PREVALENCE OF AND FACTORS ASSOCIATED WITH ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* FROM INDIGENOUS POULTRY AND FARMERS IN KITUI RURAL SUB-COUNTY, KENYA

BY

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DECLARATION

Declaration by the Candidate

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DEDICATION

I dedicate this work my sister Elizabeth Kivunzya. You have given me a reason to smile through your constant encouragement and support. To my dear parents Mr. Antony Kivunzya and Mrs. Benedetta Kavata you have lived to celebrate my achievements. To my daughter Joan Wairimu, you are the best gift I have ever received in my life. Lastly, to all my family members and friends, I thank the almighty God for making me one of your own.

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ABSTRACT

Introduction: Antimicrobial resistance (AMR) is a global public health threat amplified by inappropriate antimicrobial-use in humans and poultry. However, data on AMR in households are limited. *Escherichia coli* has been proposed as one of the pathogens to be used for AMR surveillance.
Objective: This study estimated prevalence of resistant *E. coli* and associated factors among farmers and poultry in Yatta/Kwavonza and Kanyangi Wards, in Kitui rural sub-County, Kitui County, Kenya.
Methods: Study design: A cross-sectional study was conducted between July 2017 and January 2018. **Study population** was poultry farmers and indigenous poultry in two randomly selected wards. We targeted households with ≥3 indigenous poultry and farmers' aged ≥18 years. **Sampling strategy:** Number of households sampled per ward were calculated proportionate to size and geocodes randomly generated using ArcGIS to identify household to be sampled. **Data collection tools:** Semi-structured questionnaires were used to collect data on demographics, poultry management and antibiotics. Inhibition zones were measured using a ruler and used a camera to capture indigenous poultry and zones of inhibition. **Sample collection:** In each household, stool sample from one household member was collected. Cloacal swabs from three poultry were also obtained and pooled together to form one sample for that particular household. *E. coli* was isolated and drug sensitivity testing done using disc diffusion assay. Multi-drug resistance (MDR) was defined as resistance to ≥3 antibiotics. **Data entry:** Data was entered into EPI databases. **Data analysis** done using Ms Excel 2007 and Epi Info. Proportions for individual 10 antibiotics was calculated and Odds ratio (OR) with 95% Confidence Intervals (CI) to identify factors associated with AMR in poultry and farmers. **Data presentation** was in prose and tables.
Results: A total of 134 farmers were enrolled, 91 (68%) from Yatta/Kwavonza and 43 (32%) Kanyangi with a mean age of 44 and 48 years respectively. Overall, 134 farmers' stools and 134 poultry cloacal swabs were collected. *E. coli* was isolated from 82 (62%) farmers among whom 59 (72%) were from Yatta/Kwavonza and 23 (28%) from Kanyangi. Fifty (84.7%) farmer *E. coli* isolates in Yatta/Kwavonza and 18 (78.3%) Kanyangi had resistance to at-least one antibiotic. Tetracycline was the antibiotic with the most resistant in both Yatta/Kwavonza 25 (42.4%) and Kanyangi 9 (39.1%). In poultry *E. coli* was isolated in 90 (67%) of the sample collected, 61 (68%) from Yatta/Kwavonza and 29 (32%) Kanyangi. Resistance to at-least one antibiotic was observed in 57 (93.4%) in Yatta/Kwavonza and 26 (89.7%) Kanyangi in poultry *E. coli* isolates. Amoxicillin 29 (47.5%), in Yatta/Kwavonza and streptomycin, 15 (51.7%) in Kanyangi were the most resistant antibiotics. Multidrug resistance was demonstrated in 24 (41%) and 10 (43.5%) farmers *E. coli* isolates and in 23 (38%) and 16 (55%) poultry *E. coli* isolates in Yatta/Kwavonza and Kanyangi respectively. There were no statistically significant factors associated with antimicrobial resistant *E. coli* in relation to poultry husbandry or antibiotic use in farmers and poultry.
Conclusion: The study found a high AMR prevalence in poultry and farmers with significant levels of MDR. No factors were statistically responsible for *E. coli* resistance. **Recommendation:** Genotyping of all the *E. coli* isolates in this study and further research to investigate other causes for AMR at poultry-farmer interphase.
Key words: Antimicrobial, Resistance, Poultry, Farmer, *E. coli.*

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DEFINITION OF TERMS

Antibiotics - substances that are produced by one microorganism, that act against another microorganism.

Antimicrobial - any substance of natural, semisynthetic or synthetic origin that kills or inhibits the growth of microorganism causing little or no damage to the host. Thus antibiotics will be classified as antimicrobials. Antibiotics and antimicrobials will be used interchangeably.

Antimicrobial resistance - ability of microorganism to overcome the effect of antimicrobial drug.

Emergence of antimicrobial resistance - conversion from wild-type to resistance phenotypes.

Household- a group of people who live together and have a common cooking area.

Indigenous poultry-domesticated indigenous chicken raised by humans for production of meat, eggs or both

Intermediate resistant drug: - drug that inhibits growth of organism but the therapeutic effect is uncertain.

Multidrug resistance – resistance exhibited to ≥3drugs antibiotics of different class.

Resistant drug – inability of a drug to inhibit growth of organism

Spread of antimicrobial resistance - dissemination of resistance between hosts and the environment

Susceptible drug – inhibition of organism growth when exposed to the drug & has high probability of therapeutic success.

Poultry – indigenous/ local/unimproved chicken

Zoonosis - any infection that is transmissible between vertebrate animals and humans.

ABBREVIATIONS AND ACRONYMS

AMGP	Anti-Microbial Growth Promoters
AMR	Antimicrobial Resistant
US CDC	United States Centers for Disease Control and Prevention
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
OECD	Economic Cooperation Countries Development
FAO	Food Agriculture Organization of the United Nations
FELTP	Field Epidemiology and Laboratory Training Program
FGD	Focused Group Discussion
GDP	Gross Domestic Product
GIS	Geographic Information System
GIT	Gastrointestinal Tract
GPS	Global Positioning System
HIC	High Income Country
IREC	Institutional of Research and Ethical Committee
KNBS	Kenya National Bureau of Statistics
LMIC	Low and Middle Income Countries
MDR	Multi-Drug Resistance
MERS	Middle East Respiratory Syndrome Coronavirus
MH	Muller Hilton
MTRH	Moi Teaching and Referral Hospital
PCU	Population Correction Unit
SARS	Severe Acute Respiratory Syndrome

SES	Social Economic Status
TB	Tuberculosis
US	Unites States
USD	United States Dollars
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Since the discovery and use of antimicrobials in the 1940s, morbidity and mortality in humans has reduced (Kirika, 2009), while their use in animals has contributed to food security and improved livelihoods (Marshall & Levy, 2011). However, indiscriminate antimicrobial drug use has led to selective pressure for resistance among pathogenic and endogenous flora of exposed animal and human populations causing emergence of Antimicrobial Resistance (AMR) (Marshall & Levy, 2011). *Escherichia coli (E. coli*) is a commensal bacterium of humans and animals and has also pathogenic variant that causes intestinal and extra intestinal infections (Daniel *et al*., 2012). The commensal bacterium *E. coli* is used as a sentinel for monitoring antimicrobial resistance in fecal bacteria because it is found more frequently in a wide range of hosts and acquires resistance easily (WHO, 2001).

