaxime. Further confirmation using molecular tool indicated that other isolates had no amplicon of the *bla*CTXM gene. These findings are quite interesting as this could be as a result of uneven mixing of the cefotaxime in MacConkey agar. Molecular confirmation detected isolates to contain the *bla*CTXM gene. This, therefore, confirms that ESBL-mediated plasmids are capable of carrying beta-lactamase genes and as such would result into high level presence of beta-lactam resistant phenotypes as described by Rottier *et al.,* (2012). This also confirms the presence of ESBL producing *E. coli* in fish being sold for human consumption. This is a significant public health problem. Resistance to beta-lactam/beta-lactamase inhibitor combinations in *E. coli* may be due to hyperproduction of penicillinase enzyme brought about by *bla*CTXM gene (Shaikh *et al*., 2014).

Antimicrobial susceptibility testing revealed interesting patterns with resistance rates observed in the majority of the fourteen antibiotics tested. From the antimicrobial susceptibility results, 96 percent of the isolates were resistant to ampicillin (10 µg) and ceftazidime (30 µg) , 42 percent chloramphenicol (30 µg), cefotaxime (30 µg), 47 percent ciprofloxacin (5 µg), 55 percent gentamicin (10 µg), 73 percent nalidixic acid (30 µg), 54 percent norfloxacin (10 µg), 51 percent streptomycin (300 µg), 88 percent tetracycline, 50 percent Amoxycyclave (30 µg), 97 percent Doxycycline Hydrochloride (10 µg), 65 percent Neomycin and the highest resistance were in erythromycin. This indicated that all *E. coli* isolates were resistant to one drug or another. The findings are similar to studies conducted by Kuenzli *et al.,* (2014) on food animals and water enteric bacteria where high antibiotic resistant profiles were established. For instance, a review of published and unpublished literature 44 for Democratic Republic of Congo, Mozambique, Tanzania and Zambia revealed an increased trend of resistance to ampicillin, co-trimoxazole, gentamicin, erythromycin, tetracycline and third generation cephalosporins (Mshana *et al.,* 2013). The development of high antibiotic resistance status could be attributed to the presence of extended- spectrum β- lactamases which are either transferable through mobile genetic elements or could be chromosomally mediated (Lester *et al*., 2006). Few *E. coli* isolates were susceptible to antibiotics which included 1 percent Ampicillin (10 µg) and 2 percent ceftazidime (30 µg) , 27 percent chloramphenicol (30 µg), 23 percent ciprofloxacin (5 µg), 31 percent gentamicin (10 µg), 12 percent nalidixic acid (30 µg), 36.5 percent norfloxacin (10 µg), 26 percent streptomycin (300 µg), 28 percent amoxycillin (30 µg), 20 percent neomycin and there were no susceptiblity to the following antibiotics erythromycin, tetracycline and doxycycline. This revealed that the first line of antibiotics are less effective. Resistant infections lead to increased morbidity and prolonged hospital stays, as well as to prolonged

periods during which individuals are infectious and can spread their infections to other individuals (Rubin *et al.*, 1999 and Holmberg *et al.*, 1987). The problem is particularly severe in developing countries, where the burden of infectious diseases is relatively greater and where patients with resistant infections are less likely to have access to or be able to afford expensive second-line treatments, which typically have more complex regimens than first-line drugs. In addition, these resistant isolates may escape detection with routine susceptibility testing performed by a clinical microbiology laboratory, which can result in adverse therapeutic outcomes (Tenover, 1999).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

1. Raw dry fish sold in open markets in Lusaka District of Zambia could be a source of food-borne bacterial pathogens.

2. There are high levels of contamination of dry fish with antimicrobial resistant enteropathogenic *E. coli* isolated in this study could be an indication of serious threat to Human and public health.
3. Dry Fish is a major and potential reservoir for the antimicrobial ESBL producing *E. coli* resistant genes which could be spread by the food chain.
4. The study has also shown widespread occurrence of multi drug resistance *E. coli* strains.

