# PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING *ESCHERICHIA COLI* IN INTEGRATED AGRO-AQUACULTURE IN MOROGORO, TANZANIA

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN HEALTH OF AQUATIC ANIMAL RESOURCES OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.**

**2017**

## ABSTRACT

Integrated aquaculture and other agricultural activities involve use of animal manure directly or indirectly into the pond. This farming practice increase production and minimize the production cost. Despite the benefits, this may impose diseases to fish and consumers. Use of antibiotics in poultry integrated with aquaculture may impose antimicrobial resistance to fish. Extended spectrum beta lactomase (ESBL) producing *Escherichia coli* is resistant genes that inhibit the function of third generation cephalosporins. The aim of this study was to establish the prevalence of extended spectrum beta lactamase producing *Escherichia coli* in direct and indirect integrated aquaculture in Morogoro, Tanzania. A total of 384 fish samples were collected from 26 ponds after recording water quality parameters. Isolation of bacteria was done according to standard procedures, where 174 isolates of *E.coli* were obtained out of 384 cultured samples. Positive samples were tested against Amoxicillin/Clavulanic acid, Cefotaxime, Ceftazdime, Sulfamethoxazole, Ampicillin, Amoxicillin and Ciprofloxacin antibiotics using double disc diffusion test, and then further tested using PCR for detection of ESBL resistant genes encoding CTX, SHV and TEM genes. Water quality parameters were within the normal range and suitable for culture and growth of Nile tilapia. Other pond management practices such as feed, feeding, use of manure, preparation of pond and quality of seed were poorly managed. Amoxicillin and Ampicillin antibiotics showed 100% resistivity followed by Sulfamethoxazole (93.6%). Cefotaxime (70%). High prevalence of ESBL genes shown in TEM gene 52%, followed by SHV 36% and CTX 9.7%. There were no significant differences for occurrence of ESBL genes between the direct IAA and indirect IAA ($P>0.05$). Presence of ESBL genes in fish have a direct effect to fish and consumers. They resist function of antibiotics that are used to treat diseases, therefore it is recommended that ESBL genes be screened to farmed fish to establish its prevalence before its consumption by consumers.

## DECLARATION

I, SOPHIA S SHABAN, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for any higher degree award in any other Institution.

……..…… ……..

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The above declaration is confirmed by;

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……..…… ……..

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(Supervisor)

## COPYRIGHT

## ACKNOWLEDGEMENTS

I would like to thank the Almighty God for granting me good health, courage and strength in accomplishing this work. My sincere gratitude goes to my supervisors: Prof. Mdegela, R. H. from the Department of Veterinary Medicine and Public Health and Dr. Lamtane, H. A. from the Department of Animal, Aquaculture and Range Sciences (DAARS) for giving me both moral and material support throughout this study. They worked very hard to make sure that this work is completed in a very efficient and effective way. Their suggestions and encouragement throughout this study helped me to improve and complete my work on time. I am also indebted to my colleagues Yusuph, A., Siyajali, A., Mkunde, G., Masoud, U., Wairia, J., Nantongo, M. and Njobvu, L. for their support and encouragement in my studies. Further appreciation goes to Mr. Mgusi, M. J. and Ms. Wilson, C. technicians in the Department of Microbiology, Parasitology and Biotechnology, Mr. Mkuchu, P., Ms. Mgonja, S., Mr. Makingi, G. and Mr. Ndaki, L. technicians in the Department of Veterinary Medicine and Public Health for their technical assistance during my laboratory work. Lastly, I am grateful to the Norwegian Agency for Development Cooperation (NORAD) through the Training and Research in Aquatic and Environmental Health in Eastern and Southern Africa (TRAHESA (TAN-13/0027) project for sponsoring my master's studies at Sokoine University of Agriculture and One Health for Central and Eastern Africa (OHCEA) project that helped by covering additional research expenses.

## DEDICATION

This work is dedicated to my lovely husband, Mr. Kulwa Jonathan Shimiyu for allowing me to pursue my master's degree. He has been a fruitfully, patient and a very best friend in my life as he always rendered me moral, spiritual and material support throughout my studies. Moreever, I dedicate this work to my beloved daughter, Ivana Kulwa Shimiyu whose presence has been a platform for me to work hard and make sure that I bring a better future for her. Lastly I dedicate this work to my mother, Diana Salum, my late sister, Rehema Joseph and my brother, Willium Joseph for being supportive in my life.

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| | |
|---|---|
| AML | Amoxicillin |
| AMP | Ampicillin |
| BHI | Brain Heart Infusion |
| CAZ | Ceftazdime |
| CIP | Ciprofloxacin |
| CTX | Cefotaxime |
| DNA | Deoxyribose Nucleic Acid |
| EAEC | Enteroaggregative *Escherichia coli* |
| EHEC | Enterohemorrhagic *Escherichia coli* |
| EIEC | Enteroinvasive *Escherichia coli* |
| EPEC | Enteropathogenic *Escherichia coli* |
| ESBL | Extended Spectrum Beta Lactamase |
| ETEC | Enterotoxigenic *Escherichia coli* |
| FAO | Food and Agriculture Organization |
| IAA | Integrated Agro- Aquaculture |
| IMViC | Indole, methyl red, Voges-Proskauer, citrate |
| PCR | Polymerase Chain Reaction |
| rpm | revolution per minute |
| WHO | World Health Organization |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background Information

Aquaculture sector supports life of many individuals and improves community food and nutritional availability (Ponzoni *et al*., 2007). The world status of aquaculture indicates China to be a major producer and exporter of aquatic products, contributing about 60% of the aquaculture production worldwide. Aquaculture production has increased significantly due to increased human population and decrease in capture fisheries (FAO, 2016). Fishery and aquaculture supply 17% of the global protein in the population diet as well as supporting livelihood of 12% of the world's population (World Bank, 2013).

Aquaculture in Tanzania is an emerging industry that is currently dominated by five species; the Nile tilapia (*Oreochromis niloticus*), Rainbow trout (*Onchorynchus mykiss*), African catfish (*clarias gariepinus*), seaweed (*Eucheuma cottonii, E. spinosum*) and milkfish (*Chanos chanos*). The sector is mostly dominated by small scale farmers that produce fish for household consumption and for the domestic market. Production from aquaculture increases from 4,243 metric tonnes that worth 8.5 billion in 1998 to 5,933metric tonnes worth 34 billion in 2012. Pond increased from 14,142 ponds in 1998 to 21,300 ponds in 2014. This sector contribute significantly in alleviating poverty, achieving food security, improving nutrition and promoting sustainable agriculture in the country (URT, 2015).

Integrated aquaculture agriculture (IAA) is a farming practice in which fish, animals and crops (vegetables) are grown concurrently (Little and Edwards, 2003). In IAA systems outputs from one unit are used as inputs for other unit. It is aimed at recycling of animal

wastes that serve as fertilizers and food for fish pond. It also act as an effective way of utilizing scarce resources like land and water (Bhatt *et al.,* 2011)**.** There is a growing interest in the use of organic manures in integrated systems, due to depletion in the soil fertility (Kipkemboi *et al.,* 2007). Poultry manure is excellent manure, since it contains high nitrogen, phosphorus, potassium and other essential nutrients which promote growth of phytoplankton and zooplankton a natural food for fish. It adds organic matter to soil, which improves soil structures, nutrients retention, aeration, soil moisture holding capacity and water infiltration (Nhan *et al*., 2007). Furthermore, poultry manure readily supplies phosphorous to fish ponds than other organic manure sources (Abdul-Rahman *et al*., 2011).

Although IAA contributes on the economy of resource poor communities, its mismanagement may lead to deterioration of water quality that may result into diseases outbreaks (Thi and Dang, 2012). Diseases in aquaculture have been controlled by using antibiotics that had led to the development of antimicrobial resistance in some bacteria affecting fish disease management (Kolkovski *et al*., 2003). Shaikh *et al*. (2014) reported that antimicrobial resistance to be a growing problem in both human and animal health. Another study reported the presence of ESBL genes in *E.coli* isolates from farmed animals, author suggested that presence of ESBL genes was associated with mult use of antibiotics (Seni *et al.,* 2016a; Seni *et al.,* 2016b). Katakweba *et al.* (2015) carried a study in Tanzania revealed that spread of antimicrobial resistance contributed with interaction of farmed animals in grazing areas.