Indigenous poultry account for 80% of total poultry population in the world (Sitati, 2017). In Kenya, it constitutes approximately 77% of the indigenous poultry (Sitati, 2017). Poultry numbers are increasing every year and poultry are found in 90% of rural communities (Bergevoet & Engelen, 2014; Sitati, 2017).

In poultry, antimicrobials are used to maintain health and productivity, whereas most of these antimicrobials are also used in human medicine for treatment as well as medical procedures (Laxminarayan *et.al.,* 2013). As a result, fecal flora of poultry contains high proportion of resistant bacteria and has been reported to be a source of resistant bacteria for poultry farmers (Amy *et. al.*, 2011).

Farmers are directly exposed to poultry feces daily as they feed, collect eggs, clean poultry houses and others sleep with their poultry in the same household especially in the rural areas. Antimicrobial resistance *E. coli* from poultry is normally harmless to the poultry itself but may cause infection in the flock-mates (Amy *at al.,* 2011). It may also be transferred to humans through contact, environment or via food chain. When AMR *E. coli* colonizes the intestinal tract, it may result to endogenous flora resistance genes to human (Van Boeckel *et al.,* 2015). Linking antimicrobial consumption in poultry to drug resistance is characteristically complex due to the ecological nature of selection pressure for drug resistant pathogens as well as indirect route of transmission through environmental pollution (Van Boeckel *et al.,* 2015). Therefore the epidemiology of spreading of antimicrobial resistance from food animal including poultry to human is not well understood; and more knowledge is needed to define measures that can lead to reduction of AMR *E. coli* in indigenous poultry and poultry farmers.

Projections shows that AMR will cause 100 million human fatalities and reduce global economy by $100 trillion by 2050 (O'Neill, 2014). In Europe and US, AMR infections claim at least 50,000 human lives every year and hundreds of thousands more die in other counties across the world (O'Neill, 2014; Van Boeckel *et al.,* 2015).

1.2 Problem Statement

Indigenous poultry constitutes the largest percentage of poultry in Kenya and is reared by more than 98% of farmers in Kitui with most households with 10-20 birds (Murangiri *et al.,* 2016). Being a rural setting, farmers keep indigenous poultry in the same house where people live with farmers mixing the healthy and the sick, the young and the aged poultry together. Due to inaccessibility of animal and human

health services farmers seek over-the-counter medications for both human and poultry from drug stores or from unqualified service providers, leading to drug misuse. This increases the chances of drug resistance development in poultry and farmers. Most of the poultry farmers handle their poultry without proper protection, a practice which poses a risk of spreading or transferring resistant pathogens among poultry and poultry farmers (Szmolka & Nagy, 2013; Graham, 2016). Again, in Kitui just like in other places, poultry waste is used as farm manure in most households which has also have been pointed out as one source of antimicrobial resistant bacteria that can be transferred to human (Szmolka & Nagy, 2013; Graham, 2016). However, there is dearth of information on the magnitude of the antimicrobial resistant bacterial in indigenous poultry and farmers in Kitui. While some information is available on AMR, its import to note that, these studies have been conducted mainly on hospital setting for humans (GARP-KWG, 2011) while in livestock/poultry the little data available is from market places or intensive poultry production systems with no studies conducted on rural household setup like Kitui (GARP-KWG, 2011, Kikuvi *et al.*, 2013 Ayul & Ajak, 2017).

1.3 Justification

Antimicrobial resistance (AMR) results in increased poverty, mortality, prolonged hospital stays and increased costs of treatment (WHO, 2010; Laxminarayan *et al.*, 2013). Economic losses due to reduced productivity of animals and humans suffering from infections caused by drug-resistant bacterial agents impacts negatively on food security, trade and economies of rural households. AMR in humans cannot be effectively fought without fighting AMR in animals since most bacteria pathogenic to human originate from animals and similar antimicrobial molecules are used to treat infections in humans and animals. This therefore calls for the need to generated

information on AMR in humans and livestock in Kitui that will be used to formulate appropriate interventions for prevention, slowing down and control of the resistant bacteria. It is anticipated that results from this study will informs policy and contribute to AMR surveillance locally, regionally and internationally.

1.4 Expected Outcome

The findings will establish prevalence of AMR *E. coli* in indigenous poultry and farmers in a rural set-up of Kitui County. The County Ministry of Agriculture and Livestock Development and fisheries together with Ministry of Health will utilize the information to inform policy on human and veterinary drug use for treatment and control bacterial infections. The two ministries will use the final results to strengthen AMR surveillance in the study area, improve awareness and understanding of antimicrobial resistance; strengthening the knowledge and evidence base on AMR; reducing the incidence of infection. The information will also be used to educate societies and communities on health impact due to AMR and associated factors.

1.5 Research Questions

1. What is the prevalence of antimicrobial resistant *E. coli* among indigenous poultry and farmers in Yatta/Kwavonza and Kanyangi Wards?
2. What factors are associated with antimicrobial resistant *E. coli* among indigenous poultry and their farmers in Yatta/Kwavonza and Kanyangi Wards?

1.6 Objective

1.6.1 The main objective of the study

To determine prevalence of and factors associated with antimicrobial resistant Escherichia coli from indigenous poultry and farmers in Kitui-rural Sub-county, Kenya.

1.6.2 Specific objectives

1. To estimate the prevalence of resistant *E. coli* among indigenous poultry in Yatta/Kwavonza and Kanyangi Wards.
2. To estimate the prevalence of resistant *E. coli* among poultry farmers in in Yatta/Kwavonza and Kanyangi Wards.
3. To identify factors associated with antimicrobial resistant *E. coli* at the household level among the farming community in in Yatta/Kwavonza and Kanyangi Wards.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Drug Resistance

Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial drug to which it was previously sensitive. Bacteria, viruses, fungi and parasites that become resistant are able to withstand the effects of antimicrobials (e.g. antibiotics, antivirals, antimalarials, antifungals). This makes standard treatments ineffective and the community vulnerable as drug resistant infections can spread.

Globally, AMR limits therapeutic option for treatment of infection in both human and livestock (Kimang'a, 2012) and contibutes to the global post antibiotic era where infections which used to be treated earlier can now cause death (WHO, 2010; Kimang'a, 2012). Reliable estimates of the true burden of AMR infection are scarce, though it is estimated that, AMR infections claim at least 50,000 lives every year in Europe and US alone and hundreds of thousands more dying in other counties across the world (O'Neill, 2014; Van Boeckel *et al.,* 2015). The pattern of AMR varies with different countries often experiencing different problems (O'Neill, 2014; Van Boeckel *et al.,* 2015). First generation antibiotcs are of little use today while second and third generation antibiotics are losing effectiveness in treatment of infections (GAP-KWG, 2011) and that is why WHO is in the process of putting in place interventions for reclaiming the effectiveness of antibiotics against resistant bacteria (WHO, 2010).