6.2. Recommendations

1. Public health concerns and improvements in handling and processing should be addressed to minimize the prevalence of the pathogens through urgent introduction of quality control and assurance systems.
2. Fish should be properly cooked before consumption and good quality control measures be adapted in harvesting and processing such as covering the fish to avoid flies besetting on the fish.
3. There is need for another study to improve sanitation and hygiene in markets in order to reduce *E.coli* from contaminating the environment

REFERENCES

1. Adak, G.K., Long, S.M. and O'Brien, S.J. (2005). Trends in indigenous foodborne disease and deaths, England and Wales. Gut, 51:832–41.
2. Adebayo-Tayo, B., Ody, N.N., Anyamele, L.M., Igwiloh, N.J.P.N. and Okonko, I.O. (2012). Microbial quality of frozen fish sold in Uyo Metropolis. Nature and Science, 10: 71-77.
3. Agba, A.A., Alariba, H. C., Singh, K. and Adesiyufi, A.A. (1998). Bacteriological studies and chemical analysis of public well water in samara and Zaria City in Northern Nigeria. Journal of Microbiology, 8: 88-98.
4. Basunia, M. A. and Abe, T. (2001). Thin-layer solar drying characteristics of rough rice under natural convection. Journal of Food Engineering, 47: 295–301.
5. Bala, B.K. and Mondol, M.R.A. (2001). Experimental Investigation on solar drying of fish using solar Tunnel Dryer. Drying technology, 19: 427 - 436.
6. Batchelor, M., Hopkins, K., Threlfall, E.J., Clifton-Hadley, F.A., Stallwood, A.D., Davies, R.H. and Liebana, E. (2005). *bla*CTX-M genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. Antimicrobial Agents and Chemotherapy, 49: 1319–1322.
7. Ben-Ami, R., Schwaber, M. J., Navon-Venezia, S., Schwartz, D., Giladi, M., Chmelnitsky, I., Leavitt, A. and Carmeli, Y. (2006). Influx of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* into the hospital. Clinical Infectious Disease, 42:925–934.
8. Bhandare, S.G., Sherikarv, A.T., Paturkar, A.M., Waskar, V.S. and Zende, R.J. (2007). A comparison of Microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food Control, 18: 854-868.
9. Birgy, A., Robert, C., Corinne, L., Philippe, B., Celine, C., Mohamed, B., Franck, T., and Edouard, B. (2012). Community faecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in French children. BMC Infectious Diseases. 12: 315.
10. Black, J. A., Moland, E. S. and Thomson, K. (2005). AmpC disk test for detection of plasmid-mediated AmpC-b lactamases in Enterobacteriaceae lacking chromosomal AmpC-b lactamases. Journal of Clinical Microbiology, 43: 3110-3113.
11. Bloomberg, B., Jureen, R., Manji, K. P., Tamim, B. S., Mwakagile, D. S. M., Urassa, W. K., Fataki, .M., Msangi, V., Tellevik, M. G., Maselle, S. Y. and Langeland, N. (2005). High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with

Extended-Spectrum Beta-Lactamases in Dar-es Salaam, Tanzania. Clinical Microbiology, 43:745-749.

12. Bradford, P. A. (2001). Extended-Spectrum β-Lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical Microbiology Reviews, 14:933-951.
13. Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R. and Swaminathan, B. (2007). Salmonella nomenclature – guest commentary. Journal Clinical Microbiology, 38: 2465–2467.
14. Butaye, P., Michael, G., Schwartz, S., Barett, T., Brisabois, A. and White, D. (2006). The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. Microbes Infectious, 8: 1891–1897.
15. Byan F.L (1988). Risks of Practices procedure and processes that lead to outbreak of Food-borne disease. Journal Food Protection, 51:663-673.
16. Byan. F.l, Bartleson,C.A., and Christopherson,n (1981). Hazard analysis in reference to *Bacillus cereus* of boiled and fried rice in cantonesse style Restaurants. Journal Food Protection, 44:500-512.
17. Carattoli, A. (2008). Animal reservoirs for extended spectrum b-lactamase producers. Clinical Microbiology and Infection, 14: 117–123.
18. Casellas, J.M. (1999). South America: A different continent, different ESBLs. Journal of Antimicrobial and Chemotherapy, 44: 16.
19. Chishimba, K., Hang'ombe, B.M., Muzandu, K., Mshana, S.E., Matee, M.I., Nakajima, C. and Suzuki, Y. (2016). Detection of extended-spectrum beta-lactamase-producing Escherichia coli in market-ready chickens in Zambia. International Journal Microbiology, 5275724.
20. Chopra, I., Schofield, C., Everett, M., O'Neill, A., Miller, K., Wilcox, M., Frere, J. M., Dawson, M., Czaplewski, L., Urleb, U. and Courvalin P. (2008). Treatment of health-care-associated infections caused by gram-negative bacteria: a consensus statement. Lancet Infectious Disease, 8:133-139.
21. Clarence, S. Y., Obinna, C. N., and Shalom, N. C. (2009). Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. African Journal of Microbiology Research, 3: 390-395.
22. Emikpe B.O. and Akpavie S.O. (2011). The clinicopathologic effects of Peste des petits ruminant's virus infection in West African dwarf goats. Small ruminant Research, 95: 168-173.

