HEALTH RISKS ASSOCIATED WITH URBAN FARMING: *CRYPTOSPORIDIUM* AND NON-SORBITOL FERMENTING *ESCHERICHIA COLI* AS INDICATOR ORGANISMS IN DAR ES SALAAM, TANZANIA

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ABSTRACT

Cryptosporidium and enterohaemorrhagic (EHEC) *Escherichia coli* O157:H7 are two important pathogens in humans among other common representative zoonotic pathogens carried by animals especially cattle and are discharged through their faeces into the environment. With the increasing practice of urban farming, the risk of transmission of these pathogens to humans is increased. The study aimed at determining the public health risks associated with integrated urban farming using Non-Sorbitol Fermenting (NSF) *E. coli* and *Cryptosporidium* as indicator organisms in Dar es Salaam, Tanzania. A questionnaire survey was used to collect data on knowledge and practices associated with urban farming. Livestock manure, leafy vegetables and fish samples were collected to isolate and identify NSF *E. coli* and detect *Cryptosporidium* oocysts. Confirmed isolates on biochemical tests were subjected to antimicrobial resistance testing and genetic similarities of the isolates were determined using Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR). A total of 156 samples including 63 cattle faeces, 26 poultry faeces, 53 vegetables and 16 fish samples were tested for presence of *Cryptosporidium* and NSF *E. coli*. Out of 156 samples, 36 (23.1%) yielded NSF *E. coli* and 16 (10.3%) had *Cryptosporidium* oocysts. Five samples (3.2%) had both *Cryptosporidium* oocysts and NSF *E. coli* including four from vegetables and one from fish. Out of the 48 isolates of NSF *E. coli* tested for antimicrobial resistance to six antimicrobial agents, 25 (52.1%) were resistant to at least one antimicrobial agent and of these, 12 (48.0%) showed Multidrug Resistance. ERIC-PCR profiles of the 47 isolates from different sources showed genetic similarities (74.5% - 100%) with nine major clusters identified (I - IX) determined at 90% threshold level of similarity. These results showed potential health risks that would emanate from urban integrated farming and hence the need to monitor and improve husbandry practices used in urban farming.

DECLARATION

I, **MWANZA FRANCIS**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor concurrently being submitted in any other institution.

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DEDICATION

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
CLSI	Clinical and Laboratory Standards Institute
DAEC	Diffusely Adherent *Escherichia coli*
DNA	Deoxyribonucleic Acid
EAEC	Enteroaggregtive *Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ERIC	Enterobacterial Repetitive Intergenic Consensus
ESBL	Extended-Spectrum β-Lactamase
ETEC	Enterotoxigenic *Escherichia coli*
HIV	Human Immunodeficiency Virus
HUS	Haemolytic Uremic Syndrome
IFAD	International Fund for Agricultural Development
IPM	Integrated Pest Management
NSF	Non Sorbitol Fermenter
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
SMAC	Sorbitol MacConkey
STEC	Shiga Toxin *Escherichia coli*
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Cryptosporidium and enterohaemorrhagic (EHEC) *Escherichia coli* O157:H7 are two important pathogens in humans among other common representative zoonotic pathogens carried by animals especially cattle and are discharged through their faeces into the environment (WHO, 2012). With the increasing practice of urban farming, the risk of transmission of these pathogens to humans is increased. The risk is even heightened with the husbandry practices among urban farmers that include the use of livestock or human faecal wastes in form of liquid or solid manure in vegetable or fish farming (Boischio *et al*., 2006b). Increasing poverty levels in developing nations including Tanzania is due to factors such as food insecurity, high unemployment levels and increased demand of land acquisition especially in urban areas (Mlozi, 1997). This is due to rapid population growth and urbanisation in cities of developing countries including Dar es Salaam hence survival livelihood strategies such as urban farming are usually adopted for economic sustainability (IFAD, 2007). According to the United Nations Population Fund, 60% of the world's population will live in the cities by the year 2030 (Zeeuw and Dubbeling, 2009).

Livestock including cattle and poultry contribute about 85% of animal faecal waste to the environment worldwide, a contribution less than that of human population (WHO, 2012). Since the practice of urban farming involves livestock keeping which are kept in close proximity to the residential areas due to limited land space, increased livestock manure deposition in urban areas can occur. However in most African largest cities only 20% to 50% of all solid waste produced in the urban areas are actually collected (Zeeuw and Dubbeling, 2009). Therefore, the increased practice of urban farming would eventually

result into increased rate of accumulation of livestock manure than it is collected thereby posing a health risk to the humans (Boischio *et al.*, 2006b). The livestock manure can also be used by farmers as fertilizer for crops including vegetables as well as in fish farming where integrated farming system is practiced. In this type of farming system, agricultural wastes, both solid and liquid from one farming system are used as inputs into the other and act as fertilizer within the same community (Boischio *et al.,* 2006). The manure deposited in the fish ponds acts as fertilizer that supports the growth of photosynthetic organisms on which the fish feeds (Petersen *et al.*, 2002).

Besides the use of livestock manure, chemical handling, misapplication and overuse in order to control pests in vegetable farming can pose a risk to human health resulting to impotency in men and infertility in women (Neghab *et al.*, 2014). Similarly, the irrational use of antimicrobial agents especially those intended for humans in livestock or fish can lead to development of antimicrobial resistant organisms (Guardabassi *et al.*, 2008). The risk can be increased if zoonotic pathogens including *E. coli* O157:H7 are involved and are allowed to contaminate the water or food meant for human consumption. Foodborne and water borne sources of infection of *E.* coli O157:H7 and other zoonotic enteric pathogens can occur when transmitted from livestock or livestock products including manure to humans where they could cause infections such as Haemolytic Uremic Syndrome (HUS) (Khandaghi *et al.,* 2010). *Cryptosporidium*, a protozoan parasite of public health importance especially in people who are immunocompromised can be transmitted to humans through ingestion of contaminated water or vegetables that are not properly cleaned (Ehsan *et al*., 2015).

1.2 Statement of the Problem and Justification of the Study

The increased practice of farming in urban areas including Dar es Salaam that are already overpopulated due to rapid urbanisation has resulted to increased contact by humans and

the environment to agricultural wastes including livestock manure. Livestock manure, agricultural chemicals, antibiotic residues and bad odours that are as a result of urban farming practices have a potential to pose a public health risk to humans and the environment they live in. In addition, husbandry practices such as the use of livestock manure as organic fertilizer, use of agricultural chemicals in vegetable farming and antibiotic use in livestock have a potential to pose a risk to human health and the environment (Brown and Jameton, 2000). This is due to their potential to transmit zoonotic pathogens, chemical residuals and antimicrobial resistant pathogens that would negatively affect human health. Integrated urban farming even heightens the risks as the presence of zoonotic pathogens in one component of the farming system would easily be transferred to the other and vice versa and to humans through contact or ingestion.

Currently there is limited information on studies conducted in Dar es Salaam to investigate the presence of *Cryptosporidium and* NSF *E. coli* O157:H7, its antimicrobial resistance patterns and the genetic relatedness of isolates from manure, vegetables and fish in integrated urban farming. This study therefore, aimed to determine the public health risks associated with the use of livestock manure in urban farming using NSF *E. coli* and *Cryptosporidium* as indicator organisms. It was also aimed to increase the knowledge base of people on the health risks associated with the practice of urban farming. The results of the study would strengthen the policy making platforms in the public health profession and other stakeholders in planning and adoption of an ecosystem-responsive husbandry practices in urban farming that would mitigate health risks in humans and the environment at large.

1.3 Objectives of the Study

1.3.1 Overall objective

To determine the public health risks associated with integrated urban farming using Non-Sorbitol Fermenting *E.* coli and *Cryptosporidium* as indicator organisms in Dar es Salaam,

Tanzania.

1.3.2 Specific objectives

i. To assess the knowledge and practices of farmers on the health risks associated with integrated urban farming in Kinondoni Municipality of Dar es Salaam.

ii. To determine the prevalence of *Cryptosporidium* spp. oocysts and NSF *E. coli* in livestock manure, fish and vegetable samples.

iii. To determine NSF *E. coli* antimicrobial resistance to selected antimicrobial agents.

iv. To determine the genetic relatedness among the NSF *E. coli* isolates from different sources.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 An overview on Urban Farming

Urban Farming is the growing of crops and keeping of livestock within an urban area or its surrounding areas and involves processing and distribution of the products using human and material resources within the same urban area (Addo, 2010). However, integrated urban farming involves the use of one type of output from one farming system which is otherwise supposed to be waste material and use it as an input in another sub system in order to achieve maximum efficiency of the overall expected products. There are various reasons that have been documented as to why urban farming is practiced. Food insecurity, addition to household incomes, increased poverty levels and livelihood survival strategies are among the reasons why integrated urban farming is practiced (Addo, 2010). Urban farming practice has also been attributed to a number of reasons that include, health benefits in people, improved nutrition, food security, act as an exercise, improves mental health and improves the social and physical urban environments (Bellows *et al*., 2003). It is for this reason that urban farming is not only limited to developing countries but also even in developed nations. According to Bellows *et al*. (2003), one third of the 2 million farms in the Unites States are located in the urban areas and about 35% of US ‘s vegetables, livestock and fish are produced through urban farming. In developing countries that include Tanzania, integrated farming is incorporated in urban farming for reasons such as reduction of production costs, fuel, feed and fertilizer inputs to maximize benefits from minimum capital investment (Chan, 1985). However, issues of knowledge and health risk perceptions associated with urban farming has always been a topic of research where urban farming is practiced (Drechsel and Keraita, 2014).

2.2 Knowledge and Practices on Health Risks Associated with Urban Farming

The practice of urban farming is aimed at reducing the production costs while maximizing the benefits. Cattle manure is often used to fertilize the fish ponds for the production of fish feed in the pond or as organic manure in vegetable gardens while the water from the fish ponds during harvest can also be used as fertilizer in the vegetable gardens (Little and Edwards, 2003). In this practice of urban farming, livestock can be kept at some distance away from where the fish or vegetables are but the systems can still be integrated according to Chen *et al*. (1994) cited by Little and Edwards, (2003). Due to the need by urban dwellers to have a constant supply of their products, different husbandry practices in their systems are always employed in order to sustain their production. These include application of acaricides on livestock to prevent ectoparasite infestation and also the application of insecticides on vegetables to prevent pests. In developing countries including Tanzania, urban farming is practiced by people from all social and economic status with different practices and perceptions on urban farming (Mlozi, 1997). These different groups of people practicing urban agriculture often have different levels of knowledge and perceptions on husbandry practices involved in urban farming. This is because effects of urban agriculture can have some health and environmental effects that eventually have an impact on human health. However, it has been reported in previous studies that at least all the people from these different social and economic status groups have some knowledge on the negative effects of urban agriculture despite them having more livestock in urban areas (Mlozi, 1997). It is such levels of knowledge and health risk perceptions that influence peoples practices and altitudes on urban farming (Drechsel and Keraita, 2014).