2.2 Emergence and Spread of Antimicrobial Resistance

Inappropriate exposure to antimicrobials in human or animals coupled with poor prevention and control practices to infections imposes selective pressure on the bacterial population (Van Boeckel *et al.,* 2015). This allows only resistant sub-

populations of bacteria to survive contributing to emergence and spread of antimicrobial resistance which can be intrinsic or acquired. (Alekshun & Levy, 2007; WHO, 2010; Cox & Wright, 2013). Intrinsic resistance occurs naturally in the genes in the bacterium's genome or inherent characteristics of the bacterium that allows the bacteria tolerance specific antimicrobials (Alekshun & Levy, 2007; (WHO, 2010) . This type of resistance is common to all types of a bacterial species, independent of the selective pressure from antimicrobials (Cox & Wright, 2013). In acquired resistance, bacteria acquire the ability to resist a specific antimicrobial agent to which it was previously susceptible. These traits are found in sub-populations or some traits of a bacterial species, hence the difference with intrinsic resistance (Alekshun & Levy, 2007).

2.3 Mechanisms of Acquiring Resistance

Resistance genes by bacteria are acquired either naturally or through spontaneous mutations in chromosomal genes (Alekshun & Levy, 2007; Sykes, 2010). Horizontal transfer of genes can occur within a bacterial species or over species boundaries either by uptake of naked DNA or through the integration of DNA in plasmids (Sykes, 2010 ; Alekshun & Levy, 2007). Multiple antimicrobial resistance can occur from a single gene transfer (Economou & Gousia, 2015). Antimicrobial use creates optimal environment for resistance to emerge and multiply in the absence of susceptible competitor. Exposure to low concentrations of bactericidal antimicrobials, such as betalactams, fluoroquinolones, and aminoglycosides, may stimulate bacteria to produce reactive oxygen species (Kohanski, *et al.*, 2010) which damage bacterial DNA, resulting in the accumulation of mutations. Figure 2.1 explains how resistance occurs in bacteria.

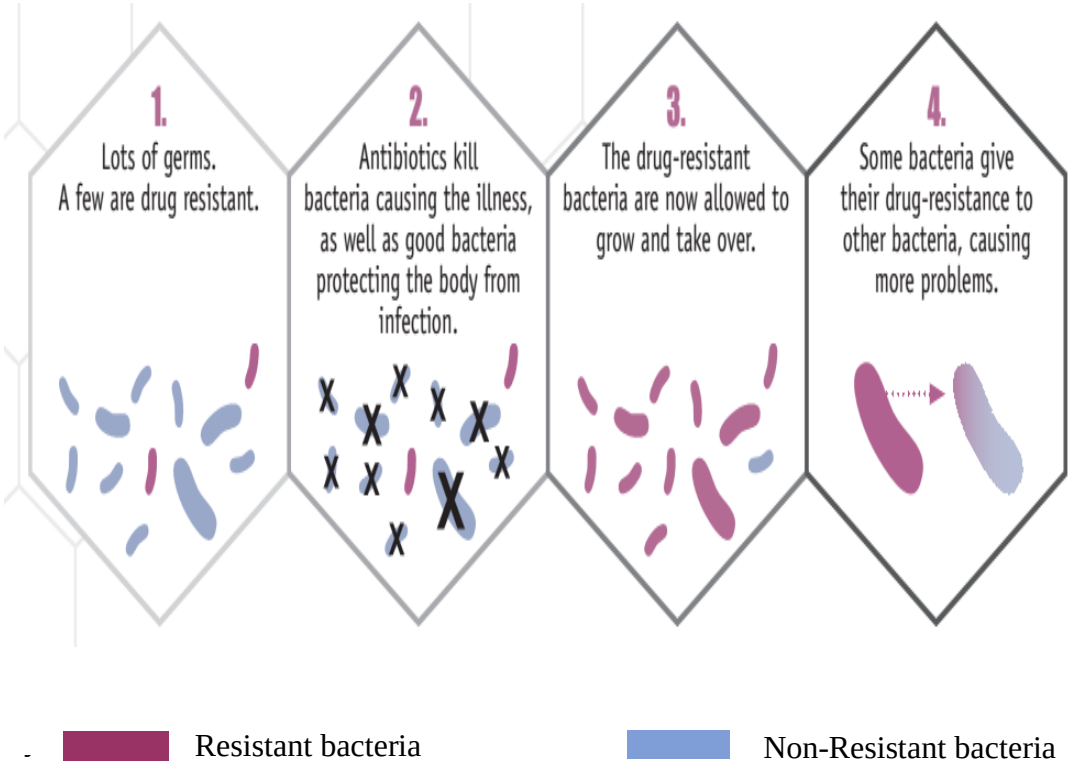


Figure 2.1: Illustration of development of resistance in bacteria.

Source **(CDC, 2013)**

2.4 Spread of Antimicrobial Resistance

Antimicrobial resistance can be spread by inheriting the resistant genes or by sharing or exchanging resistant genes with other bacteria either within or between bacteria (CDC, 2013; Hummel *et al.*, 1986). The resistant bacteria can then spread by contact (skin to skin), through excreta, through saliva or exposure to contaminated food, feed, air or water (Michael A. Kohanski, 2010). Human or animal waste containing resistant bacteria can contaminate the environment when used as manure by the farmers or washed out to water bodies (Marshall *et al.*, 2009;Wellington *et al*., 2013). Resistant bacteria then spread to human through contact, irrigation of crops, water or wildlife. In the new host the bacteria can colonize, infect and spread their resistance gene to other bacteria and also acquire other resistant genes from them (Michael A. Kohanski, 2010). Resistant bacteria can then be disseminated to other areas by

movement of animals, human or foods (Laxminarayan, *et al.*, 2013) as shown in the figure 2.2.