23. Gales, A. C., Jones, R. N., Gordon, K. A., Sader, H. S., Wilke, W. W., Beach M. L., Pfaller M. A. and Doern, G. V. (1998). Activity and spectrum of 22 antimicrobial agents tested against urinary tract pathogens in hospitalized patients in Latin America. Report from the second year of the Sentry Antimicrobial Surveillance Program.
24. Giriyapur, R., Nandihal, N., Krishna, B., Patil, A. and Chandrasekhar, M. (2012). Comparison of disc-diffusion methods for the detection of extended spectrum beta lactamase-producing *Enterobacteriaceae*. Laboratory Physicians, 3:33-36.
25. Gonzalez, C. M. and Schaeffer, A. J. (1999). Treatment of urinary tract infection. What's old, what's new, and what works. World Journal Urolo*gy*. 17:372-382.
26. Hawser, S. P., Bouchillon, S. K., Hoban, D. J., Badal, R. E., Hsueh, P. R. and Paterson, D. L. (2009). Emergence of high levels of extended spectrum-β-lactamase producing gram-negative bacilli in the Asia-Pacific region. Data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) Program. Antimicrobial Agents Chemotherapy, 53:3280–3284.
27. Hedberg, C.W., MacDonald, K.L. and Osterholm, M.T (1992). Changing epidemiology of food-borne disease: a Minnesota perspective. Clin Infect Dis. 18:671–88.
28. Holt, P.S., Geden, C.J., Moore, R.W. and Gast, R.K. (2007). Isolation of *Salmonella enterica Serovar Enteritidis* from Houseflies (Musca domestica) found in Rooms Containing *Salmonella serovar Enteritidis*-Challenged Hens. Application Environmemtal. Microbiology, 73: 6030–6035.
29. Holmberg, S.D.; Solomon, S.L. and Blake, P.A. (1987) Health and Economic Impacts of Antimicrobial Resistance. Review Infectious. Diseases, 9: 1065–1078.
30. Kaper, J.B., Nataro, J.P. and Mobley, H.L.T. (2004). Pathogenic *Escherichia coli.* Nature Reviews, 2: 123-140
31. Kayange, N., Kamugisha, E., Jeremiah, S., Mwizamholya, D.L. and Mshana, S.E. (2010). Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC Pediatrics. 10:39.
32. Koutsoumaris. K. and Nychas. G.J. (2000). Application of systemic experimental procedure to develop a microbial model for rapid fish shelf life predictions international. Journal Food microbialogy, 60: 171-184.
33. Kuenzli V., Jaeger K., Reno F., Andreas N., Susan D., Sabine H., Johannes B., Andreas F., Hansjakob F., Manuel B., Andrea E. and Christoph H. (2014). High colonization rates of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* in Swiss Travelers to South Asia–a prospective observational multicentre cohort study looking at

epidemiology, microbiology and risk factors. Biomedical Central Infectious Diseases, 14:528.