2.3 Health Risks Associated with Urban Farming

Urban farming has been associated with a number of health risks of which some have been thoroughly studied. Previous studies have categorized the health risks associated with

urban agriculture into physical, chemical, biological and psychosocial hazards (Boischio *et al*., 2006b). Out of these categories, the most common health risks that are attributed to urban farming are associated with chemical and biological hazards. Insecticides and drugs used in livestock farming are among the chemicals used in the practice of urban farming. With such, people have different risk perceptions on the impact these chemicals pose on human health. In a related study in Ghana, it was found that the use of pesticides in vegetable farming were rated highly in terms of health risks and as such some local organisations were advocating for the use of Integrated Pest Management (IPM) as the mitigating measure (Drechsel and Keraita, 2014).

Livestock including cattle and poultry produces a lot of faecal material that can accumulate within the limited land in urban areas. According to a report by World Health Organization (2012), cattle, chickens, pigs and sheep were reported to generate about 85% of the world's animal faecal waste which translate to 2.62×13^{10} kg of faecal material deposited per year in the environment. Therefore, in urban farming livestock manure is used to fertilise the soils in vegetable gardens and fish ponds. In so doing, zoonotic pathogens from livestock can be transmitted to the consumers directly or indirectly (Smit *et al*., 2001). According to a 2008 Communicable Disease Intelligence Report, zoonosis is an infection or infectious disease transmissible under natural condition from animals to humans and vice versa. There are a number of zoonotic pathogens in animal fecal waste of concern that affect humans but only 5 of these are known to cause illness in humans around the world with high frequency (WHO, 2012). These include *Cryptosporidium*, *Giardia*, *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter.* This study has focused on one of the protozoan zoonotic pathogen, *Cryptosporidium* spp. and NSF *E. coli* that include *E. coli* O157:H7.

2.4 *Escherichia coli* O157:H7

2.4.1 Overview

Escherichia coli is a gram-negative, oxidase negative and rod shaped bacterium of the family enterobacteriacea (Croxen *et al*., 2013). It was first reported by Escherich Theodor who isolated and characterised the bacterium under the name *Bacterium coli commune* in 1885 (Croxen *et al*., 2013). *Escherichia coli* over the years has been regarded as a laboratory workhorse and known as a commensal in warm blooded animals. Besides, pathogenic *E. coli* O157:H7 has been known to cause both intestinal and extra-intestinal illnesses in humans that would affect gastrointestinal, urinary tract, bloodstream and even central nervous systems as reported by Croxen *et al*. (2013). Enterohaemorrhagic *E. coli* (EHEC) in particular *E. coli* O157:H7 is one of the most common causes of Haemolytic Uremic Syndrome (HUS) in the United States that causes bloody and non-bloody diarrhoea among children and can result to death (Breuer *et al*., 2001). However, in most developing countries including Tanzania and worldwide, 1.8 million deaths in humans that occur are due to diarrhoeal diseases of which the majority are among the under five year old children according to WHO (2012) report as cited by Rochelle-Newall *et al*. (2015).

2.4.2 Sources, risk factors and transmission

2.4.2.1 *Escherichia coli* O157 in animals and manure

Enteropathogenic *E. coli* mainly resides in the gastrointestinal tract of most warm blooded animals including humans as normal flora and most transmission is through oral faecal route from one host to the other (Croxen *et al.*, 2013). The animals that usually act as reservoirs for the pathogenic strains include cattle, chickens, sheep, dogs, cats and gulls. Out of the mentioned animals, cattle is the main reservoir of pathogenic *E. coli* and can act as a source of transmission (Nataro and Kaper, 1998). In addition, calves in particular shed much more pathogenic bacteria than normal healthy cattle (Nataro and Kaper, 1998). The

transmission of the pathogenic *E. coli* is mainly through ingestion of contaminated food of animal origin especially cattle, drinking of contaminated water and also through human to human transmission (Nataro and Kaper, 1998). Practices including urban farming where there is increased contact of animals and animal waste by humans increase the risk of transmission of the pathogen to humans (Boischio *et al*., 2006a). Poor hygiene that includes consumption of raw milk or under cooked meat have also been implicated as risk factors to transmission and are among the major risk factors that have resulted in major foodborne disease outbreaks in countries including USA (Nataro and Kaper, 1998).

2.4.2.2 *Escherichia coli* O157:H7 in Humans

Several studies have been conducted where NSF *E. coli* have been isolated from humans by both specific conventional culture methods as well as molecular methods from faecal samples and other contaminated food sources (Jamshidi *et al*., 2008; Iijima *et al*., 2016; Lupindu *et al*., 2014). Therefore contamination of humans by these pathogenic strains of *E. coli* can easily result into contamination of food meant for human consumption. The Shiga-Toxin producing *E. coli* (STEC) O157:H7 had been previously isolated and reported from cattle as well as human stool in Tanzania though with relatively low prevalence which was comparable to similar studies conducted elsewhere (Lupindu *et al.*, 2014). In Kenya, the highest prevalence rates 55.9% of pathogenic *E. coli* was isolated from children suffering from diarrhoea (Iijima *et al*., 2016). Such infections in humans can result in to transmission of the pathogens to animals such as cattle or their products where they can become a source of infection especially for cattle that do not develop symptoms hence act as reservoirs.

2.4.2.3 *Escherichia coli* O157:H7 in vegetables

Contamination of farm products such as vegetables by pathogenic *E. coli* is mainly due to

husbandry practices employed during production which include application of livestock manure as fertilizer or the use of wastewater in vegetable farming (Smit *et al*., 2001). Since cattle is regarded as the main reservoir of *E. coli* O157:H7, the bacteria are shed especially by calves experiencing diarrhoea or in normal health cattle that do not show any signs of diarrhoea (Croxen *et al*., 2013). Then, depending on the environmental conditions that favour survival of *E. coli,* the bacteria can survive in the environment according to studies conducted for as long as 5 to 6 months in manure and when the manure is applied in the vegetable gardens, contamination of vegetables can occur (Avery *et al.,* 2004). The risk of contamination is increased with improper cleaning of vegetables before consumption.

2.4.2.4 *Escherichia coli* O157:H7 in fish

Integrated aquaculture systems of farming have contributed to contamination of fish with enteropathogenic *E. coli* as this practice involves the use of human or animal faecal material (Boss *et al*., 2016). Research has been conducted in countries such as Zambia, Eretria, Cameroon and found evidence of waste water being used for growing vegetables and for fish farming as reported by Smit *et al.* (2001). It is from such practices that include the use of waste water that cause contamination and results into transmission of pathogens to humans including children and the immunocompromised (Croxen *et al.,* 2013).

2.4.3 Antimicrobial resistant *E. coli* O157:H7

Antibiotics are usually administered to livestock or fish in urban farming for growth promotion, prevention and treatment of livestock or fish diseases (Guardabassi *et al*., 2008). Sometimes antibiotics are administered at under-doses or are over used resulting to development of antimicrobial resistant bacteria. The problem is worsened when the antibiotics used are intended for humans. Antimicrobial resistance complicates treatment using commonly available antimicrobial agents consequently resulting into persistence of

infections in humans (Guardabassi *et al*., 2008). In farming systems that include fish farming, the application of livestock manure in the fish pond favours development of antimicrobial resistant bacteria in the pond environment (Petersen *et al*., 2002). The health risk is heightened when zoonotic bacteria including *E. coli* O157:H7 is involved as it would easily contaminate farm products such as vegetables thereby posing a health risk to humans (Khandaghi *et al*., 2010). In Tanzania and other Eastern African regions, studies have been conducted to evaluate the transmission of resistant bacteria including *E. coli* O157:H7 within the environment among different sources such as livestock, humans, soil and water (Lupindu *et al*., 2015). The danger of pathogenic *E. coli* developing resistance against commonly used antibiotics is development of multi drug resistance. Studies have shown evidence that people who have been found to be resistant to certain antibiotics have had a history of animal farm visits, emphasizing the importance animals play in antimicrobial resistance development (Guardabassi *et al*., 2008). A review of researches in Zambia, Democratic Republic of Congo, Mozambique and Tanzania on antimicrobial resistance in both humans and animals revealed increased trends especially that of multidrug resistance (Mshana *et al*., 2013). Among the bacterial organisms that were implicated, *E. coli* O157:H7 was one of them. Antimicrobial resistance of even non-pathogenic *E. coli* is still an issue of great concern due to the tendency of the bacteria to transfer or share genes that confer resistance with the pathogenic types including *E. coli* O157:H7 through the mobile genetic elements such as plasmids (Guardabassi *et al*., 2008).

2.4.4 Clinical signs and symptoms

Pathogenic *E. coli* especially the Enterohaemorrhagic *E. coli* (EHEC) O157:H7 has been known to cause both gastrointestinal and extra-gastrointestinal infections both in humans and animals (Croxen *et al*., 2013). However, animals such as cattle can harbour it as a normal flora and would only cause disease in humans when it is incidentally ingested. Clinical infections of pathogenic *E. coli* is determined by the infecting pathotypes of the

bacteria that includes Shiga-Toxin producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregtive *E. coli* (EAEC) and Diffusely Adherent *E. coli* (DAEC) which differ in their virulence (Pérez *et al*., 2010). Generally, the common gastrointestinal infections are diarrhoeal related especially in children and can be bloody or non-bloody (Croxen *et al*., 2013). Extra-gastrointestinal infections include septicaemia, mastitis and Urinary Tract Infection (UTI) and of particular importance is Haemolytic Uremic Syndrome (HUS) caused by *E. coli* O157:H7 (Hazen *et al.*, 2015). Clinical manifestations of HUS include renal failure, thrombocytopenia, microangiopathy haemolytic anaemia and is the leading cause of renal failure especially in children (Kaper and O'brien, 2014).