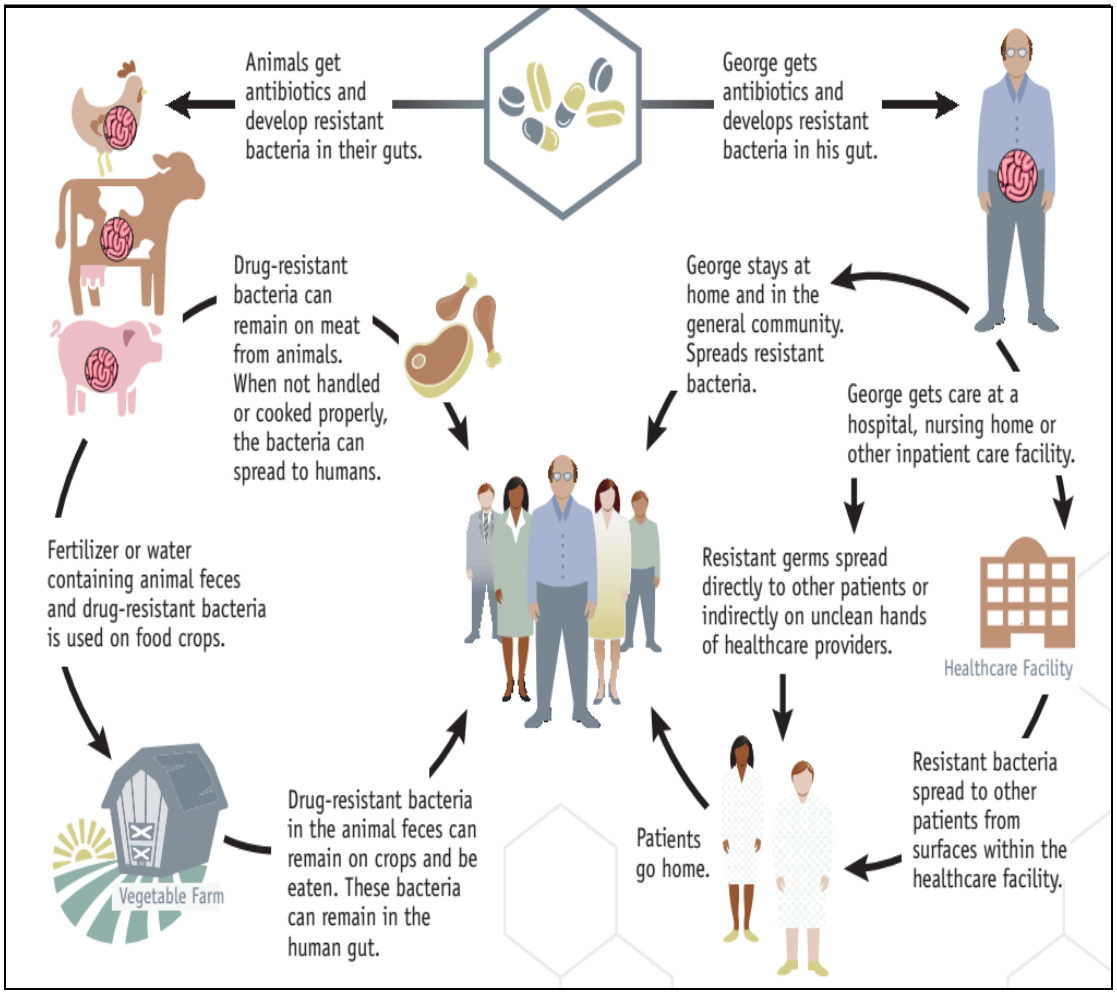


Figure 2.2: Illustration of spread of antimicrobial resistance.

***Source:*(CDC, 2013)**

2.5 Global Antimicrobial Use in Food Producing Animals

Data has shown that improper antimicrobial use in livestock leads to reduced effectiveness in humans (Laxminarayan *et al.*, 2013). Increased demand of animal products from poultry has lead to increased use of antimicrobials in animals with the aim of improving productivity. Different drugs are used by farmers for treatment or control of infections among food producing animals Figure 2.3 shows antimicrobial use in cattle, poultry and pigs.

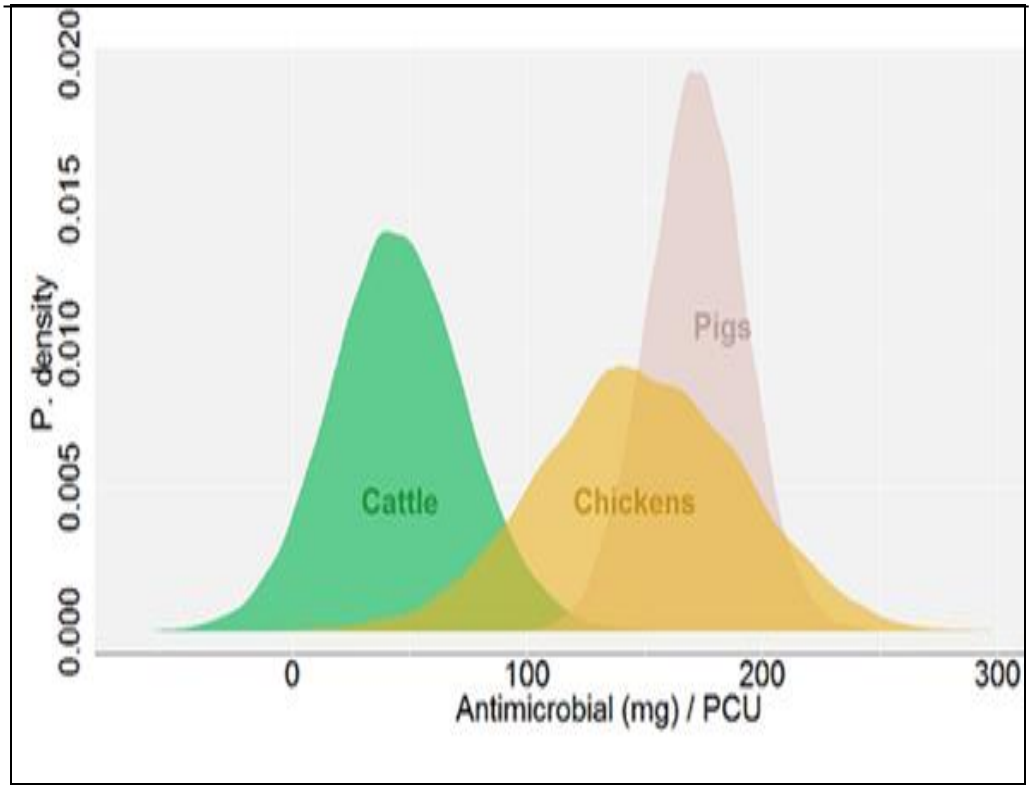


Figure 2.3: Global antimicrobial use in cattle, poultry and pigs in Organizational Economic Cooperation Countries Development (OECD)
Source: **(Van Boeckel *et al*., 2015).**

Poultry has been found to use a wide range of antimicrobials as compared to cattle and pigs globally. Such antibiotics include tetracyclines, sulphonamides, aminolycosides, betalactams, quinolones and macrolides. In 2010, China recorded the highest use of antimicrobial at 23%. Other countries included United states 13%, Brazil 8%, India 4% and Mexico 2%. However, it was projected that by 2030, antimicrobial use will increase in Myanmar to 205%, Indonesia 202%, Nigeria 163%, Peru 160% and Vietnum 157% (Van Boeckel *et al.*, 2015).

2.6 Antimicrobial Resistance in the African Region

In the African region, there is scarcity of accurate and reliable data on AMR, though it is claimed that Africa shares the worldwide trend of increasing drug resistance

(Hedberg *et al.,* 2009). For example, Mudhume and Wamae reported the increase of cotrimoxazole resistance to pneumonia infections from 19% in 2003 to 69% in 2009 (Mudhune and Wamae, 2009). Studies contacted in Zimbabwe demonstrated antibiotic resistance of >90% of the isolates to tetracycline and sulphamethaxazole/trimethoprim. *E. coli* showed resistance of 84% to ampicillin and 68% to cotrimoxazole (Mason *et al.,* 1998). For the period 1990 - 2000, Nigeria studies have shown increase of antibiotic resistance, for example ampicillin from 70% to 90%, co-trimoxazole 77% to 85%, streptomycin 71% to 79% and Nalidixic 0% to 11,3%. In South Africa, Pretoria a study over 20 years on *Neisseria gonorrhea* shows that penicillinase producing *Neisseria gonorrhea* strain increased from 4% to 16%. High levels of 36% resistance to tetracycline were reported while emergency of 7% resistance to ciprofloxacin was recorded in 2004 (Kimang'a, 2012).

Inadequate access to basic health care, limited laboratory capacity for AMR testing and reporting together with inadequate laboratory reagents complicates the issue of AMR in the African region (WHO, 2010). In addition, Africa has weak regulatory capacity, fragmented medicine supplies and distribution systems leading to substandard/counterfeit products being brought in to supply chain which increases the chances of AMR (WHO, 2010).