Antimicrobial resistance in bacteria has emerged as a threat in disease management in both human and veterinary medicine. Currently most important resistance genes has been

reported in Enterobacteriaceae, they reduces the efficacy even of the modified antibacterial with expanded-spectrum activity like cephalosporins (Chika *et al.,* 2016).

*Escherichia coli* is a Gram negative, rod-shaped bacteria belonging to Enterobacteriaceae found in soil, water, vegetable, and the intestinal tract of both humans and animals (moses *et al*., 2016). The use of animal manure in fish production may introduce this bacterium in fish production system. It is a common bacterium which usually indicates contamination of faecal origin pathogens of public health concern (Cheng *et al.,* 2016). Resistance to some antibiotics has been reported in *E. coli*. Specific genes have been identified to be responsible for antibacterial resistance development in *E. coli* such as tetracycline resistance; *tetA(A)*, tet(w) beta-lactam resistance; *bla*TEM, aminoglycoside resistance; *aadA2* and sulfamethoxazole-trimethobrim resistance; *sul1* and *sul ii* genes (Shimaa *et al*., 2016: Katakweba *et al.,* 2015). However, some *E. coli* lack any of these genes still were resistant to some antibacterial, meaning that utilizes other mechanisms to resist antibacterial activity. Some strains of *E. coli* are responsible for production of extended spectrum beta lactamase (ESBLs). Extended Spectrum Beta Lactamase genes have been reported to be responsible for development of resistance to antimicrobials used in treatment of fish diseases (Shaikh *et al.,* 2014). Study done on farmed tilapia reported occurrence of *E. coli* in fish with resistance to some antimicrobials (Rocha *et al.,* 2014).

## 1.2 Problem Statement and Justification

Integrated Agro-Aquaculture system increases farm productivity and reduce production costs as fish pond will be fertilized by animal manure and fertilized water used for vegetable/crop irrigation. However, using animal manure directly to the pond may increase the chances of disease outbreak to the system if not well managed and therefore results into poor production. Consistency use of antibiotics in animals integrated with

aquaculture may results into development of antimicrobial resistance in aquaculture (FAO, 2016). Study on prevalence of ESBL producing *E. coli* in aquaculture system with potential to cause multidrug resistance in fish and human has not yet been investigated in the study area. Findings from this study will provide baseline information on the prevalence of ESBL producing *E. coli* under integrated ago-aquaculture systems. Occurrence of ESBL producing *E. coli* in IAA systems indicate possible cross contamination between human, animals and fish making necessary to implement strict control in use of antimicrobials not only in fish production but also in human and livestock production systems.

## 1.3 Objectives

### 1.3.1 Overall objective

To determine the prevalence of ESBL and antimicrobial resistance in ESBL producing *E. coli* in integrated agro-aquaculture systems in Morogoro, Tanzania.

### 1.3.2 Specific objectives

i. To assess the effects of pond management practices in relation to distribution of ESBL genes isolated *E. coli* in IAA system.
ii. To determine the prevalence of ESBL producing *E. coli* in IAA system.
iii. To establish genetic diversity of ESBL genes isolated in *E. coli* strain in IAA system.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The Concept of Integrated Agro-aquaculture

Integrated agro-aquaculture is a linkage between subsystems in which one being aquaculture. The aim of integration is to enable effective utilization of resources (water and land). Fish farming can be integrated with poultry, livestock and crops (Prein and Ahmed, 2000: Prein, 2002). Integrated poultry-fish aquaculture utilizes chicken manure as pond fertilizers to enhance growth of plankton and other microorganisms eaten by the fish (Shoko *et al*., 2011).

The integration of organic sources and synthetic sources of nutrients not only supply essential nutrients but also have some positive interaction with chemical fertilizers to increase their efficiency and thereby reduce environmental hazards (Ahmed and Hassan, 2011). There is growing interest in the use of organic manures in integrated systems, due to depletion in the soil fertility (Kipkemboi, 2007). Poultry manure is excellent manure, since it contains high nitrogen, phosphorus, potassium and other essential nutrients. It adds organic matter to soil, which improves soil structures, nutrients retention, aeration, soil moisture holding capacity and water infiltration (Amira and Suloma, 2013). Furthermore, poultry manure readily supplies phosphorous to fish ponds than other organic manure sources (Abdul-Rahman *et al.*, 2011).

Integrated agro-aquaculture is the system that considered to be economical model of aquaculture, it may consist the link of fish, poultry and plants. Output from one system are used as input in another system and vice versa; Use of antibiotics in one system may spread to the entire system, for example use of antibiotics in livestock can be spread to fish

as well as subsequent discharge of antibiotics containing waste water. This may increase the bacterial resistance to antibiotics in the aquaculture systems and the surrounding environments (Zhang *et al.*, 2013).

## 2.2 *Escherichia coli* in Aquaculture Systems

*Escherichia coli* is a Gram negative bacteria that are normally found in the intestine of the human being, animals and fish as a normal flora. They act as probiotic in the intestine wall, however, when exceed the normal amount they may cause health problems such as diarrhea. *Escherichia coli* associated with the infections through the ingestion of edible products of marine and freshwater food origin contaminated with the bacterium. Occurrence of these bacteria is associated with fecal contamination and poor hygiene condition during processing and handling of food. Also, for fish and fishery products that are stored in the ice, poor quality of ice and food processing plant can impose contamination (Costa, 2013).

*Escherichia coli* are prokaryotic organism which is among the indicator organism for food and water contamination. The presence of *E. coli* in water or food indicates that there is particular water or food has a potential to cause harm in the health of animals and human being (Matthew *et al*., 2006). Many of confined livestock farms store wastes for several months prior to use as a fertilizer in aquaculture farms and crops. Storing the manure under confined environment can cause variation in the composition of enteric bacteria (Duriez and Topp, 2007).

There are six species in the genus Enterobacteriaceae, among others *E. coli* is of veterinary and medical significance. It causes variety of infections in many animal species. *E. coli* infection is characterized by one or more of the following: Diarrhea, Enteritis, Bacteremia

or Septicemia (Carter and Wise, 2004). In pathogenic mechanism and diseases, five major categories of *E. coli* are recognized: Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Enteroinvasive (EIEC), Enterohemorrhagic (EHEC) and Cytotoxin necrotizing factor-producing *E. coli* (Carter and Wise, 2004; Lupindu *et al.,* 2015).

Fish has been used as nutritional sources of high quality protein and supply minerals and vitamins in the diet (Costa, 2013). Fish have been threatened by bacteria diseases in the culture system that causes severe damage and high mortality leading to low yield and poor quality fish and fishery products. Most of the bacteria that affect fish in aquaculture system include enterobacteriaceae. Enterobacteriaceae in fish has been used as indicator of sewage pollution and they are opportunistic pathogens to fish (Elsherief *et al.,* 2014). These bacteria are widely distributed and mostly are found in feces of human, poultry and other animals. They are also found in contaminated water and can be present in the tissues of apparent normal fish (Rocha *et al.*, 2014).

Environmental stressors such as high temperature in the pond, high organic content such as over use of fertilizers, contribute to the occurrence of enterobacteriaceae infections in fish (Akinyemi and Ajagbe, 2013). Complex interaction between pathogen, fish and environment affects the susceptibility of the host leading to diseases development. Different pathogenic strains of *E. coli* have been identified including EPEC, ETEC and EHEC. Detection of ETEC in fish samples has been associated with food poisoning in human (Elsherief *et al.,* 2014).

High stocking density of fish in a pond has been noted to cause high chances of diseases occurrence in aquaculture. This is due to the deterioration of water quality and allows opportunistic bacteria to affect fish. Use of antibiotics in a pond to treat pathogenic

bacteria has created resistance to some strain of *E. coli*. Resistant genes carried by non-pathogenic *E. coli* may genetically be transmitted to pathogenic bacteria. By doing so resistance genes inhibit the antibiotics to cure fish diseases and therefore results in mass mortalities (Rocha *et al.,* 2014).