2.4.5 Diagnosis

2.4.5.1 Culture method

Bacteria culture for isolation of the bacterial colonies using Sorbitol MacConkey (SMAC) Agar is considered as one of the basic and presumptive method of diagnosis of pathogenic *E. coli* including *E. coli* O157:H7 (March and Ratnam, 1986). The addition of Cefixime and Tellurite increases the sensitivity of the media for the isolation of the bacteria (Jamshidi *et al.*, 2008). However, this method has a limitation in that some of pathogenic strains such as the non-motile Vero Toxic *E. coli* (VTEC) O157 strains have shown to ferment sorbitol (Karch and Bielaszewska, 2001). Diagnosis can also be complemented by molecular techniques that involve typing of the bacteria (Croxen *et al*., 2013).

2.4.5.2 Molecular Techniques – Polymerase Chain Reaction (PCR)

Molecular techniques that include Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) have been used for the identification of *E. coli* in the diagnosis of infections and contamination from different sources (Ibenyassine *et al*., 2006; Yuan *et al.*, 2010; Ateba and Mbewe, 2014). Enterobacterial Repetitive Intergenic

Consensus sequences are 127 bp imperfect conserved palindromes that were first demonstrated in enteric bacteria including *E. coli* (Wilson and Sharp, 2006). These conserved regions have been explored and used to rapidly differentiate strains of the bacteria based on their genotype hence complementary to serotyping (Isloor *et al.*, 2010). Therefore, based on the ERIC-DNA fingerprints, genetic similarity of isolates from different sources can be compared in order to study the molecular epidemiology of bacteria in geographical regions and also in outbreak investigations (Isloor *et al*., 2010). The synonymous superior alternative technique for ERIC-PCR is the Pulse Field Gel Electrophoresis (PFGE) which exhibit highly strain specificity and is a useful method in epidemiological investigations of infectious diseases (Gautom, 1997). Compared to ERIC-PCR, PFGE is costly and time consuming to run.

2.4.6 Control of *E. coli* infections in Humans

Since ruminant animals especially cattle are the most commonly associated animals that naturally harbor *E. coli* O157:H7, control of transmission should be aimed at preventing contact with manure and consumption of under cooked beef by humans (Croxen *et al*., 2013). This is because countries with the high prevalence of *E. coli* O157:H7 associated infections in children such as HUS in Argentina had also been associated with high consumption of beef per capita. In addition, the isolates from human outbreaks were epidemiologically linked to those that naturally occurred in cattle according to studies conducted by Rivas *et al*. (2008) and Louie *et al*. (1999) as cited by Croxen *et al*. (2013).

2.5 *Cryptosporidium* Species

2.5.1 Overview

Cryptosporidium the causative agent of Cryptosporidiosis is a single celled protozoan parasite that can infect humans and other mammals including livestock such as cattle,

goats, pigs and sheep (Croxen *et al*., 2013). The parasite is responsible for diarrhoeal diseases and plays a major role in food and water borne disease outbreaks around the world (Ranjbar-Bahadori *et al*., 2013). It was first demonstrated in gastric epithelium of laboratory mice in 1895 by Clark (Fayer and Ungar, 1986). After so many years of research in laboratory mice, the first bovine case caused by the parasite was reported in 1971 by Panciers *et al.* (1971) before the first human case in 1976. Since then, Cryptosporidiosis has been known to cause infections in both competent and immune competent individuals. However, the disease can be self-limiting in immunocompetent individuals but can cause life threatening infections in immunocompromised people especially those living with Acquired Immune Deficiency Syndrome AIDS (Fayer and Ungar, 1986).

2.5.2 Species and hosts affected

The common species especially in humans are *C. hominis* which was previously called *C. parvum* genotype (I) which almost occurs in humans only and *C. parvum* previously called *C. parvum* genotype (II) which affects a wide range of animals including humans (Rossle and Latif, 2013). Other species have also been reported to have been isolated from humans especially from immunocompromised individuals and these include *C. canis* (dog), *C. muris* (mice), *C. andersoni* (cattle), *C. suis* (pig), *C. meleagridis* (Turkey) and *C. felis* (cat) (Rossle and Latif, 2013).

2.5.3 Distribution of *Cryptosporidium* infections in humans

The disease has been reported to occur in many parts of the world except in the Antarctica and occurrence in both developed and developing nations is more common during warm and wet periods (Jasool *et al*., 2013). For instance, in a study of the prevalence rates in people in the Arab world from the period of 2002 to 2011 revealed that the prevalence rates ranged from <1% to 43% in immunocompetent individuals and <1% to 82% in immunocompromised patients (Ghenghesh *et al*., 2012). In the United States, the cases of

Cryptosporidium foodborne related illnesses were reported to be as high as 300,000 cases per year (Mead *et al*., 1999). Other studies have been conducted to demonstrate the infections in humans in many countries including Burkina Faso, China as well as Tanzania (Sangaré *et al*., 2015; Wang *et al.*, 2013; Tellevik *et al*., 2015).

2.5.4 Current status in Tanzania

Cryptosporidium infections are common in Tanzania especially in diarrhoeagenic children 24.2% who also have the Human Immunodeficiency Virus (HIV) and the infections are more common during the rainy season (Tellevik *et al*., 2015). In other studies within the country among children less than 5 years of age, the prevalence of the parasite was found to be relatively low. This could have been attributed to differences in methodology and seasonal variation that could have affected the infection rates of the parasite during the period of study according to Tellevik *et al*. (2015).

2.5.5 Transmission

There are several modes of transmission of *Cryptosporidium* spp. infection between humans and animals. This can occur through human to human, animal to human, animal to animal and also through vehicles such as water, food and air (Fayer and Morgan, 2000). Animals are the reservoir for the parasites and are responsible for the possible contamination of water and food meant for human consumption (Rossle and Latif, 2013).

2.5.6 Sources of infection

2.5.6.1 Animal sources

Contaminated water and food have been reported to be the main sources of transmission for *Cryptosporidium* spp. infections in humans (Fletcher *et al*., 2012). This occurs after ingestion of *Cryptosporidium* oocysts from animal faecal effluents that come in contact with food or water meant for human consumption. Cryptosporidiosis is one of the

common diseases in calves and lack of proper husbandry practices in diarrhoeagenic calves has made young calves to be a major source of infection to humans (Peter *et al*., 2015). This was confirmed by research conducted in related studies that found occurrence of *Cryptosporidium* spp. infections to be significantly high in cattle especially calves in Tanzania, Kenya and Zambia (Swai and Schoonman, 2010; Kang'ethe *et al*., 2012; Siwila *et al*., 2007). *Cryptosporidium* spp. oocysts have also been reported among wild animals in some parts of Tanzania and the results indicated positive infective rates for both diarrhoeagenic and non diarrhoeagenic animals (Mtambo *et al*., 1997).

2.5.6.2 *Cryptosporidium* spp. Oocysts in Vegetables

Farm products like vegetables and fruits can be vehicles through which *Cryptosporidium* can be transmitted to humans and the risk is increased when these products are not properly washed before sell or consumption (Tefera *et al*., 2014). The contamination can occur during production, processing and even during transportation to market places hence the need for the consumers and handlers to uphold good hygiene standards such as proper cleaning with clean water and cooking of such vegetables before consumption. Farm soils can also be a source of contamination of the parasite to the vegetables through which transmission and infection in humans can occur after ingestion (Hong *et al*., 2014).

2.5.6.3 *Cryptosporidium* spp. oocysts in Fish

Apart from fish grown under livestock-fish integrated farming, fish can also harbour *Cryptosporidium* spp. infection even from natural sources of water such as lakes (Atawodi and Bichi, 2013). Other related studies found fish from natural sources in freshwater to be contaminated with various species of *Cryptosporidium* including the zoonotic species that would easily be transmitted to humans and animals and cause disease. These species were not only found in the gastrointestinal system of fish like intestines and stomach but also

found to contaminate the edible parts of fish like fillet thereby posing a health risk humans (Certad *et al*., 2015). Therefore, husbandry practices in urban agriculture where livestock manure is used in fish ponds can be a source of transmission of *Cryptosporidium* to humans. The risk can be heightened when the edible parts of the fish are infected by the parasites.

2.5.7 Pathogenesis, Morphology and life cycle of *Cryptosporidium* spp. Oocysts

Cryptosporidium oocysts are small in size and measure about 4-6 µm in diameter and are spherical to ovoid in shape (Fayer and Ungar, 1986). The life cycle of *Cryptosporidium* is usually completed within a single host and involves both asexual and sexual stages (Rossle and Latif, 2013). The developmental stages of the *Cryptosporidium* oocysts consists of six stages which are excystation, merogony, gametogony, fertilization and zygote formation, oocysts formation and sporogony (Rossle and Latif, 2013). *Cryptosporidium* oocysts attaches and infects the superficial tissues of the intestinal epithelium in particular, the surface of the cells of the villi and crypts of small intestine (Guerrant, 1997; Fayer and Ungar, 1986).

2.5.8 Clinical signs

The major clinical manifestation of *Cryptosporidium* spp. infection is diarrhoea (Fayer and Ungar, 1986). The diarrhoea is usually more frequent in immunocompromised people living with AIDS than in immunocompetent persons and is characterised by profusion and water (Guerrant, 1997). Other less frequent clinical signs occur which can include crampy abdominal pain, nausea, vomiting and a low grade fever. Non-specific symptoms include malaise, myalgia, weakness and some headache and the severity of all these symptoms may correlate with the shedding of the oocysts in the stool (Fayer and Ungar, 1986; Guerrant, 1997).

2.5.9 Diagnosis

Microscopic staining methods can be used to identify *Cryptosporidium* oocysts in faecal smears after concentration. The differential staining methods includes Safranin-methylene blue, Kinyoun, Ziehl-Neelsen (ZN) and Diymethylsulfoxide (DMSO) Carbol fuschin that stain the oocysts red and counter stains the background (Fayer and Morgan, 2000). However, the most commonly used staining methods are the modified Ziehl-Neelsen acid fast stain and Modified Kinyoun's acid–fast stains (Rossle and Latif, 2013). These methods however require experienced persons using the microscope for a correct diagnosis, they are labour intensive and cannot distinguish between human and animal species of *Cryptosporidium* oocysts (Rossle and Latif, 2013). Immunological based methods can also be used for diagnosis though non-specific detection of antibodies due to cross reactions makes them less effective (Rusnak *et al*., 1989). Apart from the concentration techniques for detection of oocysts in water and techniques to determine oocysts infectivity and variability, molecular methods particularly PCR, have offered an alternative to diagnosis of *Cryptosporidium.* Polymerase Chain Reaction methods are rapid, highly sensitive and accurate although they have their own disadvantages (Fayer and Morgan, 2000). These include observation of false positives that can result from detection of naked nucleic acids and other microorganisms that are non-viable and could be present as a result of contamination.