2.7 Antimicrobial Resistance in East Africa

A review of studies conducted on AMR in East Africa revealed high levels of AMR to commonly used antibiotics (Lucas *et al.,* 2012). Resistance to ampicillin and cotrimoxazole ranged between 50% 100%, emerging resistance to gentamicin 20% - 47% and cefriaxone 46% - 69% in humans (Lucas *et al.,* 2012). A study on poultry in

Rwanda showed 43.3% of *E. coli isolates* had multidrug resistance to four antibiotics, gentamycin 3.7%, doxycycline 84% while erythromycin 98.8%.

In Uganda, AMR situational analysis showed that, the problem of AMR is rising marked by high hospital based mortality of 18.4% due to pneumonia, tuberculosis, and sepsis. Reports shows resistance of up to 80% to common used antibiotics such as penicillin's, tetracycline's, cotrimoxazole. Gram negative enter bacteria has reported resistance of 4% to 30% against carbapenems, a last line treatment (Uganda National Academy of Sciences (UNAS), 2016).

In Tanzania, a study on children < 5years with diarrhea in four hospitals in Dar es Salaam indicated resistance to diarroegenic *E.coli* of 56% to 100% in erythromycin, ampicillin, chloramphenicol and tetracycline (Moyo *et al.,* 2011). Challenges such as malnutrition, infectious diseases and poverty increases the chances of AMR. This is complicated by lack AMR of surveillance in the region.

2.8 Antimicrobial Resistance in Kenya

AMR in Kenya have been demonstrated in animals, humans and in the enviroment. Resistant *E. coli* isolated from Athi-river showed varying degrees of AMR and multi-drug resistance of up to seven antibiotics with most resistance to ampicilin and cefoxitin (Wambugu, *et al.,* 2015). In humans, studies and reports demonstrates increasing levels of AMR in respiratory infections, enteric infections and infections aquired in the health facility (GARP_KWG, 2011). For instance in Kilifi 2005, *E. coli* recorded 85% resistance to cotrimaxole,78% resistance to amoxicillin and 42% to chloramphenicol which are common antibiotics (GARP_KWG, 2011). In poultry, a study on farmers practising intensive commercial chicken farming found 72% resistance to tetracyclines (Kariuki *et al.,* 1999) while Kikuvi and others reported

AMR of 74% in poultry samples collected from martket outlets. Antibiotic resistance was recorded in common drugs such as ampicillin, streptomycin, tetracycline, sulphamethaxazole/trimethoprim and kanamycin ranging between 42.5% to 11.9% (Kikuvi *at al.*, 2013).

2.9 General Aspect of Antimicrobial Resistance

Emergence of antimicrobial resistance is a natural biological phenomenon in bacterial evolution, as the survival of bacteria with the phenotypical traits best adapted to the current environment (Sykes, 2010). Indiscriminate use of antimicrobial causes selective pressure on the microbial population killing most infectious microbes as immune systems deals with the rest (Sykes, 2010). This gives opportunity to resistant mutants in the population to multiply and increase in numbers putting the patient at higher risk of a resistant infection in the future (WHO, 2010). More bacteria are becoming resistant to common antibiotics and this is the most significant future threats to public health (WHO, 2010). Antimicrobial resistant occurs mostly in microorganisms that are likely to be transmitted in the community such as pneumonia, diarrheal diseases and tuberculosis (TB) (WHO, 2010).

2.10 Economic Cost of Antimicrobial Resistance

Given the lack of data, economic impact of AMR estimated that 10 million people would be dying every year leading to reduction of Gross Domestic Product (GDP) by 2% to 3.5%, costing the world about 100 trillion United States Dollars (USD) by the year 2050, based on drug resistant bacteria and public health issues (O'Neill, 2014). It is evident that according to the estimation, deaths due to AMR infections would be more than those caused by cancer in 2050 (O'Neill, 2014). It is estimated 28.3 million people are extremly poor of which 26.2 million are in low income countries however,

AMR impact negatively on the efforts to reduce extreme poverty by < 3% by 2030. (World Bank, 2016).

With antimicrobial resistance, the medical cost of treating a patient increase due to use of more expensive antimicrobials, labour cost, laboratory cost, cost of extra days stay in hospitals due to failure of initial theraputics, cost of isolation and control of other infections. Estimatimations of the care of patient shows that 77% of the total cost goes to bed, 2% to labotarory services and 21% to drugs and the cost of hospital stay reduced by 3% if patients staying for four or more days reduce their days in hospital by one (Howard *et al.*, 2018) Again, increase in resistance to first line drugs increases treatment cost up to 8 folds (GARP-KWG, 2011) while AMR, in livestock production could decline (World Bank, 2016). Figure 2.4 shows showing estimated deaths due to antimicrobial resistance globaly by the year 2050.

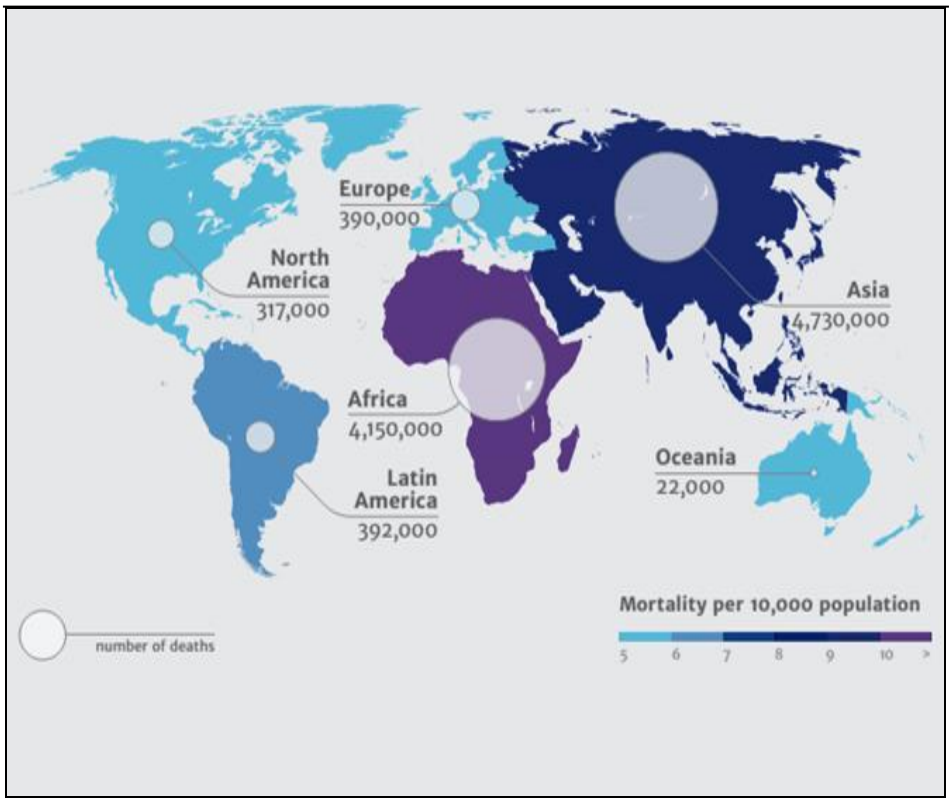


Figure 2.4: Estimation of deaths by continent due to AMR by the year 2050.
Source: (Van Boeckel et al., 2015)

Today, cancer is the leading cause of deaths (Figure 2.5). However, antimicrobial resistance is estimated to be the leading cause of deaths by 2050. This increase of deaths caused by AMR will be 14.2 times higher than today (O'Neill, 2014). Figure 2.5 shows comparison of death due to AMR and other causes.