## 2.3 Anti-Microbial Resistance

Antibiotics are used in aquaculture to control diseases and promote growth to maximize production of the sector (Komar, 2008). The use of antibiotics in aquaculture industry has been increased the need for consideration for public health concern due to presence of antibiotic residuals that can impose pressure in bacteria population and antibiotic resistance. Tetracycline, Sulfonamides and beta lactam antibiotics are widely used in aquaculture due to their wide spectrum activity (Zhang *et al.,* 2013). Antibiotic resistance has been a global problem, it occurs due to the over use of antibiotics. More prevalent is observed in low and middle income counties that include South and South East Asia (Boonyasiri *et al*., 2014). Asia has been reported as the major producer and consumer of antimicrobial resistance agent (ESBL), high prevalent of 65% has been reported, Thailand 58.2-69.3%, and Viet Nam 51% (Nguyen *et al*., 2016). Use of antibiotics has now become threat as they can be harbored by food, animals, fish and plants and pass through food chain to human being. The use of antibiotics is a global challenge and preventing the use of these antibiotics need a strategy not only at nation level but also at global level (Shaikh *et al.,* 2014).

Use of antibiotics is associated with presence of resistance genes in animals and food. All food, animals and plants acts as reservoirs of antimicrobial resistance. Transmissions of these genes are both direct and indirect from one ecosystem to another (Founou *et al*., 2016). The widespread agricultural use of antimicrobial has long been considered a crucial

influence on the prevalence of resistant genes and bacterial strains. It has been suggested that antibiotic applications in agricultural settings are driving force for the development of antimicrobial resistance (Akinyemi and Ajagbe, 2013). Epidemiologic evidence supports the view that there is a direct link between resistant human pathogens, retail produce, farm animals, and farm environments. Several enzymes are known to ruin antibiotic activity by targeting and cleaving the bonds (Walk *et al.,* 2007).

## 2.4 Extended Spectrum Beta Lactamase

Extended Spectrum Beta Lactamase genes (ESBLs) are drug resistant that have ability to hydrolyze third generation cephalosporins which are commonly used to treat serious infections caused by members of the enterobacteriaceae family, these genes are likely acquired by *E. coli* (Nguyen *et al.,* 2016). Extended spectrum beta lactamases CTX-M type known to cause antibiotic resistance in Gram negative bacteria, have emerged at several locations in the World. These genes are the major threat to modern medicine (Hernandez *et al.,* 2013). Extended spectrum beta lactamase (ESBLs) mediate resistance to all penicillins, third generation cephalosporins (ceftazidime, cefotaxime and ceftriaxone) and aztreonam and in doing so they inactivate the function of antibiotic in the body (Shaikh *et al.,* 2014). Also, Morris *et al*. (2014) reported that ESBLs causes resistance to the expanded spectrum cephalosporins, penicillins by limiting the options available for treatment of serious bacterial infections. Infection with ESBL producing *E.coli* results in significant increases in morbidity, mortality and healthcare costs. The prevalence of antibiotic resistance among ESBL producing *E.coli* has increased markedly in recent years. These have been observed in number of patients due to infection of ESBL-producing *E. coli* (Lautenbach *et al.,* 2001; 2008). Extended-spectrum β-lactamase producing *E.coli* might spread from farm, animals to human through food chain (Börjesson *et al.*, 2016; Katakweba *et al.,* 2015). Studies in recent years documented the

prevalence and occurrence of ESBL producing *E. coli* in food products such as meat, poultry and raw milk (Elhadi and Alsamman, 2015). Extended spectrum beta lactamase have ability to hydrolyze third generation cephalosporins antibiotics that are used to treat diseases caused by enterobacteriaceae family. Among the enterobacteriaceae agent include *E. coli* that can be transmitted to human through food producing animals such as fish and fishery product (Nguyen *et al.,* 2016). Bacteria resistances to beta lactam among the gram negative bacteria are mostly frequently related to the production of β-lactames (Zhang *et al*., 2013).

Mechanism of β- lactam antibiotics is such that they all contain β-lactam ring in their structure that have similar features of chemistry, mechanism of action, pharmacological and clinical effects. Antibiotic that are used in treating animals work by inhibiting cell wall synthesis of bacteria and similarly the antimicrobial resistant genes in the bacteria they have similar mechanism of inhibiting the activity of antibiotics. All beta lactam bacteria have ability to produce beta lactamase enzymes, trapping mechanism, modification of target binding protein, impair penetration of drug to target binding protein and the presence of an efflux pump. These properties give them ability to resist activity of antibiotics to kill particular bacteria possessing these genes (Singh *et al.,* 2015).

### 2.5 Detection of Antimicrobial Resistance in the Laboratory

Antimicrobial drug resistance (AMR) arises if micro-organisms such as bacteria or viruses survive exposure to a drug that would normally kill them. Many studies has been done worldwide but in Tanzania, there is limited information on the issue of AMR (Seni *et al.,* 2016a; Seni *et al.,* 2016b). The determination of antimicrobial susceptibility of a clinical isolate, especially with increasing resistance, is of great potential for the optimal antimicrobial therapy of infected patients. They can be detected by using Nucleic acid-

based assays (Fluit *et al.*, 2001). In integration system there are different way where antimicrobial resistance can be controlled. Proper management of the farm is one of the proper way of controlling AR in the system.

Presence of antimicrobial in integrated aquaculture can originate from many sources, one been from chicken manure and feed residues from poultry that harbor antimicrobial resistance. Poultry have been exposed to drugs that are used to treated different diseases, and resulted into accumulation of drugs residues that can be transmitted to other agricultural systems then to human (Komba *et al.,* 2015). Control of AMR can be done through surveillance and monitoring of the environment and use of drugs in treating diseases. Also through doing research on new emerged resistant that can affect the effectiveness of antibiotics from treating diseases (Tacconelli *et al.,* 2017).

## 2.6 Water Quality Parameters and Pond Management Practices

Nutrient enrichment of the pond is essential management practice in aquaculture, however this can lead into deterioration of water quality. Desirable water parameter described as the condition of water at which fish survive and grow well, while undesirable water quality parameters are those parameter that cannot support fish growth; author narrated that desirable water temperature for cultured Nile tilapia in captivity ranged from 28$^{o}$C to 32$^{o}$C, dissolved oxygen ranged 4mg/l-6.5mg/l and pH of 6.5-8.5 respectively (Lin *et al.*, 2001). Water exchange is important in the pond because zero water exchange contribute in outbreak of disease and development of antimicrobial resistant as disease occurrence stimulate application of antibiotics that in turn the residues will cause antimicrobial resistance (Pruder, 2004). Poor water quality from rivers and streams can cause problem in raising fish, increased suspended solid in water affect water quality parameters that include temperature, dissolved oxygen and water pH. High level of nutrient can lead to

growth of algal bloom that can cause fish mortalities, also diseases in aquaculture encourage application of antibiotics that can lead to accumulation of antimicrobial residues favoring antimicrobial resistant and ESBL genes in fish pond (Omitoyin, 2005).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study Area