2.5.10 Treatment and control

Due to the double thick walled nature of the parasite, *Cryptosporidium* oocysts are resistant to environmental conditions including a number of disinfecting agents such as chlorine. The most effective chemical that would inactivate *Cryptosporidium* oocysts is ozone (Korich *et al*., 1990). In humans, Cryptosporidiosis is usually self-limiting in immunocompetent individuals and might just require little or no treatment but is difficult

to treat in immunocompromised individuals as none of the drugs that have been studied are curative (Derouin *et al.*, 2008). Nitrazoxanide is reported to be the only drug that showed some evidence to reduce both parasitic loads and symptoms in immunocompetent people and can only be considered for possible treatment in immunocompromised people at higher doses (Abubakar *et al.*, 2007). Otherwise, support therapy that includes rehydration, electrolyte replacement and anti-motility agents remain the treatment strategies currently in place. Similarly, some scholars such as Joachim *et al*. (2003) cited by WHO (2012) have reported that halofuginone, an antiprotozoal agent decreases the parasitic load in animals but does not eliminate the shedding of *Cryptosporidium* oocysts by calves. However, when used together with good sanitation and proper disinfection it might help to limit the infection in calves hence reduce the risk of transmission to humans.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Dar es Salaam region of Tanzania which is located on the Western coast of the Indian Ocean. It lies between Latitudes 6° 33′ and 7° 0′ South of the Equator and Longitudes 33° 33′ and 39° 0′ East of the Greenwich (PMO and RALG, 2014). The region has a population of 4 364 541 according to the National Bureau of Statistics (2015) and has three municipal councils that include Ilala, Temeke and Kinondoni. These are further divided into 80 Constituencies, 10 divisions, 90 wards and 542 streets (Mitaa). Kinondoni municipality where the study was conducted has the highest number of people at 1 775 049 covering an area of 522.3 square kilometres with four divisions namely Kinondoni, Magomeni, Kibamba and Kawe according to 2012 population census. The study was conducted in two of the four divisions and covered Bunju and Wazo wards in Kawe division with Mbezi and Kibamba wards in Kibamba division (Fig. 1).

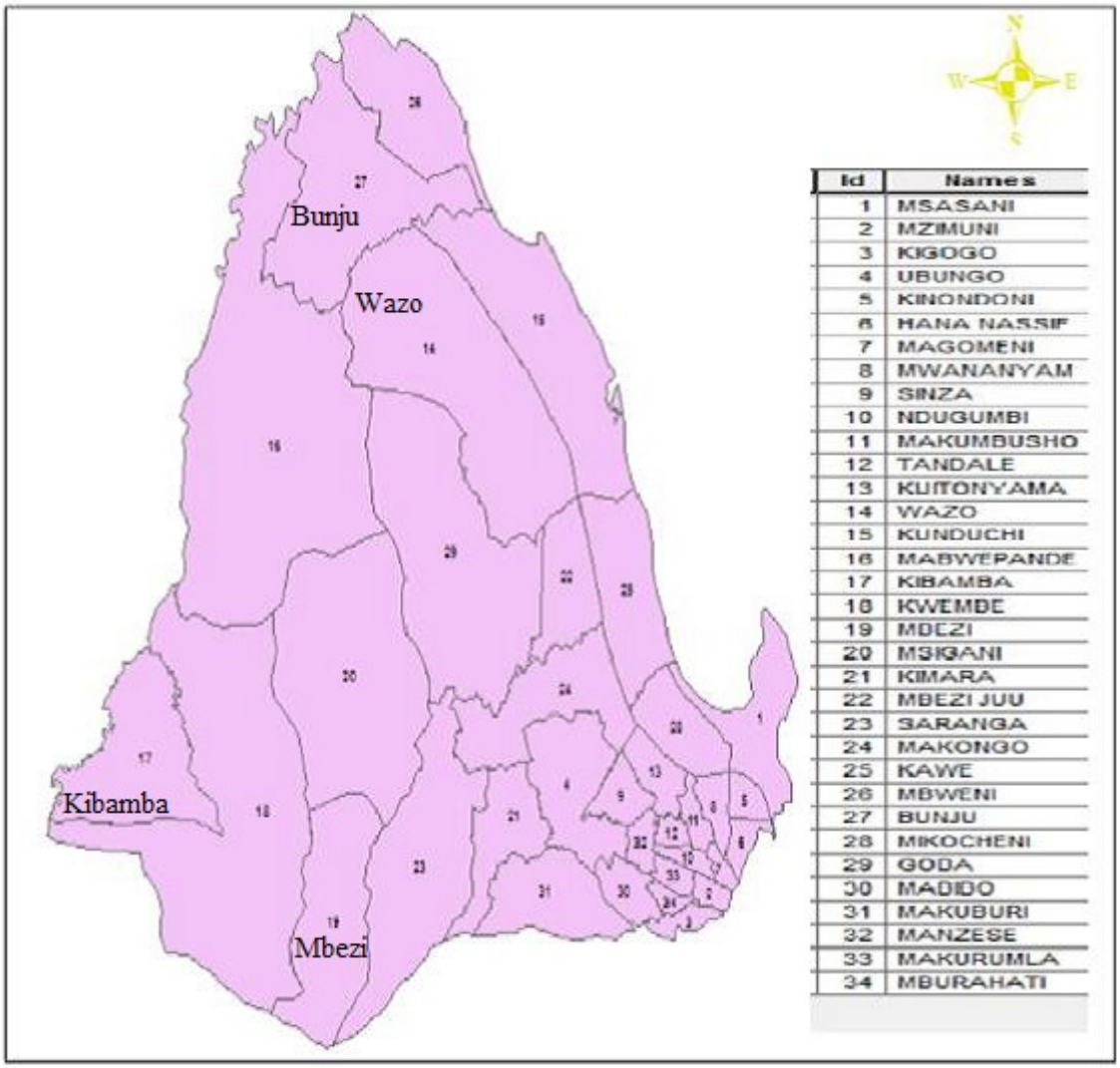

Figure 1: Map showing Kinondoni municipality and location of wards included in the study in Dar es Salaam.

Source: Kinondoni Municipal profile (2014)

3.2 Study Design and Sample Size Determination

A cross sectional study was used to determine health risks associated with integrated urban farming. Faecal samples from livestock, fish and vegetable samples were collected from the randomly selected households involved in urban farming in the study area. The households that were involved in the study were randomly selected from the list of all the households within the four wards involved in urban farming.

The sample size estimation for livestock manure, vegetables mainly comprising of the commonly grown leafy vegetables including *African spinach* (*mchicha*) and fish were calculated using the approach based on sample size formula;

$$n = \frac{Z_{\alpha/2}^2 \, p \, (1-p)}{d^2}$$

Whereby;

$Z_{\alpha/2}^2$ = Confidence Level (CL) at 95% which is equivalent to 1.96,

α = Probability of type I error equivalent to 0.05 (two sided),

p = Expected proportion in a population based on known or unknown prevalence

d = Absolute error or precision at 5% (Charan and Biswas, 2013).

Therefore, known prevalence of NSF *E. coli* in manure according to a study reported by Lupindu *et al.* (2014) was 0.9%, in vegetables 0.35% reported by Khangdaghi *et al*. (2010) and Fish at 50% for unknown prevalence. Hence the calculated expected numbers of samples to be collected were 138 for manure, 350 for vegetables and 384 for Fish within the study households. A total of 93 households were randomly selected for the study. A questionnaire interview was conducted at each household and a total of 156 samples were collected from these households comprising of 89 manure, 51 vegetables and 16 fish samples. The reduced number of samples was due to absence of some farming activities at some households as most farmers had their vegetable gardens and fish ponds dried up due to lack of water supplied by rain at the time of the study.

3.3 Data Collection

3.3.1 Questionnaire survey and sample collection

A structured questionnaire was administered to each individual owner or representative of

the households that were randomly selected. The questionnaire was pre tested before the study in Morogoro among peri-urban farmers. The questionnaire covered questions on the demographic data of the respondents such as age, sex, religion, education level and occupation. The husbandry practices included the name and frequency of chemicals commonly used, farm product-processing and the use of livestock manure as organic fertilizer in either vegetables or fish farming. From each household visited for interviews, samples either fresh fecal sample or manure sample from cattle or poultry, vegetables and fish were collected for laboratory examination. Approximately, 100g of fresh faecal sample was collected using a sterile examination glove and placed in a cooler box containing ice packs before being transported to the Public Health Laboratory at SUA in Morogoro for analysis. The fresh faecal samples were collected at least within five minutes of being deposited on the ground in cases where direct collection per rectum was difficult. Three to Five faecal deposits were collected and pooled to make one household sample for the fresh manure. Dry faecal samples were collected from the deposition area at the household before being used i.e. in the garden. Similarly, three to five sites of the dry manure were collected to make a pooled faecal sample. Fish samples were collected from the fish ponds by using the available fishing equipment such as fishing nets and scoop nets. The samples were stored in a sealable plastic bag and transported in cooler box with ice packs and stored at refrigeration temperature before being analysed. The edible leafy vegetables such as *African Spinach* (*mchicha*) that were ready for sell or consumed in the gardens were randomly collected and placed in the sterile sealable plastic bag before being placed in a cooler box with ice and transported to the laboratory. Approximately three to five edible parts of the available leafy vegetables were collected from a garden at a household to avoid sampling error. After sampling, the samples were transported on ice packs in a cooler box from Dar es Salaam to the Public Health Laboratory at SUA in Morogoro for laboratory processing and examination and all the samples were processed in the laboratory within 48 hours after sample collection.