34. Kuroda, K., Suzuki, R., Ihara, K., Miyagi, H., Watanabe, H., Sato, K., Hang'ombe, B.M., Mubita, C., Isogai, N., Mulenga, E. and Isogai, E. (2014). Detection of virulence genes of *Escherichia coli* and *Salmonella* spp. from fecal samples of Kafue lechwe (*Kobus leche kafuensis*) and pastoral cattle in the interface areas of Zambia. African Journal Microbiology Research, 7: 504–508.
35. Lester C. H., Frimodt M. N. and Sorensen T. L (2006). In vivo transfer of the van A resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers. Antimicrobial Agents Chemotherapy, 50: 596-599.
36. Levine, M. M. (1987). *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, interoinvasive, enterohemorrhagic and enteroadherent. Journal of Infectious Diseases, 155: 377-389.
37. Lihan, S., Radu, S., Rusul, G., Karim, M. I. A., Ling, O. W., and Maisin, N. (1999). Diversity of DNA sequences detected by PCR-based DNA fingerprinting, antibiotic resistance and plasmid profiles of *Escherichia coli* occurring in raw milk. Asia Pacific Journal of Molecular Biology and Biotechnology, 7: 127-133.
38. Ministry of Finance and National Planning, (2005). Lusaka District Draft Development Plan 2006 to 2011, Ministry of Finance and National Planning, Planning and Economic Management Department, Lusaka, Zambia, 2:49.
39. Mshana, E. S., Mecky, M. and Mark, R. (2013). Antimicrobial resistance in human and animal pathogens in Zambia, Democratic Republic of Congo, Mozambique and Tanzania: an urgent need of a sustainable surveillance system. Annals of Clinical Microbiology and Antimicrobials, 12:28.
40. Mshana, S. E., Hain, T., Domann, E., Lyamuya, E. F., Chakraborty, T. and Imirzalioglu, C. (2013) Predominance of *Klebsiella pneumoniae* ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania. BMC Infectious Diseases, 13:466.
41. Mshana, E. S. (2011). Molecular Epidemiology of Extended Spectrum Beta -Lactamases (ESBL) Producing Enterobacteriaceae from the Bugando Medical Centre, Mwanza, Tanzania and the University of Giessen Medical Hospital, Germany. Weill Bugando University College of Health and allied Sciences. Tanzania.
42. Mshana E. S. (2009). State of the Globe: Evaluating the Existence of Extended Spectrum Beta Lactamases. Journal of Global Infection Diseases, 1: 85–86.

43. Mwansa, M.S., Hang'ombe B.M., Knight-Jones T.J.D. and Grace, D. (2017). Antimicrobial Resistant Enteropathogenic *Escherichia coli* and *Salmonella* spp. in Houseflies Infesting Fish in Food Markets in Zambia. International Journal Environmental. Research. Public Health, 14: 21.
44. Nakhjavani, F.A., Emaneini, M., Hosseini, H., Iman-Eini, H., Aligholi, M., Jabalameli, F., Haghi-Ashtiani, M.T., Taherikalani, M. and Mirsalehian, A. (2013). Molecular analysis of typical and atypical enteropathogenic *Escherichia coli* (EPEC) isolated from children with diarrhea. Journal Medicine Microbiology, 62: 191–195.
45. Nataro, J.P. and Kaper, J.B. (1998). Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews, 11: 142-201.
46. Ndugulile, F. R., Jureen, S. H., Stig, H., Willy, U. and Nina, L. (2005). Extended Spectrum β-Lactamases among Gram-negative bacteria of nosocomial origin form an Intensive Care Unit of a Tertiary Health Facility in Tanzania. BMC Infectious Diseases, 5:86.
47. Orskov, I., Orskov, F., Birch-Andersen, A., Kanamori, M., Svanborg-Edén, C. (1992). O. K, H and fimbrial antigens in *Escherichia coli* serotypes associated with pyelonephritis and cystitis. Scandal Journal Infectious Diseases Supplement, 33:18–25.
48. Paterson, D.L. and Bonomo, R.A. (2005). Extended-spectrum beta-lactamases: a clinical update. Clinical Microbiology Review, 18: 657–686.
49. Paterson, D. L., Hujer, K. M. and Hujer, A. M. (2003). Extended-spectrum beta-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries. Dominance and widespread prevalence of SHV- and CTX-M-type beta-lactamases. Antimicrobial Agents and Chemotherapy, 47: 3554–3560.
50. Public Health England. (2013). Annual Report and Accounts.
51. Public Health England. (2014). Annual Report and Accounts.
52. Rayamajhi, N., Kang, G.S., Lee, Y.D., Kang, L.M., Lee, I.S., Park, Y.K., Lee, S.H. and Yoo, S.H. (2008). Characterization of TEM, SHV and *AmpC*-type beta-lactamases from cephalosporin-resistant Enterobacteriacea isolated from swine. International Journal Food Microbiology, 124: 183–187.
53. Reich, F., Atanassova, V. and Klein, G. (2016). Extended spectrum beta-lactamase and *AmpC*-producing Enterobacteria in healthy broiler chickens, Germany. Emerging Infectious Diseases. 19: 1253–1259.
54. Rodriguez, B. J., Navarro, M.D., Romero, L., Martinez, M. L., Muniain, M. A., Perea, E. J., Perez, C. R. and Pascual, A. (2004). Epidemiology and clinical features of infections

caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. Journal of Clinical Microbiology, 42:1089-1094.

55. Rodriguez, B. J., Juan, C. A., Jose, M., Cisneros, F. G., Antonio, O., Juan, P., Horcajada, T. T., Beatriz, M., Gemma, N., María, C., María E., Carmen, P., Ana, C., Llanos, R. and Canton, A. P. (2008). Community Infections Caused by Extended-Spectrum β-Lactamase–Producing Escherichia coli free. Archives of Internal Medicine, 168:1897-1902.
56. Rombout, F.M. and R. Nout. (1994). Food Microbiology and hygiene. Encyclopedia of Human Biology. Academic Press, 111: 661-665
57. Rossolini, G. M., D'Andrea, M. M. and Mugnaioli, C. (2008). The spread of CTX-M-type extended-spectrum beta-lactamases. Clinical Microbiology Infections, 1: 33 – 41.
58. Rottier W.C., Ammerlaan H.S. and Bonten M. J. (2012). Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum betalactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. Journal Antimicrobial Chemotherapy, 67: 1311-1320.
59. Rubin, R.J., Harrington, C.A., Poon, A., Dietrich, K., Greene, J.A. and Moiduddin, A. (1999). The Economic Impact of *Staphylococcus aureus* in New York City Hospitals. Emerging Infectious Diseases, 5: 9–17.
60. Rupp, M. E. and Fey, P. D. (2003). Extended spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* considerations for diagnosis, prevention and drug treatment. Drugs. 63: 353 – 365.
61. Ryan, K.J. and Ray, C.G. Sherris. (2004). Medical Microbiology: An Introduction to Infectious Diseases, 4th ed.; McGraw Hill:New York, NY, USA. 362–368.
62. Sader, H. S., Jones, R. N. and Gales, A. C. (2006). The SENTRY participants group (Latin America). SENTRY antimicrobial surveillance program report: Latin American and Brazilian results for 1997 through 2001. Brazilian Journal of Infectious Disease, 8:25–79.
63. Shaikh, S., Fatima J., Shakil S., Rizvi S.D. and Kamal, M.A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi Journal of Biological Sciences, 22: 90–101.
64. Shukla, I., Tiwari, R. and Agrawal, M. (2004). Prevalence of extended spectrum β-lactamase producing *Klebsiella pneumoniae* in a tertiary care hospital. Medical Microbiology, 22:87-89.