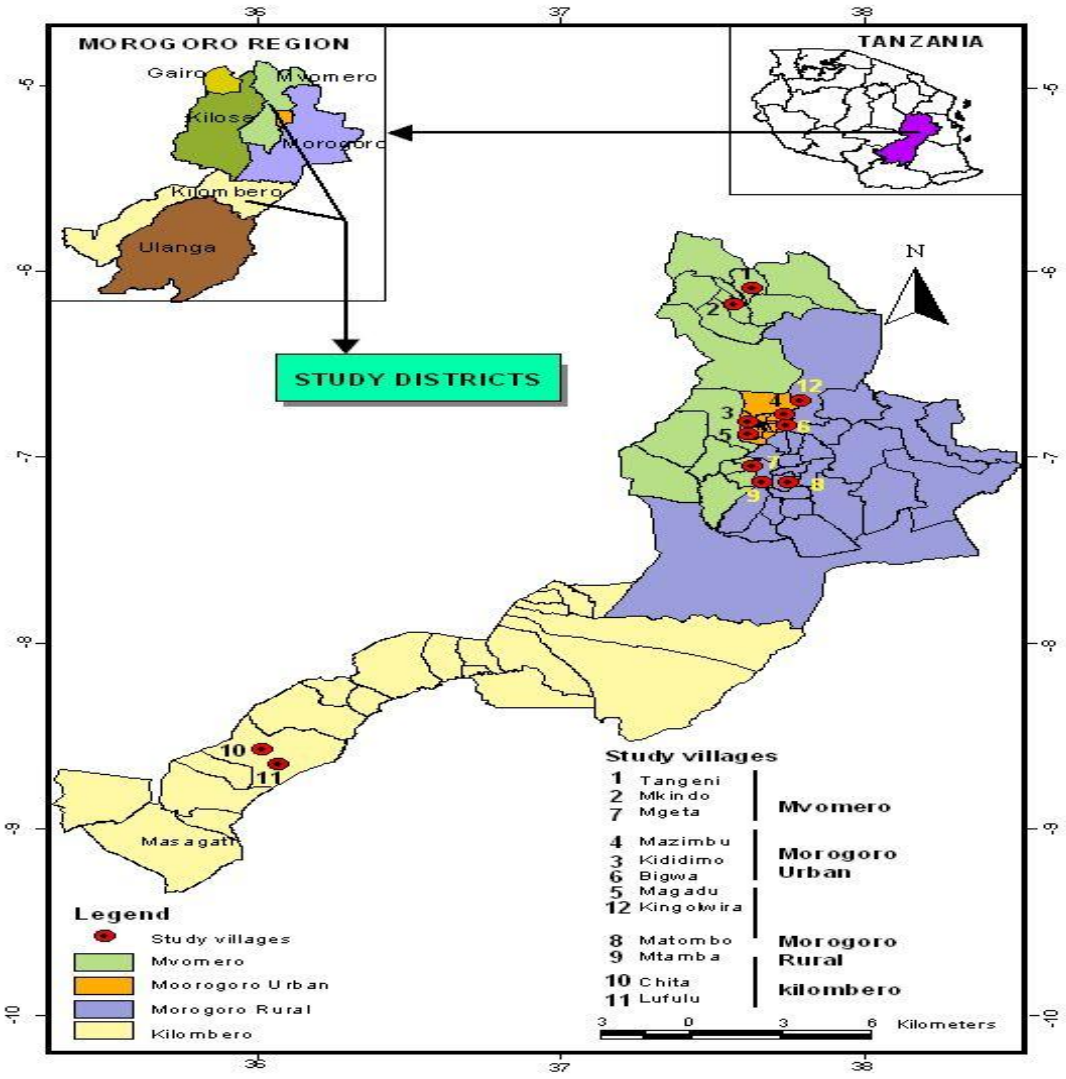


**Figure 1: Map showing sampling site**

**Source: Land management and remote sensing laboratory, at SUA**

This study was conducted in Morogoro region in which Kilombero, Mvomero, Morogoro rural and Morogoro urban districts were involved. It was conducted in areas where chicken and fish integration system is well established. Morogoro is located at 8° 00' 00" S, 37° 00' 00" E. The total population of 2,218,492 (NBS, 2012). The region is

characterized by a rainy season from November to May and a dry season from June to October (Balama *et al.,* 2016). The region receives an annual rainfall of between 1200mm and 1800 mm and the temperature ranges from 25 to 32°C (Magehema *et al*., 2014). The main economic activities at the site include agriculture, fishing, fish farming, agro-forestry and livestock keeping.

## 3.2 Sample Size Estimation and Sampling Techniques

### 3.2.1 Sample size estimation and study design

Sample size was estimated using the following formula;

$n=Z^2P\ (1-P)/D^2$: Whereas n= sample size (number of isolates), Z=1.96 (confidence interval), P= 0.5 (Prevalence), D= 0.05 (Precise) (Niang *et al.,* 2006). $n=1.96^20.5\ (1-0.5)/0.05^2$: n=384.

Fifty percent prevalence was selected because there is no published prevalence used for the same study in fish, available prevalence is for cattle and poultry.

Fish were collected from 26 ponds, two ponds from direct integration system and 24 ponds from indirect integrated system. From direct IAA 32 fish were collected where by 16 fish were taken from each pond, while 168 fish were collected from indirect IAA in which seven fish were taken from each pond. Therefore 200 fish were sampled and from each fish two swabs were taken, that makes a sample size of 400 isolates.

### 3.2.2 Setup of Integration Agro- Aquaculture (IAA)

Direct integration agro-aquaculture system is an integration system were pond and poultry or animal hut is linked together in such a way that animal waste enter in a pond without any transport, On the other hand indirect integrated agro-aquaculture refers to a system in which animal wastes (manure) collected from elsewhere fertilizes the pond. Figure 2A

demonstrates a direct integrated aquaculture system in which poultry hut built on top of the pond and remnants from the poultry hut drops direct to the pond that are used as source of food and fertilizer to fish and pond respectively. Effluents from the pond can be used to irrigate vegetables around the pond. This system enhance effective utilization of natural resources and increase production, also production cost is reduced as fish feed only on phytoplankton, nutrients from manure and remains of vegetables.

Indirect integrated agro- aquaculture system is an integration system were pond and poultry hut or animal hut is linked in such a way that wastes and manure from animal need to be transported from the animal hut to the pond, the two systems ( animal hut and pond) are not directly linked. This system has the same role as in system 2A however, manpower is employed to manage activity of taking manure from the animal hut to pond. Two photos were taken in the study site to describe the setup of the systems.


**Figure 2: (A) - Direct integrated agro-aquaculture system, (B) - Indirect integrated agro-aquaculture systems**

### 3.2.3 Field data collection and sampling technique

During farm visit, mixed questionnaires were used to interview fish farmers so as to gather information about farm management practices such as management of dissolved oxygen, water temperature, pH, feed storage, stocking density, feeding regime and feeding frequencies, manure application and preparation of pond before stocking. Water parameters such as dissolved oxygen, water temperature and pH were recorded before fish sampling, measurement of Ph, DO and temperature was done using D.O meter.

A total of 400 isolates were collected from farmed Nile tilapia (*Oreochromis niloticus*). The isolates were swabbed from fish gills and intestine that were taken from cultured Nile tilapia. Fish were dissected according to standard procedure to obtain a required isolate, isolates were collected direct at the field in Kilombero district because of lack of resources to transport live fish from the field to laboratory which was at Sokoine University of agriculture (SUA). Live fish samples from Morogoro municipal, Morogoro rural and Mvomero district were taken to SUA for laboratory analysis this was due to the fact that transport of live fish these areas were easy costless. Purposive sampling procedure was employed in collecting the fish from different districts since farmers were unevenly distributed within and between districts.

Gross examinations of the fish were done to detect if there were any abnormal features or appearance of fish before samples collection. Weight, total length and standard length of Nile tilapia were determined before taking swabs from the intestine and gills. Swabs were taken in the second gill, to avoid external contamination, as in the first gill was more exposed to external surface. In the intestine, swab was taken in the small intestine. Collected swabs were stored in the Stuart transport medium agar for microbiological procedures in the laboratory.

## 3.3 Laboratory Work

### 3.3. 1 Bacteria identification and confirmation

Swabs were first cultured into the MacConkey medium agar (Oxoid) and blood medium agar (Oxoid), and then incubated at 37$^{o}$ C for 24 hours under aerobic condition. Colonies with appearance suggestive to be *E. coli* were then sub cultured on the MacConkey medium agar and incubated at 44$^{o}$ C for 24 hours in aerobic condition. Colonies with the appearance suggestive to be *E. coli* were subjected to gram stain, examined and results were recorded accordingly. Positive results from gram stain reaction were then subjected to IMViC test for the confirmation of *E. coli*. Isolates that were positive to Indo reaction and Methyl red reaction and negative to pros and citrate reaction were confirmed as *E. coli* positive.

## 3.4 Extended Spectrum Beta Lactam Screening and Confirmation

Extended Spectrum Beta Lactamase producing *E. coli* were screened using MacConkey medium agar plate supplemented with 1 mg/L cefotaxime at 37$^{o}$C for 24hrs. Confirmed colonies of *E. coli* were streaked onto MacConkey medium agar plate supplemented with 1 mg/L cefotaxime and incubate at 37$^{o}$C for 24h. One to four (1-4) typical colonies for *E. coli* from MacConkey agar plates were selected and inoculated into selective brain heart infusion broth and incubate at 37$^{o}$C for overnight. Grown culture of *E. coli* in BHI (Oxoid) were transferred in 15% glycerol and stored at -80$^{o}$C for ESBL screening.