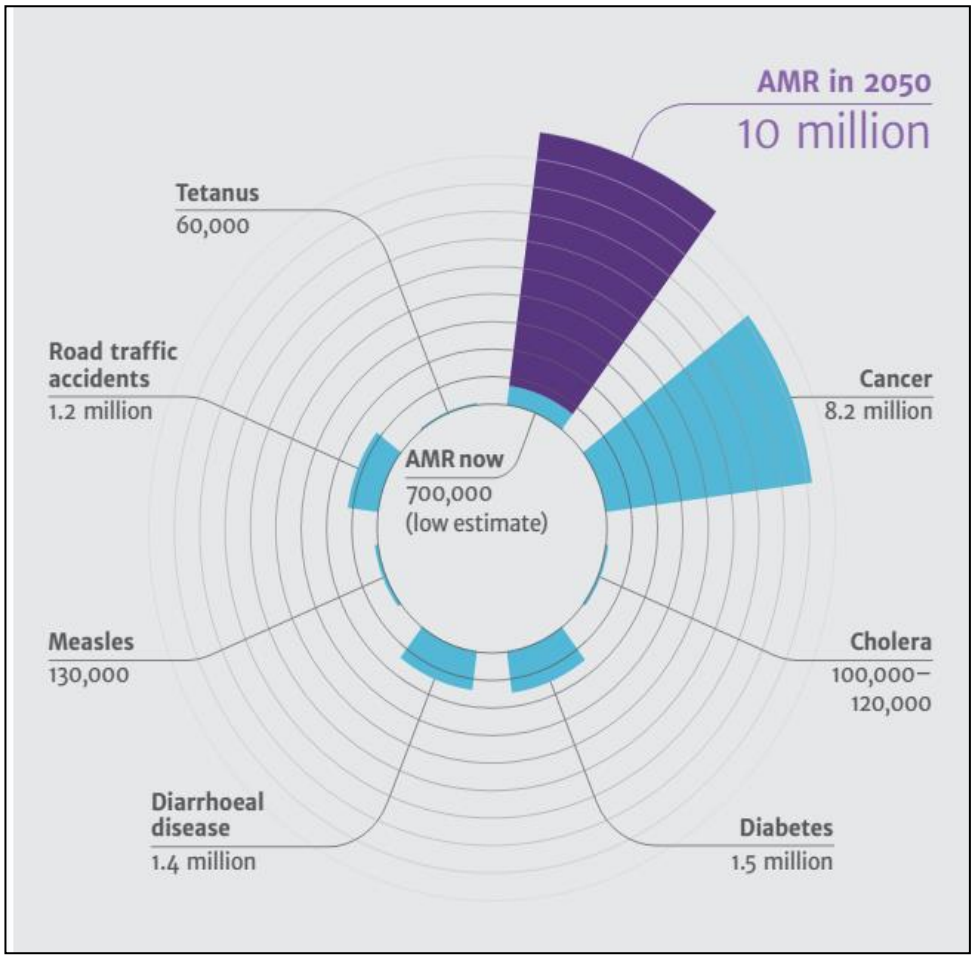


Figure 2.5: Comparison of causes of deaths (globally) attributed to AMR and other infections every year by 2050
***Source:*O'Neill, 2014)**

2.11 Factors Promoting AMR in Human

Antibiotic use is the major factor selecting for antimicrobial résistance in bacteria. Studies described limited capasity to diseases diagnosis, over prescription at health facilities due to fear of drug failure, severe and nasocomial infections as drivers to AMR at the health facilities (Omulo, *et al.*, 2015). Health professional may subscribe

unjustified prescription, for example in cases of diarrhoea and common cold or may give under-dose or short duration treatment. At the personal level factors such as self-medication, non-compliance to treatment or premature discontinuation of medication when a patient feels better may lead to AMR (Roess *et al.*, 2015; WHO, 2010). Social economic status (SES) (Roess *et al*., 2015) of the patient can also influence how the health providers prescribe their drugs, for instance, people in crowded areas normally experience infections more frequently leading to more often treatment with antibiotics. Infections can also be promoted by movement of people from one place to another while distance to health facilities and poor sanitation increases the chances of antibiotic use and is associated with SES (Roess et al., 2015). For example a study by Kariuki and others reported high prevalence of non-typhoidal *Samonella* in children living in informal settlement compared to those from high socio-economic class (Samuel Kariuki *et al.,* 2006). People living in rural areas have limited access to health facilities and fewer of effective antibiotics (Omulo *et al.,* 2015). Sharing housing with poultry, sharing water sources with animals and use of poultry waste as fertilizer has also promoting antimicrobial resistance in human (Roess *et al.*, 2015)

2.12 Factors Promoting AMR in Poultry

Antimicrobial use in poultry has been associated with emergency of antimicrobial resistance in poultry (Vuuren, 2001; Roess *et al.*, 2015). Antibiotics have been used for prophylaxis and treatment in poultry (Roess e*t al.*, 2015). The list of antibiotic used in poultry includes all major classes of antibiotics used in human medicine. The emergence of quinolone-resistant *Campylobacter* species has been linked to the use of quinolone enrofloxacin in poultry (Vuuren, 2001). Owning other livestock or owning ≥ three poultry has been associated with use of antibiotics which in turn may lead to AMR (Roess *et al.*,2015).

Again, soil can be a major source of antimicrobial resistance to poultry. This is based on the fact that soil is the original source of antibiotics used in veterinary and human health (Woolhouse *et al.,* 2015a). A study in Kiambu, Kenya has demonstrated the most classes of antibiotics used in poultry with tetracycline leading at 64%-52.4%, sulphonamides 25.2% -38.5%, quinolones 6.7%- 7.9% and nitrofurans 2.4%-2.9%. (Wanjiru, 2014).

2.13 *Escherichia coli* as an Indicator of Antimicrobial Resistance

Escherichia coli is one of the many bacterial (commensal) species that normally colonizes the gastrointestinal tract (GIT) of warm-blooded animals and humans (Wellington *et al.,* 2013). Although commensal bacteria may not cause harm to the host, depending on certain conditions or individuals, they may cause infections. When drugs are used for any reason, the commensals are also exposed to selective pressure. Hence the belief that, resistance emerges from commensals and spreads to other pathogens through conjugative plasmids (Wellington *et al.*, 2013). The level of AMR among commensal bacterial is considered a good indicator of selective pressure for antimicrobials hence it predicts the emergence of resistant pathogens. The largest commensal population in the body are found in the GIT, thus acting as the main reservoir for multidrug resistance (MDR) bacteria (Wellington *et al.,* 2013). Due to its ubiquity, ease with which it acquires conjugative plasmids and relevance to human medicine, *E. coli* from feces are often used in resistance monitoring programmes as an indicator for acquired antimicrobial resistance by gram-negative bacteria (Swedres-Svarm, 2013).