3.3.2 Determination of Prevalence of *Cryptosporidium* Oocysts and NSF *E. coli*

3.3.2.1 Detection of *Cryptosporidium* Oocysts

Cryptosporidium oocysts were screened from manure, vegetable and fish sample suspensions using the modified Kinyoun's acid fast stain as described by Ezzaty Mirhashemi *et al*. (2015). Each of the previously prepared sample suspension that was stored at -20°C and for each sample suspension made, one drop was collected to make a thick smear on a clean glass slide using a plastic pasteur pipette. The smear was allowed to air dry before being fixed with absolute methanol for 1-2 minutes. The slides were then flooded with Kinyoun's Carbol-fuchsin for 5 minutes after which they were rinsed briefly with 50% ethanol for 3-5 seconds before being thoroughly rinsed with tap water. The slides were decolourised with 1% sulphuric acid for 1-2 minutes until no more colour ran from the slide. The slides were rinsed again with tap water and drained. Methylene blue was used to counter stain the smear for 1 minute and after the final rinse, the slides were allowed to air dry, ready for microscopic examination. Examination of the slides was done at magnification of ×100 with oil immersion. The positive slides were proof checked with a calibrated eyepiece graticule of the microscope to check for the size of approximately 4-6 µm of *Cryptosporidium* oocysts and morphology.

3.3.2.2 Isolation of NSF *E. coli*

Standard methods were used to isolate NSF *E. coli* where 1g of manure was added to 4 ml of Maximum Recovery Diluent (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 37 °C for 12-18 h, after homogenization for one minute. Intestinal contents were used for the fish samples. One loop full of the suspension was collected and streaked on to MacConkey Agar (Oxoid Ltd., Basingstoke and Hampshire, UK). This was followed by incubation for 24 h at 37 °C.

The vegetable samples were manually cut into small pieces using a sterile blade and 10 g was measured and placed in a stomacher bag. Then 90 ml of MRD solution was added to the sample and the mixture was stomached for 1 minute at low speed. All the suspension samples were transferred into clean bijou bottles where samples for NSF *E. coli* and *Cryptosporidium* isolation where collected. A loop full of the suspension was collected to be streaked onto the MacConkey agar for incubation at 37 °C for 24 hours. About 5-6 pure typical and atypical *E.* coli colonies were sub cultured on to Sorbitol-MacConkey agar (Oxoid Ltd., Hampshire, UK) and incubated at 37 °C for 24 hours in order to obtain the NSF colonies of *E. coli*. Since only pure colonies were picked, the yielded confirmed NSF *E. coli* colonies even from one sample were treated as different strains of an isolate for further antimicrobial resistance testing and genetic relatedness determination. These sub-isolates were labelled for identification purposes.

3.3.3 Confirmation of *E. coli* isolates

All the NSF colonies were subjected to biochemical tests screening. The Indole, Methyl-Red, Voges Proskauer (Sigma-Aldrich Co., Switzerland) and Citrate (IMViC) tests were used for the confirmation of *E. coli* isolates according to the manufacturer's instructions. Isolates that were Indole positive, Methyl red positive, Voges Proskauer negative and Citrate negative were confirmed to be *E. coli.* The confirmed NSF *E. coli* isolates on biochemical tests were stored Muller Hinton Broth containing 15% v/v glycerol at -20 °C for future antimicrobial susceptibility testing and molecular analyses.

3.3.4 Antimicrobial Resistance Testing

The antimicrobial resistance testing of all NSF *E. coli* isolates was performed using the disc diffusion method as described by Clinical and Laboratory Standards Institute (CLSI, 2013). The pure culture of NSF *E. coli* colony was suspended into test tube containing

10ml of 0.9% Sodium Chloride solution and its concentration (turbidity) was compared to the standard 0.5 McFarland. The sterile swab was used to completely streak the previously prepared Mueller Hinton (MH) Agar (Oxoid Ltd, Hampshire, UK) plates. After placing the antimicrobial susceptibility test discs (Oxoid Ltd., Hampshire, UK), the agar plates were incubated overnight at 37 °C and the inhibition zone diameters produced around the antimicrobial discs were measured using a ruler and interpreted using the Kirby-Bauer chart. Six antimicrobial agents commonly used in both livestock and humans were used to test for resistance. These included Amoxicillin (AML-10 µg), Tetracycline (TE-10 µg), Azithromycin (AZM-15 µg), Ampicillin (AMP-25 µg), Ceftriaxone (CRO-30 µg) and Ciprofloxacin (CIP-1 µg)

3.4 Determination of Genetic Relatedness of the Isolates from Different Sources

3.4.1 DNA extraction

Genomic DNA was extracted by heat lysis method where all pure isolates were grown overnight at 37 °C on Nutrient Agar (Thermo Scientific, UK) to obtain fresh cultures (Englen and Kelley, 2000). Approximately 3-5 colonies were picked and mixed with 100 µl of sterile water in the sterile Eppendoff tube to obtain turbid suspension of bacterial cells. By using a Techne® Dri-Block® heater (Bibby-Scientific Ltd., Staffordshire, UK), the bacterial suspension was heated at 95 °C for 15 minutes. The suspension was centrifuged at 12 000 × g for 10 minutes to obtain the supernatant that was used as DNA template. The recovered DNA was stored at -20 °C until needed for amplification and 5 µl was used as template for PCR.

3.4.2 DNA amplification by ERIC-PCR

The amplification of genomic DNA was carried out in a 25 µl total volume containing 3 µl of Deionised water, 3.5 µl each of a forward and reverse primer, 10 µl of master mix and 5 µl of the DNA template. The primers that were used to amplify ERIC sequences in the

NSF- *E. coli* isolates were forward primer 1R, 5'-ATG TAA GCT CCT GGG GAT TCA C-3' (TAG Copenhagen A/S) and reverse primer 2R, 5'-AAG TAA GTG ACT GGG GTG AGC G-3' (TAG Copenhagen A/S). Using a Takara PCR Thermal Cycler Dice® (Takara Bio, Japan), the reactions were conducted with an initial denaturation temperature of 94 °C for 7 minutes followed by 34 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 38 °C for 1 minute, extension at 72 °C for 5 minutes and a single final extension step at 72 °C for 15 minutes before the holding temperature at 4 °C. The PCR products were observed by performing Agarose Gel electrophoresis. The 1.5% Agarose gel was made by mixing 1.5 g of 1.5% Agarose with 100 ml solution of 1 x Tris-borate-EDTA (TBE) buffer and stained with 6 µl ethidium bromide before pouring in the gel tray. For each tube of PCR products, 8 µl of each PCR suspension was mixed with 2 µl of 6x blue loading dye. Electrophoresis was conducted at 100 V for 45 minutes before visualising the amplicons under UV light and the results were recorded. A 100 bp DNA molecular weight maker Promega™ (Fisher Scientific, UK) was used.

3.5 Data Analysis

3.5.1 Questionnaire and laboratory data analysis

All data were recorded in Microsoft excel sheet file and were exported onto IBM SPSS Version 20 statistical software package for analysis. Descriptive statistics, frequencies and cross tabulations were used to determine the public health risk based on the responses from the respondents to determine proportions for different variables. The chi-squared test was used to calculate statistical significance between different proportions with significance considered at $p \leq 0.05$. The data on the prevalence of *Cryptosporidium* and *E. coli* was also compared with the sources of various samples including manure, vegetables and fish to check for significance of association between occurrence and the source. Further, antimicrobial resistance patterns were determined for the NSF colonies of *E. coli* to determine the public health risk of the pathogen.

3.5.2 Determination of genetic similarity of *E. coli* isolates

The genetic relatedness of NSF *E. coli* isolates from different sources was determined by using the Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) according to a study by Ali *et al*. (2014). The resulting DNA banding patterns were used to generate a similarity phylogenetic dendogram with optimization and tolerance coefficient both set at 1 % to determine the genetic relatedness of the NSF- *E. coli* isolates from different sources using the BioNumerics software, GelCompar II Version 6.6.11 (Applied Maths, Sint-Martens- Latem, Belgium).

CHAPTER FOUR

4.0 RESULTS

4.1 Knowledge and Practices on Health Risks

A total of 93 households within the 4 wards were visited and interviewed. Of the 93 households, 30.1% (28/93) were females and 69.9% (65/93) males. Majority of the respondents, 88.0% (81/92) were involved in livestock keeping out of which 72.0% had cattle as their major livestock while 25.6% kept poultry as a major livestock.

4.2 Knowledge and Practices

Out of the 88 respondents 58% (51/88) of the respondents had knowledge on the health risks associated with integrated urban farming. Out these who had knowledge, 64.7% (33/51) had no formal education. About 87.7% (71/80) of respondents used livestock manure from either cattle or poultry as organic fertilizer in their vegetable gardens, 8.6% (7/81) sold their manure to others and only 3.7% (3/81) used livestock manure for other uses. The results also indicated that 79.8% (67/84) used chemicals in their farming systems especially in vegetable farming. Pyrethroids were the most common used chemicals 53.1% (34/64) and Carbamates, Diacylhydrazine, Carbamides, Bipiridines and Corticosteroids were the least used chemicals by farmers 1.6% (1/64) for each as shown in Table 1.

Table 1: Distribution of respondents on common chemical (insecticides) used (N= 93)

Chemical Name	Number of respondents (%)
Organophosphate	12 (18.8)
Avermectins	2 (3.1)
Formamidine	3 (4.7)
Diathiocarbamates	5 (7.8)
Pythreoid	34 (53.1)
Carbamates	1 (1.6)
Diacylhydrazine	1 (1.6)
Carbamides	1 (1.6)
Bipiridines	1 (1.6)
Tetracycline	3 (4.7)
Corticosteroids	1 (1.6)

4.3 Prevalence of NSF- *E. coli* and *Cryptosporidium*

Out of the total number of 156 samples collected, NSF- *E. coli* was demonstrated in 36 (23.1%), (95% CI: 16.7-30.5) of the samples. Seventeen isolates from both manure (19.1%) and vegetables (33.3%) yielded NSF *E. coli* while two (12.5%) isolates were from fish. The overall prevalence of *Cryptosporidium* in manure, vegetables and fish was 10.3% (16/156) (95% CI: 6.0-16.1). From the total of 16 samples of fish, six (37.5%) demonstrated presence of *Cryptosporidium* oocysts with five (9.8%) from vegetables and five (5.6%) from manure (Table 2). Five (3.2%) samples had both *Cryptosporidium* oocysts and NSF *E. coli* including four from vegetables and one from fish.