## 3.5 Antibiotics Sensitivity Test

The isolates that were resistant to Cefotaxime (CTX (30µg)) were taken for antimicrobial susceptibility test. Isolates were cultured into Muller Hilton agar and double disc diffusion method was used to test antimicrobial sensitivity. Seven antibiotics were used; Amoxicillin/clavulanic acid (AUG (30µg)), Cefotaxime (CTX), Sulphamethoxazole

/Trimethoprim (SXT (25µg)), ceftazidime (CAZ (30µg)), Ciprofloxacin (CIP (5µg)), Amoxicillin (AML (10µg)) and Ampicillin (AMP (10µg)). CTX and CAZ were selected because they belong to broad spectrum beta lactam third generation cephalosporins antibiotics used in treating infection caused by bacteria, on the other hand amoxicillin, ampicillin and ciprofloxacin are the most common antibiotics used in veterinary medicine and human medicine. Isolates that showed resistance to any of the third generation cephalosporin were taken for further confirmation of ESBL production. Confirmation of ESBL producing *E. coli* was done using the Double-Disc Synergy Test (DDST). Different generations of Cephalosporin were applied next to a disc with clavulanic acid (amoxicillin + clavulanic acid). The antibiotic disc was arranged at a distance of 20 mm apart with Amoxicillin/Clavulanic acid (AMC) disc that was put at the center of the agar plate. Positive results were to be indicated when the inhibition zones around any of the cephalosporin discs were augmented in the direction of the disc containing clavulanic acid (Pichichero and Zagursky, 2014).

## 3.6 Detection of ESBL Genes using Polymerase Chain Reaction

### 3.6.1 DNA extraction

Isolates from 15% glycerol were re-cultured in the nutrient medium agar (Oxoid) and incubated at 37$^{o}$C for 24 hours. Colonies from nutrient medium agar were then cultured on Muller Hilton medium agar (Oxoid) and incubated at 37$^{o}$C for 24 hours. Pure and single colonies of confirmed ESBL producing *E. coli* were taken and inoculated into Eppendorf tubes containing 300 µl of DNA water for DNA extraction. Boiling method was used in extraction of DNA, in which isolates were exposed to water bath-Julabo (SW23) at 100$^{o}$C for 15 minutes. After boiling samples were exposed to centrifugation process at 1500 rpm for 5 minutes to separate DNA materials and other residues, 50µl volume of supernatant was extracted but only 5µl of the total volume (supernatant) used for PCR work.

### 3.6.2 Primer sequence

Multiplex PCR test for beta lactam genes were carried out for the family SHV, TEM and CTX-M type of genes. All isolates that were confirmed as ESBL producers were exposed to PCR test. Primers were obtained from South Africa (Inqaba biotech) that were used for detection of SHV, TEM and CTX-M genes from the isolates. Primers sequences are shown in Table 1.

**Table 1: Sequence of oligonucleotide primers used for the detection of extended spectrum beta lactamase genes**

| Primers | ($^{o}$C) | Nucleotide sequence(5'-3') | Amplicon size(bp) |
|---|---|---|---|
| SHV-F | 60 | CGCCTGTGTATTATCTCCCT | |
| SHV-R | 62 | CGAGTAGTGTCCACCAGATCCT | 293 |
| TEM-F | 60 | TTTCGTGTCGCCCTTATTCC | 403 |
| TEM-R | 62 | ATCGTTGTCAGAAGTAAGTTGG | |
| CTX-M-F | 60 | CGCTGTTGTTAGGAAGTGTG | 569 |
| CTX-M-R | 62 | GGCTGGGTGAAGTAAGTG | |

F=forward primer, R=reverse primer, bp=base pair (Mohammed *et al*., 2016)

### 3.6.3 DNA amplification

DNA amplification was carried out in the PCR machine, Takara PCR Thermal cycler Dice (Gradient PCR), and the amplification was carried out under the following conditions for the mentioned genes: Initial denaturation 94$^{o}$C for 3minutes, denaturation 94$^{o}$C for 45 seconds of 35 cycles, annealing at 60$^{o}$C for 30 seconds of 35 cycles, extension at 72$^{o}$C for 3 minutes of 35 cycles and final extension at 72$^{o}$C for 2 minutes. PCR fragments were analyzed at 1.5% agarose gel at the voltage of 110V for 60 minutes using Electrophoresis tank. Observation of Amplicon bands were done in trans-illuminator device (Mohammed *et al.,* 2016).

### 3.7 Data Analysis

Mixed structured questionnaire were used to gather the information on pond management practices. Collected data were subjected to Microsoft excel spread sheet version 2013 to compute prevalence. Frequencies tables were computed for descriptive data. Bio data of fish (mean and standard deviation) were compared using student *t* test, statistical differences was established at 95% confidence interval. Chi- square ($\chi^2$) test were used to test association of pond management practices gathered using questionnaire and occurrence of ESBL genes.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Pond Management Practices and Bio-data

#### 4.1.1 Water quality parameters

Means and ranges of water quality parameters are given in Table 2.the table show minimum and maximum recorded temperature, Dissolved oxygen and pH. All the three water quality parameters were within the recommended levels that support normal growth of Nile tilapia (*Oreochromis niloticus*).

**Table 2: Mean and range of water quality parameters from ponds in which fish were sampled**

| Water parameter | Mean±SD | Range |
|---|---|---|
| pH | 8.24±0.97 | 6.48-9.96 |
| DO (mg/l) | 6.84±2.32 | 2.08-9.77 |
| Temperature ($^{o}$C) | 28.09±3.39 | 21.7-36.68 |

Note: n=26 which is the number ponds

### 4.2 Water Parameters and Occurrence of ESBL

Water temperature, pH and dissolved oxygen were categorized in two groups, desirable and undesirable parameters for fish survivals in a pond. Desirable range for dissolved oxygen was considered from 4mg/l to 6.5mg/l and undesirable dissolved oxygen was below and above the mentioned range. Desirable pH was considered between 7.0 and 8.0 while undesirable pH were considered being below or above the desirable pH. Desirable water temperature ranges from 27$^{o}$C to 30$^{o}$C while undesirable water temperature ranges above and below the desirable range (Bhatnagar and Devi, 2013). The information on

desirable and undesirable water parameters in association of the ESBL genes occurrence are given in Table 3.

**Table 3: Association of water parameters and occurrence of ESBL genes**

| Water parameter | Status | Percentage of ponds (n =26) | PCR positive (n =174) | Percentage of positive PCR isolates |
|---|---|---|---|---|
| pH | Desirable | 78.3 | 146 | 83.9 |
| | Undesirable | 21.7 | 28 | 16.1 |
| Dissolved oxygen | Desirable | 87 | 157 | 90.2 |
| | Undesirable | 13 | 17 | 9.8 |
| Water temperature | Desirable | 87 | 162 | 93.1 |
| | Undesirable | 13 | 12 | 6.9 |

### 4.3 Pond Management Practices

Among the farm management practices that were observed during sampling include adequate supply of quality water, monitoring of water parameters (dissolved oxygen, water temperature and pH), management of effluent, availability of good quality and quantity feed, proper feeding, feeding frequency and feeding regime, appropriate stocking density and pond preparation. According to the responded Most of the ponds visited use water from stream and rivers (84.6%), while only 15.4% uses underground water (wells). Quantifying pond management practices were not well followed. Farmers had no or less knowledge on proper pond management practices.

Water change during and after harvesting were not considered due to the scarcity of water for aquaculture activities. Only 34.6% of responded changed water in the ponds, while 65.4% were not doing this activity. Use of antibiotics to treat fish was not a common practice, only 15.4% used antibiotics to treat fish when disease outbreak occurs. Flooding was not common, 7.7% of the area experiences flooding. Feeds and feeding were also not managed well, 19.2% of the responded provided the right feeds and feeding practices

while 80.8% were not. Pond management practices were linked with ESBL results, in addition to that findings showed high statistical significant difference to all variables (P<0.001) except for ponds that were cleaned before stocking (P>0.001) as summarized in table 4.