65. Smith, M. E., Hanson N.D, Herrera, V.L., Black, J.A., Lockhart. T.J. and Hossain A. (2007). Plasmid-mediated, carbapenem-hydrolysing blactamase, KPC-2, in *Klebsiella pneumoniae* isolates. Journal Antimicrobial Chemotherapy, 51:711-4.
66. Son, R., Ooi, W. L., Rusul, G., Karim, M. I. A. and Nishibuchi, M. (2001). Detection of *Escherichia coli* O157:H7 by multiplex-PCR and their characterization by plasmid profiling, antimicrobial resistance, RAPD and PFGE analysis. Journal of Microbiological Methods, 46: 131-139.
67. Son, R., Rusul, G., Sahilah, A. M., Zainuri, A., Raha, A. R. and Salmah, I. (1997). Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, Telapia (*Telapia mossambica*). Letters in Applied Microbiology, 24: 479-482.
68. Son, R., Nasreldin, E. H., Zaiton, H., Samuel, L., Rusul, G., Nimita, F., Yuherman. and Endang, P. (1998). Characterization of *Vibrio vulnificus* isolated from cockles (*Anadara granosa*): antimicrobial resistance, plasmid profiles and random amplification of polymorphic DNA analysis. FEMS Microbiology Letters, 165: 139-143.
69. Son, R., Sahilah, A. M., Rusul, G., Zainori, A., Morigaki, T., Asai, N., Kim, Y. B., Okuda, J. and Nishibuchi, M. (1998). Detection of *Escherichia coli* O157:H7 in the beef marketed in Malaysia. Applied and Environmental Microbiology, 64: 1153-1156.
70. Son, R., Samuel, L., Gulam, R. and Zuraini, M. I. (1999). RAPD-analysis, antibiotic resistance and plasmid profiles of *Escherichia coli* isolated from village and broiler chicken. Malaysian Applied Biology, 278: 51-60.
71. Stamm, W. E. and Stapleton, A. E. (1998). Approach to the patient with urinary tract infection. International Infectious Diseases, 1270-1272.
72. Steinke, D. T., Seaton, R. A., Phillips, G., MacDonald, T. M. and Davey, P. G. (2001). Prior trimethoprim use and trimethoprim-resistant urinary tract infection. A nested case-control study with multivariate analysis for other risk factors. Journal of Antimicrobial and Chemotherapy, *47:781-787.*
73. Szulmayer, W. (2001). Food Drying Science and Technology: Microbiology, Chemistry, Applications.edited by Yiu H. Hui. Destech Publications Inc. Lancaster, pennisylvania, 781.
74. Tenover, F. C., Mohammed, M. J., Gorton, T. S. and Dembek, Z. F. (1999). Detection and reporting of organisms producing extended-spectrum ß-lactamases. Survey of laboratories in Connecticut. Journal Clinical Microbiology, 37:4065-4070.
75. Ternhag, A. T., Åke, S., Karl E. and Johan, G. (2008). Short- and Long-term Effects of Bacterial Gastrointestinal Infections Prevalence of microbial contamination of fresh

seafood product sold in Constantine, Algeria. Emerging Infectious Diseases, 14:143 - 148.

76. Thampuran, N., Surendrara, J.A. and Surendran, P.K. (2005). Prevalence and characterization of typical and atypical *Escherichia coli* from fish sold at retail in Cochin, India Journal Food Protection, 68: 2208-2211.
77. Villegas, M. V.,Kattan, J. N., Quinteros, M. G. and Casellas, J. M. (2007). Prevalence of extended-spectrum β-lactamases in South America. Clinical Microbiology Infection, 14: 154 – 158.
78. Vu-Khac, H., Holoda,E., Pilipcinec, E., Blanco, M. and Blanco, J.E. (2007). Serotypes, virulence genes, intimin types and PFGE profiles of *Escherichia coli* isolated from piglets with diarrhea in Slovakia. Veterinary Journal, 174: 176-87.
79. Wan, K. F., Son, R., Cheah, Y. K., Patrick, G. B. and Michael, C. M. V. L.(2003). Antibiotic resistance, plasmid profile and RAPD-PCR analysis of enteropathogenic *Escherichia coli* (EPEC) clinical isolates. Southeast Asian Journal of Tropical Medicine and Public Health, 34: 620-626.
80. Wang, F., Jiang, L., Yang, Q., Han, F., Chen, S., Pu, S., Vance, A. and De, B. (2011) Prevalence and antimicrobial susceptibility of major foodborne pathogens in imported seafood. Journal of Food Protection, 74: 1451- 1461.
81. Yayue Li., Boyang, C., Bin, L., Dan, L., Qili, G., Xia, P., Junli. W., David, A., Bastin, L.F. and Lei, W.I. (2006). Isolation, identification and serotyping of *Escherichia coli* isolates from estuarine, seafood and clinical sources. Volume: 44. Publisher: American Society for Microbiology.