2.14 Antibiotics of Interest

Several bacterial phenotypes have emerged with resistance against certain antibiotics of interest include trimethoprim-sulfamethoxazole (TMP-SMZ), quinolones/ fluoroquinolones, extended-spectrum cephalosporins, aminoglycosides and other commonly used drugs. These agents are used in the treatment of human *E. coli* infections, as well as poultry. Quinolones group of drugs are very important medicine in human (WHO, 2010) and resistance to them is of great concern and normally resistance emergence of resistance against antibiotics can be due to frequent exposure to them (WHO, 2010). More than 50% of antibiotics used in livestock are tetracycline, followed by sulphonamide at 21% while the rest is aminoglycosides, beta-lactams, quinolones and macrolides. A fifth of the mean consumption per year is in poultry (GARP-KWG, 2011).

2.15 Detection of Antimicrobial Resistance

Antimicrobial resistance can be detected using phenotypic or molecular method. Disc diffusion is an example of the phenotypic method.

2.15.1 Disc diffusion test

Disc diffusion is a method of antimicrobial susceptibility testing manifested by inhibition of bacteria in Muller Hinton Agar. Its commonly used for *E. coli* and *Staphyloccocus aureaus*. The bacteria of interest is streaked on the media to produce confluent growth. Antimicrobial-impregnated disc are then place on the streaked agar using a forceps and incubated for 18-24 hours at 37^0 C. Antimicrobial diffuses from these disc into the media inhibiting growth of susceptible bacteria which manifest as clear zone. The diameter of respective inhibition zone is directly proportional to concentration of the tested antimicrobials. The zone diameter are measured using a

ruler and interpreted according to Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2010).

2.16 Antimicrobial Resistant *E. coli* in Poultry

Antimicrobials are used in both animals as in humans for therapy and control of bacterial infections. However, antimicrobial agents may be continuously fed to food animals such as poultry as antimicrobial growth promoters (AMGP) (Roess *et al.*, 2015). If not professionally used, antimicrobial drugs can cause selection pressure to both enteric pathogenic bacteria and endogenous flora in exposed individual humans and animals (London *et al.*, 2001). In Poultry, mostly antibiotics are administered to whole flocks rather than individual birds.Van den Bogaad and Stobberingh, (2000) stated that, antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant phenotypes. Amaechi *et al.*, (2015) found that the resistant strains from the gut of poultry could soil the carcass during slaughter while Zahraei and Bonab, (2006) stated that the eggs from poultry are contaminated when hens are laying. Therefore the resistant *E. coli* can infect human directly via food. Research by Momtaz *et al.*, (2012) on human volunteers showed that *E. coli* resistant bacteria from gut of poultry may colonize the human intestinal tract and may also contribute to resistance genes to human endogenous flora. The spreads of an antibiotic resistance is through plasmid pSL222-6, in *E. coli* from poultry to humans Marshall & Levy, (2011). In high income countries, studies done by Van den Bogaad and Stobberingh, (2000) and Kohanski *et al.*, (2010) showed that poultry were source of antibiotic resistance for poultry farm workers while London (2001) and others, agreed that poultry farm workers together with their family members in low- and middle-income countries (LMIC) are at an increased risk for carriage of antibiotic-resistant bacteria and

diarrhoeal pathogens than the general population London *et al.*, (2001). This was explained at the National Academies press workshop by Wegener, (2012) that in LMIC, people generally live in close contact with livestock than in high income countries (HIC) and this is linked to increased risk for diarrhoeal diseases.

In Jamaica, increased resistance to fluroquinolones, tetracycline, kanamycin and Nalidixin has been noted on healthy broiler poultry (Miles *et al.*, 2006), and that resistance to more than one drug was also noted in both human and poultry in the same region (Miles *et al.*, 2006). In Korea, a study comparing *E. coli* isolate resistance among poultry and swine workers and non-livestock workers as control group showed a high bacterial resistance to ampicillin, tetracycline and gentamycin, and that the resistance was higher in the livestock workers than in non-livestock workers and the assumption was that, AMR transferred from animal to human (Cho, Lim, & Kang, 2012).

2.17 Zoonotic Aspects of Antimicrobial Resistance *E. coli*

There is a link between antibiotic use in food producing animals and the occurrence of AMR infection in humans. Studies by Call *et al.*, (2013) and Marshall & Levy, (2011) showed that many pathways exist for the spread of resistant *E. coli*: According to Marshall & Levy, (2011), *E. coli* can cause infections in humans by itself and also has the potential of zoonotic AMR in the food-chain, due to transfer of resistance genes from animal origin commensals to human pathogenic bacteria. Though proper cooking of livestock product can inactivate bacteria, improper handling of the faecal contaminated food pose risks to humans to be colonised by AMR *E. coli* (Marshall & Levy, 2011). According to Hammerum & Heuer, (2009) the colonization of the human gut by animal derived strains may be transient, however, there may be need

sufficient time for transfer of resistant genes to them. High prevalence of AMR *E. coli* is a good indicator for selective pressure to antibiotic use and resistance to be expected in pathogenic bacteria while low prevalence of resistance in the intestinal flora in food-animals indicates quality and safe products (Van den Bogaad and Stobberingh, 2000).

2.18 Conceptual Framework

The study framework (Figure 2.6) demonstrates known factors that influence the emergence of AMR in both poultry and farmers. Household variables such as income levels, education levels and sanitation determine people's living standards and ability to access health care. Persons with low income levels are likely to be found in crowded areas with poor sanitation while accessibility to health care providers in human and availability of veterinary services in poultry impacts on antibiotic use. Poultry husbandry influences the emergency of AMR in poultry and the risk of transferring the resistant bacteria to humans.