Table 2: Distribution of proportions for NSF *E. coli* and *Cryptosporidium* from different sources

Source	Total samples	Number of positive samples (%)	
		E. coli	**Cryptosporidium**
Manure	89	17 (19.1%)	5 (5.6%)
Vegetables	51	17 (33.3%)	5 (9.8%)
Fish	16	2 (12.5%)	6 (37.5%)
Total (Overall)	156	36/156 (23.1%)	16/156 (10.3%)

4.4 Association between NSF *E. coli* and *Cryptosporidium* from sources

There was a statistical significance difference between the overall occurrence of *E. coli* and *Cryptosporidium* in manure, vegetables and fish p = 0.0040 (95% CI; 4.14- 21.37). The results obtained indicated no statistical significance difference in the occurrence of NSF- *E. coli* in manure, vegetables and fish p > 0.05 (Table 3). However, comparison between different sources revealed that proportions of *Cryptosporidium* oocysts in vegetables p = (0.0262) (95% CI; 2.56-55.54; df = 1) were significantly higher than in fish p= 0.0007 (8.53-59.23) and there was no statistical significant difference between occurrence of *Cryptosporidium* in manure *p*= 0.5565 (95% CI; -5.38-16.4; df = 1) and that in vegetables (Table 4).

Table 3: Comparison of prevalence of *E. coli* in different sources

Sources	CI	X^2	p- value
Manure and Vegetable	-1.68-30.63	2.826	0.0927
Manure and Fish	-20.33-21.23	0.0777	0.7805
Fish and Vegetable	-7.94-39.04	1.672	0.1960

Table 4: Comparison of prevalence of *Cryptosporidium* in different sources

Sources	CI	X^2	p-value
Manure and Vegetable	-5.38-16.4	0.346	0.5565
Manure and Fish	8.53-59.23	11.525	0.0007
Fish and Vegetable	2.56-55.54	4.942	0.0262

4.5 Antimicrobial Resistance

Out of the 48 NSF *E. coli* isolates subjected to antimicrobial resistance testing, 25 (52.1%) were resistant to at least one antimicrobial agent. Resistance of the test isolates was more common to Amoxicillin (35.4%) while Ciprofloxacin had the least proportion of resistant isolates (2.1%). Proportions of resistant isolates to other antimicrobial agents are as displayed in Table 5 and Fig. 1. However, out of the total number of isolates that were resistant to at least one antimicrobial agent, 12 (48.0%) displayed multidrug resistance (MDR). Multi-drug resistance was considered as resistance of an isolate to two or more classes of antimicrobial agents (Magiorakos *et al*., 2011). The most common overall pattern of MDR involved AML, AZM and AMP (Table 6). The most commonly observed MDR pattern for isolates from manure was AML, TE and AZM while those from vegetables included AML, AMP and AZM.

Table 5: Antimicrobial resistant profiles of 48 NSF *E. coli* isolates

Antibiotic (Drug concentration)	Proportion of resistant isolates n (%)
Amoxicillin (10 µg) - AML	17 (35.4%)
Tetracycline (10 µg) - TE	13 (27.1)
Azithromycin (15 µg) - AZM	11 (22.9)
Ampicillin (25 µg) - AMP	12 (25.0%)
Ceftriaxone (30 µg) - CRO	6 (12.5%)
Ciprofloxacin (1 µg) -CIP	1 (2.1%)

Table 6: Multi-Drug Resistance patterns of NSF *E. coli* isolates

Isolate ID	Source	Antimicrobial resistant pattern
143M	Manure	AML, TE, AZM, AMP, CRO, CIP
24V	Vegetable	AML, TE, AZM, AMP, CRO
9M	Manure	AML, TE, AZM, CRO
14M	Manure	AML, TE, AZM, AMP
21V	Vegetable	AML, TE, AMP, CRO
25V	Vegetable	AML, AZM, AMP
78M	Manure	AML, TE, AMP
108V	Vegetable	AML, AZM, AMP
112aV	Vegetable	AML, AZM, AMP
113aV	Vegetable	AML, AZM, AMP
113cV	Vegetable	AML, AZM, AMP
128aM	Manure	TE, AZM, CRO

4.6 ERIC-PCR Results

The ERIC-PCR profiles produced after gel electrophoresis revealed highly polymorphic DNA fragments both in number and size among the isolates. This variation was demonstrated even between isolates from the same sample type and source (Figure 2). All isolates that were subjected to ERIC-PCR produced a range of 1 to 9 bands with an average of 4 bands per isolate. The proportion of the isolates with a particular band size among the sources ranged from approximately 100 bp 17.0% (8/47) to 1700 at 2.1% (1/47). The most common band size shared among the isolates was 300bp and was observed in 61.7% (29/47) of the isolates as shown in the representative gel image (Figure 2).

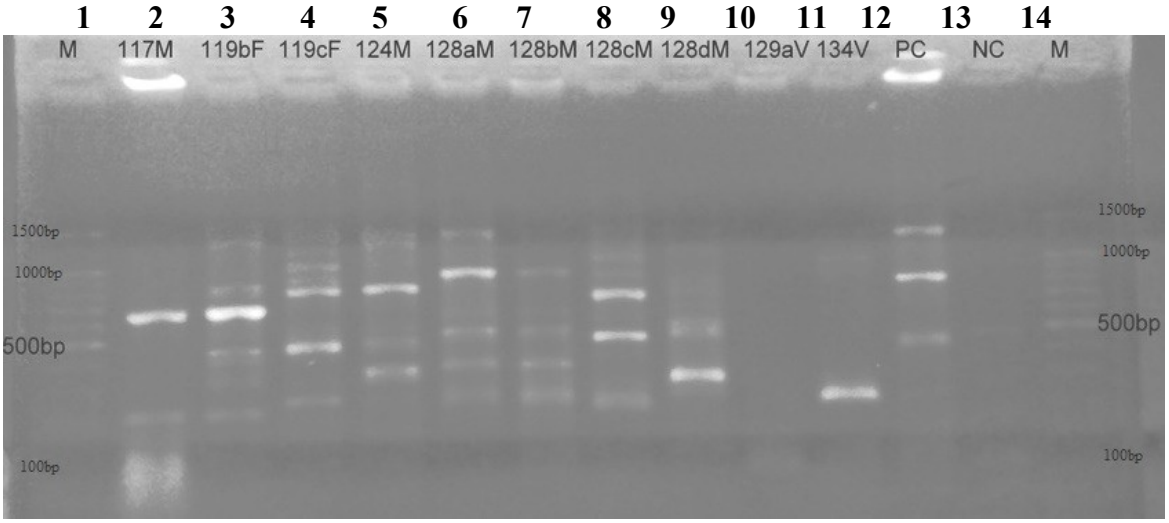

Figure 2: **Results showing representative DNA fingerprints from ERIC-PCR of NSF *E. coli* isolates from manure, vegetable and fish samples; Lanes 1 and 14, 100bp DNA ladder; Lanes 2, 5, 6, 7, 8 and 9, isolates from manure; Lanes 3 and 4, isolates from Fish, Lanes 10 and 11, isolates from vegetable; Lanes 12 and 13, positive control (*E. coli* K-12) and negative control respectively.**

4.7 Determination of Genetic Relatedness (Similarity) among the NSF- *E. coli* Isolates

The ERIC DNA fingerprints were used to construct a similarity dendrogram to demonstrate the genetic similarity among the isolates from different sources. Nine clusters were identified and designated as (I) to (IX) for identification purposes of this study (Table 7). The similarity level among isolates ranged from 74.5% to 100%. However, clusters were identified based on a similarity threshold of 90% and above. Cluster (I) was the largest while clusters (IV) and (IX) were the smallest in size with only one isolate making a cluster for each of them (Figure 3). Isolates from the same household which included, 117M from manure, 112V from vegetable with sub isolates 112aV, 112bV, 112cV, 112dV and 119F from fish with sub isolates 119bF and 119cF exhibited different antimicrobial resistance patterns and were grouped in different clusters (Figure 3). Isolate

112aV produced a MDR pattern with 112dV resistant only to TE and both were in cluster (II) with a genetic similarity level of 96.5%. Isolate 112dV from vegetable showed 100% similarity with isolate 93M from manure. The isolates from fish were not 100% similar but were grouped in cluster (I) and did not exhibit MDR except for isolate 119bF which showed resistance only to AML. Isolates 117M from manure was more similar to sub isolates 112cV (98.0%) and to 112bV (94.8%) and were in same cluster (III) with no MDR pattern observed.

Table 7: Distribution of similarity of 47 isolates by proportions in Clusters

		Proportion of isolates n (%)		
Cluster	**Similarity %**	**Manure**	**Vegetable**	**Fish**
(I)	90.6%	8 (17.0)	1 (2.1)	2 (4.3)
(II)	96.5%	2 (4.3)	2 (4.3)	0 (0.0%)
(III)	93.9%	1(2.1)	10 (21.3)	0 (0.0%)
(IV)	81.0%	1 (2.1)	0 (0.0)	0 (0.0%)
(V)	94.3%	5 (10.6)	1 (2.1)	0 (0.0%)
(VI)	91.8%	1 (2.1)	6 (12.8)	0 (0.0%)
(VII)	98.0%	2(4.3)	0 (0.0)	0 (0.0)
(VIII)	100%	0 (0.0)	3 (6.4)	0 (0.0)
(IX)	89.7%	0 (0.0)	0 (0.0)	1(2.1)