**Table 4: Association of different management practices and occurrence of ESBL genes**

| Variable | Management option | %of pond | PCR Positive | P-Value |
|---|---|---|---|---|
| Pond location | Resident areas | 73.1 | 140 | 0.0001 |
| | other areas | 26.9 | 34 | |
| Water source | Streams/rivers | 84.6 | 156 | 0.0001 |
| | wells | 15.4 | 18 | |
| Water change | normal change | 34.6 | 33 | 0.0023 |
| | no change | 65.4 | 141 | |
| Antibiotic | used | 15.4 | 38 | 0.0001 |
| | not used | 84.6 | 136 | |
| Flooding | occurred | 7.7 | 13 | 0.0001 |
| | not occurred | 92.3 | 161 | |
| Cleanness before stocking | Yes | 46.2 | 72 | 0.4045 |
| | No | 53.8 | 102 | |
| Appropriate feeding | Yes | 19.2 | 40 | 0.0001 |
| | No | 80.8 | 134 | |
| Application of fertilizer | Yes | 30.8 | 90 | 0.0001 |
| | No | 69.2 | 84 | |
| Appropriate feed storage | Yes | 19.2 | 41 | 0.0001 |
| | No | 80.8 | 133 | |
| Diseases outbreak | Yes | 23.1 | 45 | 0.0001 |
| | No | 76.9 | 129 | |

### 4.3.1 Bio-data of fish size and condition factors

Measurements of fish conducted in this study were total length and body weight. Condition factors of the fish were calculated after getting the b-value (slope) from the regression analysis between length and body weight. Condition factor is normally used to indicate the well-being of the fish. For both systems, the condition factors were greater

than one, however in the direct IAA condition factor were higher than indirect IAA (Table 5). Weight and length of the fish samples from direct integrated and, weight and length of fish samples collected from indirect integrated systems including their ranges are summarized in table 5 below. Statistical analysis showed high significant difference body weight, total length and condition factor between direct integrated system and indirect integrated system at 5% significance level.

**Table 5: Summary of bio data and their implication on growth status of the fish**

| **Parameter** | **Direct IAA (n=32)** | **Indirect IAA (n=168)** | **P –Value (α =0.05)** |
|---|---|---|---|
| | Mean±SD | Mean±SD | |
| Total length (cm) | 9.52±0.47 | 16.44±0.26 | 0.0001 |
| Body weight (g) | 24.96±4.05 | 75.21±3.26 | 0.0001 |
| Condition factor | 2.19±0.05 | 1.58±0.03 | 0.0001 |

### 4.4 Isolation of *Escherichia Coli* and Characterization

A total of 384 isolates were processed for identification and characterization of *E. coli* species, missing samples were died during laboratory work. Two hundred and twelve (55%) isolates which were pink, medium colonies on MacConkey medium agar and medium grey beta haemolitic colonies on blood agar were selected for biochemical test. None lactose fermenter bacteria on Mac Conkey agar were not included in the study. Also, samples that shown smooth circular, purple colonies on brilliance *Escherichia coli* agar were included in the study. Gram stain reaction demonstrated Gram negative, rods shaped in single. In Indo reaction and methyl red reaction, isolates appears positive while in vogues proskaur test and Simon's citrate test isolates appears negative, also isolates were tested catalase positive. From the above characteristics 212 samples were confirmed *Escherichia coli*. Tables 6 and 7 summarize the characteristics and biochemical test for *Escherichia coli.*

**Table 6: Culture characteristics of *E. coli* in different media**

| Media Used | Culture Character |
|---|---|
| MacConkey agar | Medium smooth, circular colonies with pink colour |
| Blood agar | Hemolytic, smooth, circular grey colonies |
| Brilliance *E. coli* agar | Small smooth, circular purple colonies |
| Muller Hilton agar | Colorless, smooth circular colonies in the entire edge |

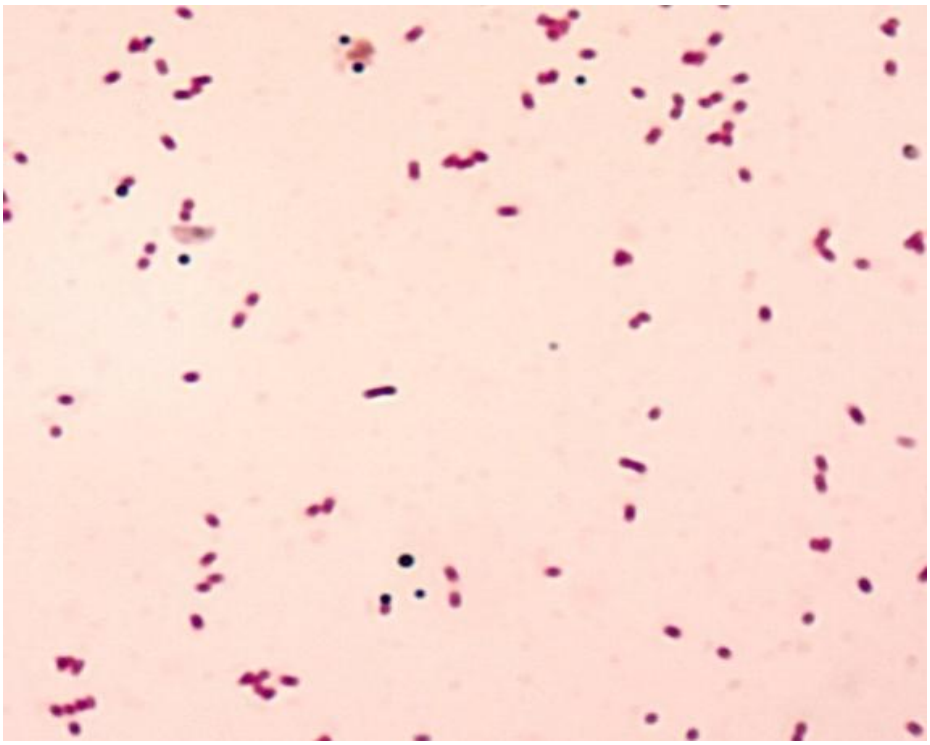

**Figure 3: Gram stain reaction of *E. coli* isolates**

**Figure 4: IMViC test of *E.coli* isolates**

**Table 7: Morphological and biochemical characteristics of *E.coli* isolates**

| Characteristics | Results |
|---|---|
| Gram stain | Red rods in single (Gram negative) |
| Cell morphology | rods in single |
| Lactose fermentation | + |
| Growth at 44$^{o}$C | + |
| Catalase test | + |
| Purple colour in brilliance *E coli* agar | + |
| Indole reaction | + |
| Methyl red test | + |
| Vogues proskaur test | - |
| Simon's citrate test | - |

Key: +: positive results; -: negative results

### 4.6 Antimicrobial Susceptibility of *Escherichia coli*

All 212 *E.coli* isolates were sensitive to ciprofloxacin while all 212 *E.coli* isolates were resistant to AMP (10µg), SXT (25µg) and AML (10µg) for both systems. For indirect integrated system, amoxicillin/clavulanic acid 63, isolates (36.6%) were resistant, 69 isolates (40.1%) were intermediate resistant and 40 samples (23.3%) were sensitive. In

direct integrated system there were only 40 isolates that were confirmed positive *E. coli* and out of that 16 (40%) were resistant to Amoxicillin/clavulanic acid, four samples (10%) were intermediate resistant and 20 samples (50%) were sensitive to amoxicillin/clavulanic acid.

Sensitivity of Cefotaxime antibiotic in indirect integrated system, 97 isolates (56.4%) were resistant, 57 samples (33.1%) were intermediate resistant and 18 samples (10.5%) were sensitive. While in direct integrated system 28 samples (70%) were resistant, eight samples (20%) were intermediate and 4 samples (10%) were sensitive. Ceftazidime drugs in indirect integrated system demonstrated resistance and complete resistance in 62 samples (36%), 52 samples (30.2%) intermediate resistant and 58 samples (33.7%) were sensitive. In in direct integrated system 12 samples (30%) were resistant, 28 samples (70%) demonstrate intermediate resistance, and there were no sample that was sensitive to this antibiotic.

There was no statistical significant difference ($P>0.05$) observed in AUG resistant, CTX resistant, CTX intermediate and CTX sensitive, SXT sensitive and resistant while in CAZ only resistant group shown no statistical significance different. On the other hand highly statistical significant difference ($P<0.05$) was observed between CIP, AMP and other group of sensitivity (Table 8).