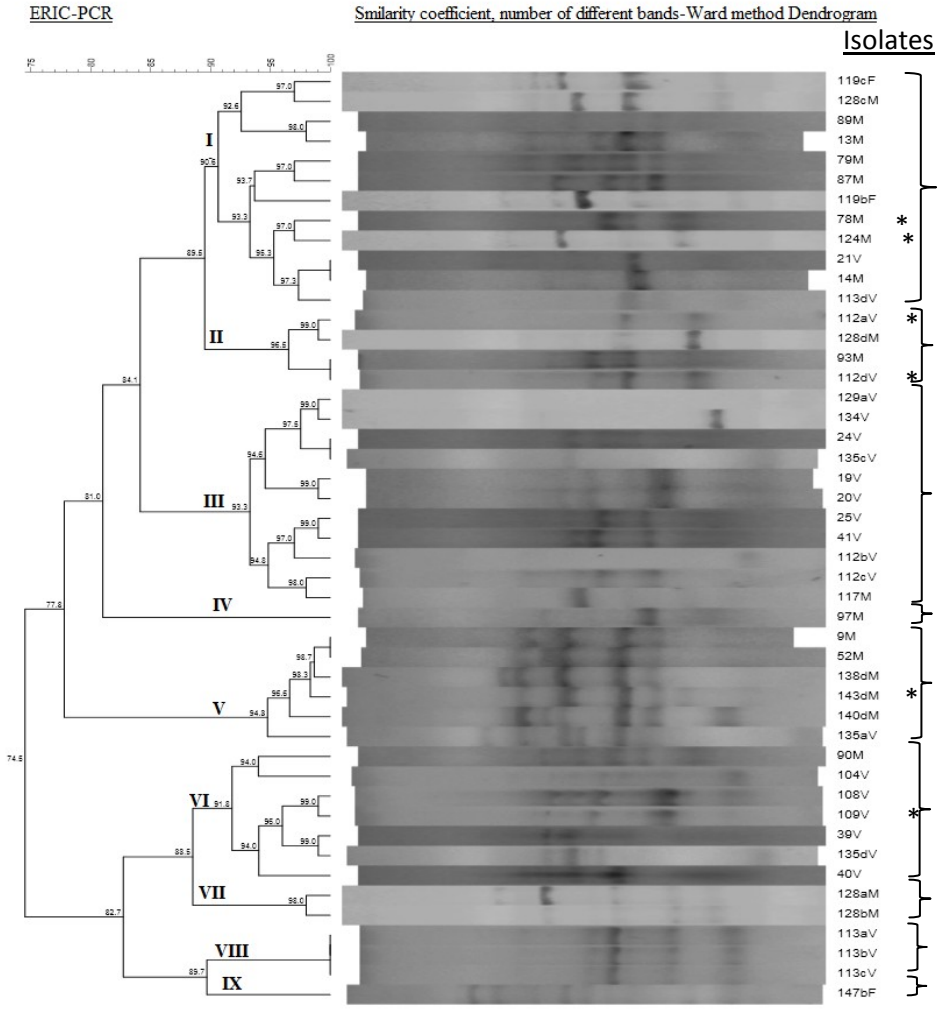

Figure 3: Dendrogram showing ERIC-PCR band patterns, clusters and genetic similarity of NSF *E. coli* isolates from manure, vegetables and fish samples. Notes (Key); Isolate number = Isolate ID; M = Manure source; V= Vegetable; F= Fish; * = Poultry source

CHAPTER FIVE

5.0 DISCUSSION

5.1 Knowledge and Practices of Farmers on the Health Risks Associated with Urban Farming

The results indicated that out of 92 respondents, 58.0% of the farmers interviewed had knowledge on the health risks associated with urban integrated farming out of which 64.7% (33/51) had no formal education. The health risks mentioned that were associated with chemical use in urban agriculture included laboured breathing 40%, skin rashes 22% and blindness 2%. The risks associated with use of manure were, bad smells 20%, infections 14% and increased pests 2%. This proportion is similar to what has been previously reported in a related study in Dar es Salaam. In a related study, 51.7% (n=404) of the respondents had knowledge on the health risks associated with the practice of livestock keeping in urban farming in Dar es Salaam, Tanzania (Mlozi, 1997). Additionally, pathogenic microorganisms that include *E. coli* O157:H7 strains have been isolated and characterised in urban areas within Tanzania where livestock keeping was practiced (Lupindu *et al*., 2014). This demonstrates the association of urban farming that involves livestock keeping with the spread of potential pathogenic organisms from livestock and the environment to humans. This is in agreement with other studies conducted in Kenya that indicated a potential public health risks of integrated urban farming that involve livestock keeping (Nyanjuru, 2007). Apparently many people who are aware of the health risks associated with integrated urban farming have been reported to be the ones who are also involved in the practice (Boischio *et al.*, 2006b). The practice of using livestock manure as fertiliser and the use of chemicals such as insecticides in this study was also reported in Ghana where the use of waste water in vegetable farming was found to pose a public health risk to consumers due to contamination by pathogenic microorganisms (Obuobie *et al*., 2006).

5.2 Prevalence of NSF *E. coli* and *Cryptosporidium* spp. Oocysts

A number of studies have demonstrated the potential spread of zoonotic pathogens including pathogenic *E. coli* O157:H7 and *Cryptosporidium* from livestock to humans through water and foodborne sources (Smit *et al*., 2001; Lupindu *et al*., 2014; Ingham *et al*., 2004; Boischio *et al*., 2006a) especially where integrated urban farming is being practiced. In this study, out of 156 samples collected, the overall prevalence of NSF *E. coli* and *Cryptosporidium* from urban farming areas of Dar es Salaam was 23.1% and 10.3% respectively. The prevalence of NSF *E. coli* reported by Lupindu (2014) from a similar study in urban and peri-urban areas of Morogoro region of Tanzania was 13.7% from a total of 1 046 samples collected from different sources.

This prevalence was significantly low $p= 0.0031$ when compared to the one obtained in this study despite the large sample size from which the isolation was done. However, significantly high occurrence of NSF *E. coli* isolates $p< 0.0001$ was also reported in the same study from cattle manure samples as compared to other sources that included soil and water samples. The overall significant high prevalence reported in this study could be due to the inclusion of NSF *E. coli* isolates from poultry manure samples that were collected from some households and used them as fertilizer in their gardens and fish ponds. In addition, this study was also conducted among urban farmers practicing integrated farming with an inclusion of vegetable samples which were not part of the previously reported study. Livestock manure is commonly used as organic fertilizer in vegetable gardens in integrated farming system. However, similar numbers of NSF E. coli isolates were isolated from vegetables and manure despite the number of vegetable samples collected being less than that of manure. This finding indicated the possible persistence of enteropathogenic *E. coli* contamination in vegetables due to the use of livestock manure. Despite the study being a cross sectional study, this result is in

agreement with previous studies that revealed the likelihood of *E. coli* to persist in vegetables grown in gardens supplied with contaminated water in Morocco (Ibenyassine *et al.,* 2006).

Studies have previously been conducted that have indicated occurrence of *Cryptosporidium* in cattle among smallholder farmers within Tanzania (Swai and Schoonman, 2010; Swai *et al.,* 2007; Chang'a *et al.,* 2011). The prevalence of *Cryptosporidium* in this study was similar to the results reported in other studies in cattle within Tanzania (Swai *et al*., 2007). However, the overall prevalence in this study was lower than 54.5% reported in previous studies conducted in Tanzania. This could have been due to the inclusion of diarrhoeagenic cattle including calves despite using the same Ziehl-Neelsen staining method for detection. In this study, the health risk impact for *Cryptosporidium* spp. oocysts on human health is even more heightened with the demonstration of the oocysts not only from livestock manure, but also from vegetables and fish. In this study, the prevalence of *Cryptosporidium* from different sources was within the range of 5.3% to 62%, which is the prevalence range of *Cryptosporidium* infections in animals previously reported in Tanzania (Swai *et al.*, 2007).

Occurrence of *Cryptosporidium* in cattle has also been reported in other African countries including Ghana, where the prevalence ranged from 16.0% to 61.0% (Duedu *et al*., 2014). In Ethiopia, the parasite had been isolated from fruits and vegetables where washing of these products with clean water was found to be significantly associated with reduced parasitic contamination (Tefera *et al*., 2014). The parasite had also been isolated from vegetables in other studies outside Africa including Iran and Korea (Ranjbar-Bahadori *et al*., 2013; Hong *et al.*, 2014). Other studies conducted in Tanzania and Kenya had also found that livestock manure especially from cattle including calves are the most common

cause of vegetable and environmental contamination with *Cryptosporidium* that may pose a public health risk (Chang'a *et. al.*, 2011; Peter *et al*., 2015). The occurrence of the parasite in fish from ponds in Tanzania has not been much documented. However, studies on the natural occurrence of *Cryptosporidium* spp. in fish from the pond intended for human consumption have been reported and an overall prevalence of 10.5% was found in a related study in Nigeria (Atawodi and Bichi, 2013). This finding was similar to the prevalence of 10.3% found in this study. The similar results might be due to the same Ziehl-Neelsen staining technique that was used although, apart from the intestinal contents collected for analysis as in our study, gills were also analysed. But the proportions in this study are much generally lower 10.3% compared to other similar studies reported in fish 39.3% and 37.0% in Nigeria and France respectively (Atawodi and Bichi, 2013; Certad *et al*., 2015). However, these results were recorded in fish from the lake and molecular technique including nested PCR was used for analysis. In addition presence of developmental stages of *C. parvum* were observed in the digestive epithelial cells of the fish, a result that confirms potential contamination of the fish by the parasite hence increased risk of human transmission (Certad *et al*., 2015).

5.3 Antimicrobial Resistance Patterns of *E. coli* Isolates from Different Sources

Different studies have reported antimicrobial resistance among NSF *E. coli* from livestock manure, vegetables and fish (Lupindu *et al.*, 2014, 2015; Mainda *et al*., 2015; Boss *et al.*, 2016). The levels of resistance for the isolates to selected antimicrobial agents in this study were not very different from those obtained in other studies from within Tanzania and in other regions worldwide. Tetracycline and Amoxicillin had the highest proportions of resistant isolates while Ceftriaxone and Ciprofloxacin yielded the least number of resistant isolates 2.1%. Other studies have also reported lowest antimicrobial resistance rates among *E .coli* isolates including *E. coli* O157:H7 as low as 0.0% in countries including

Costa Rica, Belgium and Zambia, (Pérez *et al*., 2010; Holvoet *et al.*, 2013; Mainda *et al*., 2015). The highest level of antimicrobial resistance observed in this study among NSF *E. coli* isolates from different sources pose a health risk that is likely to be associated with urban integrated farming. Further, the finding in this study of NSF *E. coli* isolates being resistant to Ciprofloxacin, a quinolone which is mainly used in human medicine is of great public health concern. This study found that livestock manure especially from cattle still act as the potential reservoir for antimicrobial resistant NSF *E. coli* strains. Just as reported by Holvoet (2013), when MDR patterns of isolates from manure and vegetable sources where compared in this study, isolates from vegetables had a similar MDR pattern to isolates from manure. This finding clearly showed the similar commonality of origin of the isolates despite being isolated from different sources. The sharing of a common source of zoonotic pathogens including *E. coli* O157:H7 can equally result into sharing of genes that confer antimicrobial resistance ability, hence a public health risk to humans (Kelch and Lee, 1978).

The MDR isolates reported in this study is of great public health especially with resistance extended to cephalosporins that include Ceftriaxone and a quinolone, Ciprofloxacin. This finding in this study may indicate the likelihood of presence of *E. coli* strains that produce Extended-Spectrum β-lactamases (ESBLs) that are being reported in increased frequency in many countries (Guardabassi *et al*., 2008). In addition, food of animal origin including fish have been reported to be an important vehicle through which MDR including ESBLs *E. coli* producing strains would be spread to humans hence the risk.

5.4 Genetic Relatedness of NSF *E. coli* isolates from Different Sources

There are many studies previously conducted to determine genetic relatedness of *E. coli* isolates by using ERIC-PCR. ERIC PCR is one of the PCR based typing method used to

determine the genetic similarities of different strains of enterobacteriacea including *E. coli* O157:H7 originating from different sources (Ibenyassine *et al*., 2006; Ateba and Mbewe, 2014; Ali *et al*., 2014). Alternatively, other researchers have used more superior methods like the Pulse Field Gel-Electrophoresis (PFGE) which is like a gold standard method to achieve this objective (Gautom, 1997a; Lupindu *et al*., 2015). In this study, ERIC-PCR successfully discriminated NSF *E. coli* strains of isolates from manure, vegetable and fish into clusters based on their ERIC- PCR DNA profiles produced. Nine clusters were identified with the overall similarity ranging from 74.5% to 100%. These results showed increased discriminatory power by ERIC-PCR among isolates than what was reported by Ateba and Mbewe (2014) who used the same method and found that, out of 94 of *E. coli* O157:H7 isolates, 8 clusters were identified with the overall similarity ranging from 71% to 91%. However, the study results are in agreement with what was reported by Lupindu (2015a) despite the use of PFGE method that yielded isolates with an overall similarity of 53.3% to 100% with 8 clusters identified.

Therefore, these findings also confirm the discriminatory power of bacterial clones by use of ERIC-PCR method to be equivalent to that of PFGE as reported by Ibenyassine *et al*. (2006). Some clusters contained isolates that exhibited 100% genetic similarity and displayed similar MDR patterns despite having been isolated from different sources. An example of such was cluster (I) where isolate 21V from vegetable and 14M from manure had a similar MDR pattern of AML, TE and AMP. This was likely an indication of NSF *E. coli* flow between the two integrated farming components although there is lack of concrete conclusion on the direction of flow as reported by Lupindu *et al.* (2015). Another interesting result in this study showed some isolates from the same household isolated from different sources including manure, vegetable and fish were found not to share 100% ERIC-DNA profiles despite the use of livestock manure as organic fertilizer by the

household. In addition, such isolates even exhibited different drug resistance patterns and were clustered differently in the dendrogram. From the results, some isolates from different sources were observed to be 100% genetically similar. This observation was an indication of pathogen flow between components of the farming system. The results for isolates from the same households and were observed together in clusters (II), (III) and (VIII) agrees with other studies that indicated that strains of the same subtype would lie within the 95% and 100% similarity interval regardless of the optimization and tolerance settings which were both set at 1% in this study as reported by Carrico *et al*. (2005).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study determined the potential health risks that might be associated with urban integrated farming illustrated by presence of *Cryptosporidium* spp. and NSF *E. coli* as indicator organisms in livestock manure, vegetables and fish. The risk would be heightened by the flow of antimicrobial resistant and genetically diverse zoonotic pathogens between different components of the farming systems through husbandry practices employed.

The food sources including leafy vegetables may be a source of transmission for not only of zoonotic, but also of MDR pathogens, which are an indication of faecal contamination especially from animal sources such as cattle which are a reservoir hence posing a health risk to humans. The risk can be heightened especially when the use of essential antimicrobial drugs meant for human medicine is not monitored and controlled.

ERIC-PCR proved to be alternative and cost effective method to separate bacterial isolates including *E. coli* O157:H7 from different sources in urban integrated farming and determine their genetic relatedness.

6.2 Recommendations

In view of the conclusions based on the results of this study, the following are the recommendations;

i. Sensitization of communities by local authorities to raise awareness on the health risks of urban farming and promote good husbandry practices such as treatment of

livestock manure before applied as manure to reduce the risk of transmitting zoonotic pathogens to humans.

ii. Recognising the antimicrobial resistance current findings in this study, there is apparent need to develop national plans to monitor and regulate the use of chemicals and antimicrobials used in the country.

iii. Mitigation measures in urban integrated farming to reduce transmission of antimicrobial resistant bacteria that may pose a public health risk be emphasised.

iv. Further molecular studies should be conducted that would identify and characterise common circulating zoonotic pathogens including *Cryptosporidium* spp. and *E. coli* O157:H7 in livestock, humans, vegetables and fish to determine the species, their associated risk factors of flow, survival and transmission in an integrated urban farming system.

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APPENDIX

Appendix 1: Structured Questionnaire

Date of interview……/……/……

Questionnaire number……..

My name is..... (Name of Enumerator), and I am A Postgraduate Student from Sokoine University of Agriculture in Morogoro. We are conducting a study for the assessment of communities' knowledge, practices and perceptions on the health risks associated with integrated urban farming system in your community.

I would be glad if you allow me to speak to you for a few minutes.

1. SOCIO-DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS SAMPLED POPULATION

SECTION 1: IDENTIFICATION PARTICULARS

DISTRICT:	
DIVISION:	
WARD:	
VILLAGE/STREET:	
NAME OF HHH:	
MARITAL STATUS: 1. Married, 2.Single, 3. Divorced 4. Divorced 5. Widow/Widower	
SEX	1. Male…… 2. Female…….
NAME OF RESPONDENT	
AGE	1=15-19 [ ] 2=20-29 [ ] 3= 30-39 [ ] 4=40-49 [ ] 5= More than 60yrs
RESPONDENT/HH PHONE NUMBER	

1. **Educational status** 0= None [ ], 1= primary [ ], 2= secondary [ ], 3= Bachelors [ ], 4= Post graduate
2. **Religion** 1= Muslim [ ], 2= Christian [ ], 3= other [ ] (Specify) ……
3. **Occupation** 1= Farmer [ ], 2= Entrepreneur [ ], 3= Government worker (Civil Servant) [ ], 4= Private Worker [ ], 5= House wife [ ], 6= Urban Farmer [ ], 7= other [ ] specify……..

2. KNOWLEDGE OF THE RESPONDENT ON THE INTEGRATED URBAN FARMING SYSTEM

4. Have you ever heard of integrated farming system? (If No Skip to 6)
   1= Yes [ ] 2= No [ ]
5. If the answer to Q4 was yes, what is involved in this type of farming?
   1= Keeping of livestock only 2= Keeping of fish only 3= Keeping of vegetables only 4= Keeping the combination of any of the above
6. Are you involved in any type of farming system that involves Livestock in this household?
   1= Yes [ ] 2= No [ ]
7. If your answer to Q 6 was yes, what major type of Livestock do you have?
   1= Cattle [ ], 2= Pigs [ ], 3= Chickens [ ], 4= Sheep [ ], 5= Goats [ ]
8. How do you dispose the manure that comes from your livestock?
   1= Sell [ ], 2= Use as manure [ ], 3= Other (Specify) [ ]……

3.0 ASSESMENT OF THE RESPONDENT ON THE HUSBANDRY PRACTICES ON INTEGRATED URBAN FARMING.

9. Do you process livestock, fish or vegetable products before selling or using them?
   1= Yes [ ], 2= No [ ]

10. If yes, in what form do you sell these products?

    1= Processed and packed [ ], 2=Semi-processed [ ], 3= other (specify) [ ]…

11. Do you process livestock, fish or vegetable by products such as manure, effluent, silt and other solid wastes? 1= Yes,[ ] 2= No [ ]

12. If yes, in what form do you process these by products? 1= Biogas production [ ], 2= Slurry making [ ], 3= Composite manure [ ], 4= Fish feeds [ ], 5= other [ ] (specify) ……

13. Do you administer chemicals or drugs such as antibiotics and pesticides to livestock, fish or vegetables?

    1= Yes [ ] 2= No [ ]

14. If your answer to Q. 13 was yes, what is the reason for doing that?

    1= to treat livestock when they are sick 2= to prevent livestock diseases

    3= to improve or boost productivity 4= other [ ] (Specify)……..

15. What type drugs or chemicals (Names) of do you administer?

    1=Livestock……..

    2=Fish……...

    3=Vegetables……

16. What is the source of your fingerlings?

    1= Own fingerlings [ ] 2= Commercial fingerlings [ ]

17. How often do you administer these drugs/chemicals?

    1= Daily [ ], 2= Weekly [ ], 3= Monthly [ ], 4= only when required

18. Do you observe the withdrawal period indicated for the drugs or chemicals you use before using you farm products?

    1= Yes [ ] 2= No [ ]

19. If No, what mitigation measure do you use to prevent the drugs or chemicals be transferred.to the consumers……...?

20. How do you dispose of the containers for drugs or chemicals after use?

    1= Burn them [ ], 2= Dump them at one site for collection [ ], 3= Burry them [ ], 4= throw them any how [ ]

21. Do you have access to extension services? 1= Yes [ ] 2= No [ ]

22. If yes, what is the distance to the nearest service provider? (In km) [ ]

23. Where do you get your veterinary and agricultural drugs?

    1= Government veterinary officer [ ] 2= Extension officer [ ] 3= Agro-agriculture shops [ ] 4=Agro-veterinary shops [ ] 5= Farmers' groups [ ] 6= NGOs [ ] 8= Own knowledge and experiences [ ] 9= other (specify)…….

24. Who usually administers the drugs to livestock?

    1= Vet [ ] 2= Owner [ ] 3=Other [ ] (Specify)…….

4.0 ASSESMENT OF THE RESPONDENT ON THE PERCEPTIONS ASSOCIATED WITH HUSBANDRY PRACTICES ASSOCIATED WITH URBAN INTEGRATED FARMING

25. Are there any health risks emanating from the management practices of livestock, fish or vegetables farming? 1= Yes [ ], 2= No [ ]

26. If yes, what are the health risks

    ……

    ……

I WOULD LIKE TO THANK YOU SO MUCH FOR YOUR TIME AND THIS IS ALL I HAD TO FIND OUT FROM YOU.