**Table 8: Sensitivity of the isolates for different antibiotics**

| Antibiotics | Sensitivity | Indirect IAA (n) | % | Direct IAA (n) | % | P- Value |
|---|---|---|---|---|---|---|
| AUG | R | 63 | 36.6 | 16 | 40 | 0.690 |
| | I | 69 | 40.1 | 4 | 10 | 0.001 |
| | S | 40 | 23.3 | 20 | 50 | 0.001 |
| CTX | R | 97 | 56.4 | 28 | 70 | 0.115 |
| | I | 57 | 33.1 | 8 | 20 | 0.104 |
| | S | 18 | 10.5 | 4 | 10 | 0.930 |
| AML | R | 172 | 100.0 | 40 | 100 | 0.001 |
| | I | 0 | 0.0 | 0 | 0 | 0.001 |
| | S | 0 | 0.0 | 0 | 0 | 0.001 |
| CAZ | R | 62 | 36.0 | 12 | 30 | 0.469 |
| | I | 52 | 30.2 | 28 | 70 | 0.001 |
| | S | 58 | 33.7 | 0 | 0 | 0.001 |
| SXT | R | 161 | 93.6 | 40 | 100 | 0.10 |
| | I | 0 | 0.0 | 0 | 0 | 0.001 |
| | S | 11 | 6.4 | 0 | 0 | 0.100 |
| AMP | R | 172 | 100.0 | 40 | 100 | 0.001 |
| | I | 0 | 0.0 | 0 | 0 | 0.001 |
| | S | 0 | 0.0 | 0 | 0 | 0.001 |
| CIP | R | 0 | 0.0 | 0 | 0 | 0.001 |
| | I | 0 | 0.0 | 0 | 0 | 0.001 |
| | S | 172 | 100.0 | 40 | 100 | 0.001 |

Note: AUG=Amoxicillin/Clavulanic acid (30µg), CTX=Cefotaxime (30µg), AML=Amoxicillin (10µg), CAZ=Ceftazidime (30µg), SXT=Sulphamethoxazole/Trimethoprim (25µg), AMP=Ampicillin (10µg) and CIP=Ciprofloxacin (5µg)

**Figure 5: Arrangement of antibiotic discs on the culture plate for sensitivity test**

### 4.7 Polymerase Chain Reaction Results

Overall results showed that in direct integrated system; CTX genes were represented in 15 isolates (8.9%), TEM genes represented in 46 isolates (27.4%), SHV genes represented in 19 isolates (11.3%). *Escherichia coli* isolates that harboring both SHV and TEM were 52 isolates (31%), isolates that harboring both TEM and CTX were six (3.6%) while only 30 isolates were negative to all primers (Table 9).

Results from direct integrated system that have poultry and other agricultural activities included the following; twenty *E. coli* isolates (50%) were represented by TEM genes, 13 isolates (32%) were represented by SHV genes and only one isolate (2.5%) was represented by CTX gene. Isolates that expressed in both SHV and TEM were two (5%) while four isolates were negative to all genes. There were no statistical difference ($P>0.05$) between the two integration agro-aquaculture systems. Occurrence of ESBL genes in

direct IAA and indirect IAA had no significant difference. Therefore, having direct or indirect system has no influence on the occurrence of ESBL genes.

Table 10, demonstrated amplification of more than one gene in one *E. coli* isolates. In indirect integrated system 52 isolates were harboring both SHV and TEM genes and six isolates harboring CTX and TEM, while in direct integrated system only five isolates harbored TEM and SHV only.

**Table 9: Table showing percentages of samples amplified for the gene encoding ESBLs for SHV, TEM and CTX-M genes**

| **Primers** | **Direct IAA (n=42)** | **%** | **Indirect IAA (n =227 )** | **%** | **P- Value** |
|---|---|---|---|---|---|
| TEM | 22 | 52.4 | 104 | 45.8 | 0.4334 |
| SHV | 15 | 35.7 | 71 | 31.3 | 0.5718 |
| CTX | 1 | 2.4 | 22 | 9.7 | 0.1195 |
| Negative | 4 | 9.5 | 30 | 13.2 | 0.5091 |
| **Total** | **42** | **100** | **227** | **100.0** | |

Note; n= number of *E. coli* isolates

Table 10 shows statistical significance difference observed in CTX, TEM+CTX. These happen due to the fact that CTX genes were less in both system that bring insignificances.

**Table 10: Percentage of the sample showing interaction of the amplification of genes encoding SHV, TEM and CTX-M genes**

| **Primers** | **Direct IAA (n)** | **%** | **Indirect IAA (n)** | **%** | **P - Value** |
|---|---|---|---|---|---|
| TEM | 20 | 50 | 46 | 27.4 | 0.006 |
| SHV | 13 | 32.5 | 19 | 11.3 | 0.001 |
| CTX | 1 | 2.5 | 15 | 8.9 | 0.17 |
| SHV+TEM | 2 | 5 | 52 | 31.0 | 0.001 |
| TEM+CTX | 0 | 0 | 6 | 3.6 | 0.225 |
| Negative | 4 | 10 | 30 | 17.9 | 0.227 |
| **Total** | **40** | **100** | **168** | **100.0** | |

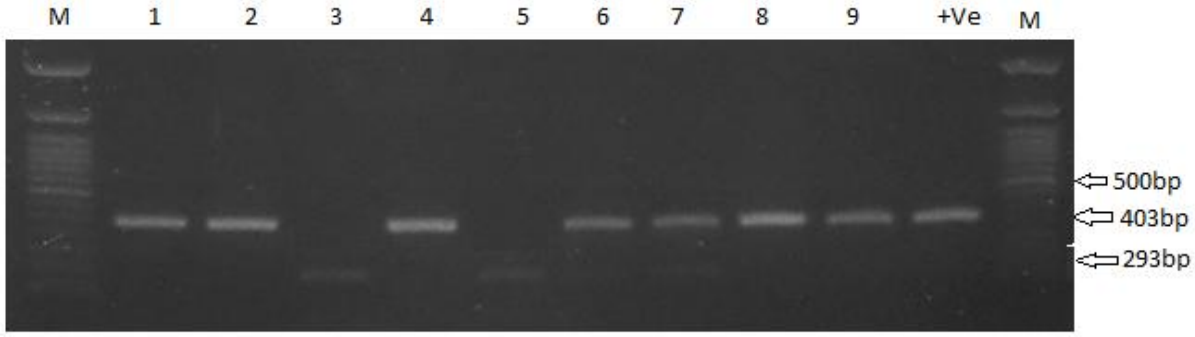


**Figure 6: Photograph of PCR products for TEM genes among the ESBL positive isolates from farmed Nile tilapia**

Note: SHV=bp 293: TEM= bp 403 and CTX= bp 569; M=ladder, +ve = positive control, 1- 9 are isolates loaded for amplification.

# CHAPTER FIVE

## 5.0 DISCUSSION

The aim of this study was to determine the prevalence of Extended Spectrum Beta Lactamase producing *E. coli* in integrated aquaculture system. Findings showed that ESBL genes are prevalent in the mentioned study site and pond management practices are not well captured by farmers. Furthermore, sensitivity testing showed ciprofloxacin being sensitive to all *E. coli* isolates in both direct IAA and indirect IAA, while Amoxicillin, Ampicillin and Sulfamethoxazole shown to be resistant to all *E.coli* isolates from both systems.

Water quality is the most limiting factor in pond fish production, the most difficult parameter to understand, predict and manage. Deterioration of water quality in aquaculture affects feeding efficient, growth rate and survival of fish and sometimes leads to outbreak of diseases (Bhatnagar and Devi, 2013). Water quality parameters can be affected by feeding frequencies, amount of feed, type of feeds and feeding regime in the pond. When pond management practices such as feeding frequencies, feeding regime, fertilization, stocking density, management of both influent and effluent entering in the pond are not well understood by farmers (farm manger) may contribute into poor performance of fish and even an outbreak of diseases (Sanders, 2009).

Results from interview with fish farmers regarding pond management practices revealed that there is improper management of feeding practices, preparation of ponds and poor management of water entering into the pond. It has been reported that improper management of feeds, feeding practices as well as pond preparation during and after harvesting of fish may lead to poor growth and yield of the fish (Bhatnagar and Devi,

2013). Poor management of water quality parameters, control of manure in a pond, and preparation of pond before stocking may contribute to high prevalence of enteric bacteria and susceptibility of different antibiotics to these bacteria. High organic content in the pond, decreased dissolved oxygen and stocking density can affect life of fish in the pond. Also, presence of high organic matter and low dissolved oxygen give room for pathogenic and nonpathogenic bacteria to proliferate in the pond. *Escherichia coli* is exclusive fecal bacteria and therefore using manure in the pods results into high concentration of *E. coli* that harbor extended spectrum beta lactamase genes that affect drugs efficiency in treating diseases. When pond management practices are poor high prevalence of ESBL genes in the pond is possible (Soliman *et al.,* 2010). Water temperature on growth of Nile tilapia have many implications on tilapia culture, temperature affect feeding response, reproduction rates, age and growth rate of fish (Nguyen *et al.,* 2014). Change in water parameters and environment conditions of the pond tends to stress fish health and therefore opportunistic pathogens may proliferate and affect the production of fish. El-Sherif and El-Feky (2009) reported that high pH contributed to low growth performance of fish and increases the chances of diseases in a pond. Also unstable pH cause proliferation of opportunistic and pathogenic bacteria in a pond. Limbu *et al*. (2016) reported, water temperature, dissolved oxygen and pH ranging from $25^{o}$C-$30^{o}$C, 4.0-8.0mg/l and 6.5-9.5 respectively in aquaculture integrated systems. This favor optimal growth of fish under captivity, when water quality parameters are optimal the yields in the pond increases and create profit to farmers.

Findings from this study showed that probably high prevalence of ESBL was highly contributed by poor pond management practices such as poor control of water entering into the pond, improper use of organic fertilizers, and poor management of practices. When poor water quality is used for fish farming it can lead to proliferation of

enterobacteriaceae that may result to outbreak of diseases. Also, applying fertilizers without following the amount of fertilizer recommended lead to deterioration of water quality that may harm the fish. Furthermore, poor management of feeding practices can result into under feeding or over feeding fish, also formulation of feed with improper inclusion for fish health. It seems that fish farmers apply manure without even knowing the amount required based on the pond size. Sometimes fish farmers use kitchen leftover as supplementary which may contain pathogenic bacteria into a pond that may introduce pathogenic bacteria into the pond and; these bacteria are opportunistic, when water parameters deteriorate they attack fish and cause diseases.

The majority of fish farmers use water from streams and rivers for their aquaculture activities. Some of these streams and rivers are passing through the rice paddles where they can wash out pesticide and other antibiotic residues that were used to control weed and pests in the farm, these residues may get into the pond and increases the chance of resistance in the pond. There is high possibility of collecting all the possible environmental pollutants and introduces them into the pond, that may cause deterioration of water quality and affect fish growth and survive of fish. Water change in fish ponds may help to reduce *E. coli* contamination. In this study water change was rarely practiced by fish farmers therefore increases chance for proliferation of bacteria that can harbor ESBL genes. In the current study water parameter in some pond showed poor water quality that associated with high prevalence of ESBL genes. In some ponds dissolved oxygen concentrations were below recommended levels that were caused by water scarcity and excessive fertilization.

Antibiotic residues from poultry manure may cause *E. coli* to develop resistance thus when fertilized in the pond, it can be transferred to fish. Integrating fish and poultry probably may contribute highly to the presence of resistance genes in fish, through pond fertilization. It has been reported that causes of bacteria to be resistant to some antibiotics is probably due to multi drug application during treatment of fish or/and poultry diseases (Shaikh *et al.,* 2014). Results from present study showed that all isolates were resistant to ampicillin, amoxicillin and sulphamethoxazole while ciprofloxacin being the most sensitive antibiotic to all *E.coli* isolates. Findings obtained from the present study concur with the findings by Kajeguka *et al.* (2015). List of antibiotics used in the present study and its resistivity is in agreement with the study conducted by Pavlickova *et al*. (2016), in which *E. coli* isolates were tested against several antibiotic one being sulfamethoxazole, ciprofloxacin and ampicillin.

Currently there is high resistance of *E. coli* to antibiotics such as tetracycline, sulfamethoxazole/trimethoprim and ampicillin. High resistant in integrated aquaculture has been suggested to be caused by high use of antibiotics as well as interaction of different systems (Zhang *et al.,* 2013). Also, use of waste water has been considered to cause presence of resistant genes in aquaculture (Mhongole *et al.,* 2016). Katakweba *et al.* (2015) did a study on prevalence of antibiotic resistance in health adults, foods, food animals and the environment and reported a high resistance in ampicillin, tetracycline and cephalothin antibiotics that were used to test the sensitivity to *E. coli.* Hernandez *et al.* (2013) tested sensitivity in *Escherichia coli* using tetracycline, Trimethoprim/ Sulfamethoxazole and ampicillin that are used in veterinary and human medicine.

Different studies has reported presence of ESBL genes in poultry, fish and fishery products (Lupindu *et al.,* 2015). Founou *et al*. (2016) conducted on antibiotic resistance in food chain reported prevalence of ESBL genes producing *Escherichia coli* in broiler (36%) and broiler meat (39%). Extended spectrum beta lactamase has been reported to be present in food, chicken, pork fresh meat, fish and remnants from slaughter areas (Nguyen *et al.,* 2016). High prevalence of ESBL genes has been reported in chicken meat (92%), beef (34.3%) and fish (29.3%) (Boonyasiri *et al.,* 2014). Nguyen *et al*. (2016) reported the following ESBL genes reported as CTX-M-9 (31.2%), CTX-M-1(29.8%) and CIT (34.5%) in integrated aquaculture system. Boonyasiri *et al*. (2014) reported high prevalence of ESBL genes producing *Escherichia coli* of 75.5% and 77.3% from food factory workers and health animal, and 40% from broiler chicken respectively.

Study on extended spectrum beta lactamase on chicken meat and human by Overdevest *et al*. (2011) found that all isolates of *E. coli* were ESBL positive and isolates were tested against different antibiotics including ampicillin, cefotaxime, ceftazidime, Cefpodoxime, Cefoxitin, amoxicillin-clavulanic, ciprofloxacin, gentamicin, streptomycin, chloramphenicol, tetracycline and trimethoprim; these authors demonstrated that high prevalence of resistance genes among the isolates of *E. coli* were associated with high use of antibiotics to treat chicken. Resistant genes found in chicken are similar to those found in human and therefore transfer of resistant genes from chicken, fish to human being is probably through food chain (Morris *et al*., 2014).

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

Generally, pond management practices such as proper use of fertilizers (organic fertilizers), pond preparation before stocking, feeding practices, were poorly managed in both systems. Lack of proper pond management practices were thought to be the major cause ESBL genes as there were no monitoring of water entering into the ponds and poultry manure used for fertilization of pond. On the other hand water quality parameters such pH, temperature and DO were in a range that support growth of Nile tilapia in captivity.

Sensitivity testing showed three antibiotics (Amoxicillin, sulphamethoxazole and Ampicillin) being 100% resistant to confirmed isolates of *E.coli*, and only ciprofloxacin been sensitive to all isolates. For direct IAA the prevalence of extended spectrum beta lactamase producing *Escherichia coli* were 2.4%, 35.7% and 52.4% for CTX, SHV and TEM gene, and indirect IAA the prevalence were 9.7%, 31.3% and 45.8% for CTX, SHV and TEM respectively.

### 6.2 Recommendations

In aquaculture farming, pond management practices should be improved, farmers should be provided with enough knowledge on how to manage integrated aquaculture farming. Also, water quality parameters such pH, temperature and DO are key to high growth performance and survival of fish, therefore they must be maintain through the farming season.

Use of antibiotics should be avoided where possible as antibiotics has shown to contribute in antimicrobial resistance. Use of poultry waste should be managed to minimize the spread of antimicrobial residues to fish and water, this can be done by minimizing the use of antibiotics in poultry. Also water source for fish farming should be screen to check and minimize any contamination that could lead to deterioration of water parameters and proliferation of enterobacteriaceae bacteria as well as reducing the chance of accumulation of antimicrobial residuals from the environment to the pond.

Extended spectrum beta lactamase producing Escherichia coli have projected to be prevalent in the system, however, sequencing of the isolates should be done so that it can indicate clearly specific strain of e. coli that harbor ESBL genes.

Further study should be conducted on aquaculture to get enough knowledge on how to reduce or eliminate antimicrobial resistance in aquaculture system and environment in general.

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