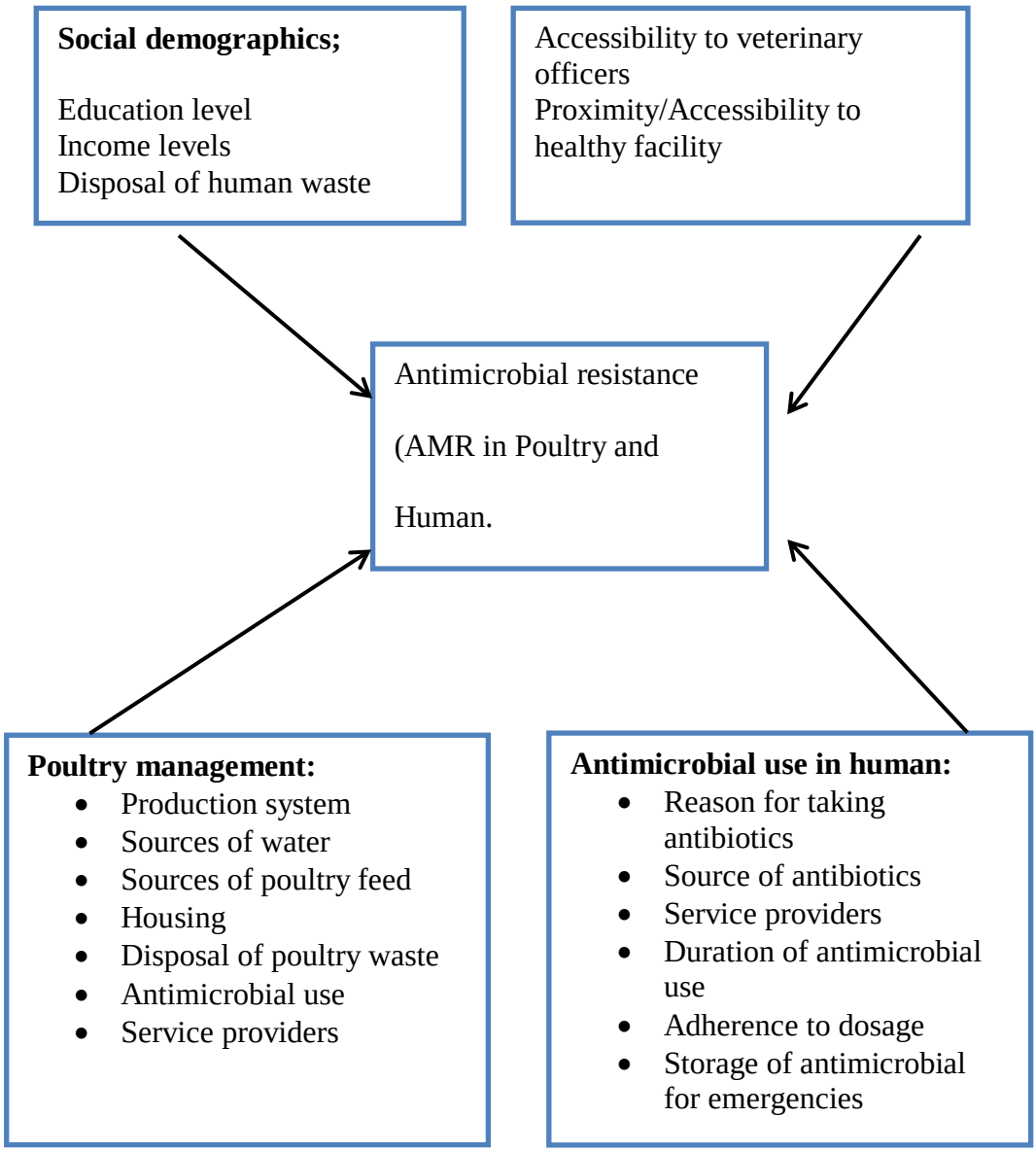


Figure 2.6: Conceptual Framework

Adopted from Roess *et al.*, 2015

CHAPTER THREE

METHODS AND MATERIALS

3.1 Study Area

The study was conducted in Kanyangi and Yatta/Kwavonza Wards in Kitui rural Sub-County, in Southern Eastern part of Kenya. Kanyangi covers approximately 418.70 km^2 while Yatta/Kwavonza covers 757.40km^2. The area is a semi-arid with most of the population living in the rural areas. Projected population of Kanyangi and Yatta/Kwavonza Ward is 25,806 and 35,895 respectively (KNBS 2010a). The wards are primarily a livestock rearing area, where livestock enterprises are a major source of livelihoods. Poultry farming has a great potential and according to 2009 (Census, 2009b). Studies done in other areas in rural area, Katulani district, in Kitui showed that over 98% of the rural households kept indigenous poultry (Murangiri *et al.,* 2016) at an average of 17 chicken per household (Murangiri *et al.,* 2016) while in Mutomo and Kyangwithya Wards, > 95%of the households kept indigenous chicken (Kivunzya *et al.,* 2018). Figure 3.1 shows map of Kitui County.

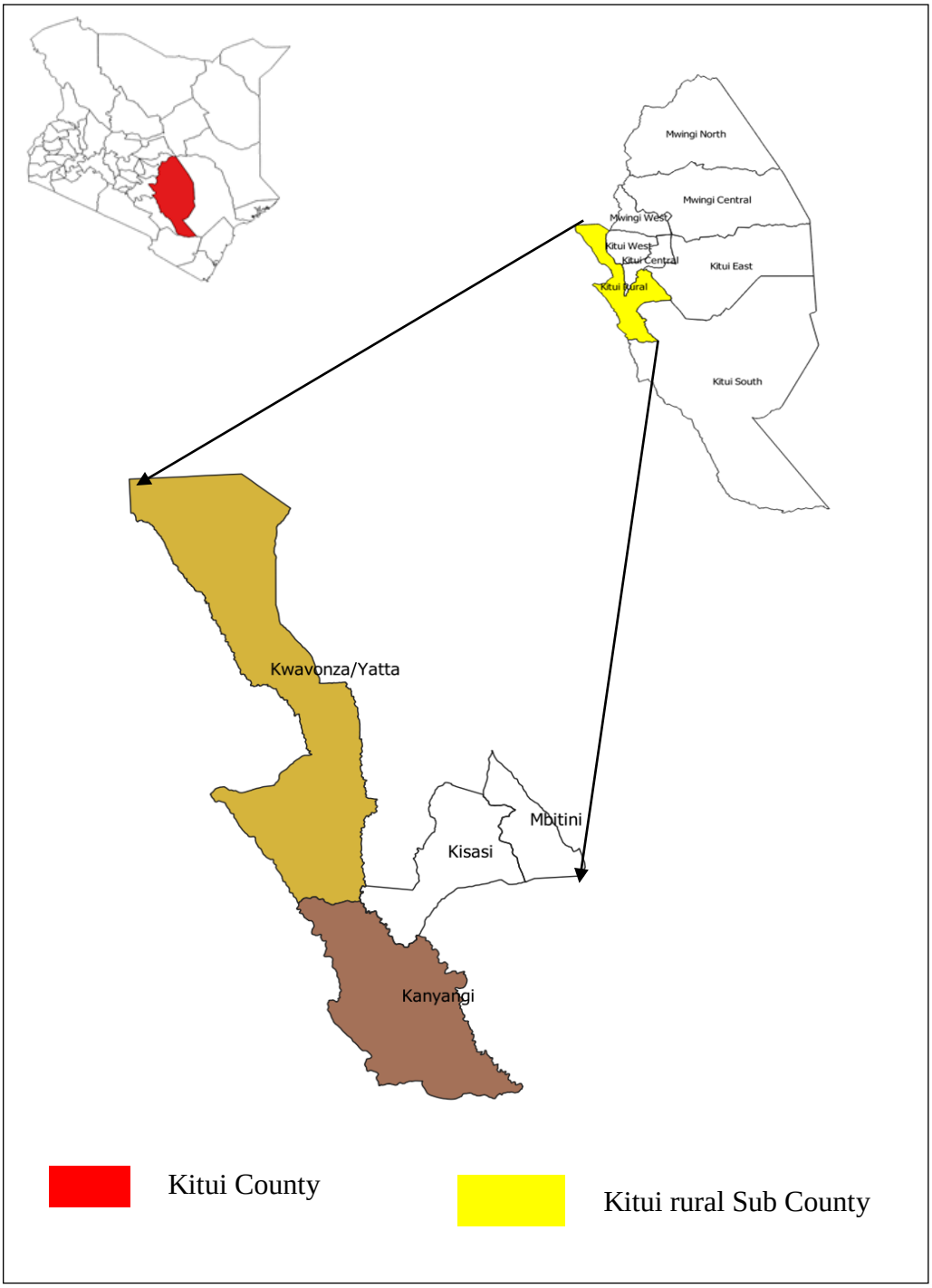


Figure 3.1: Map of Kitui County showing Kitui rural Sub County and the randomized coordinates

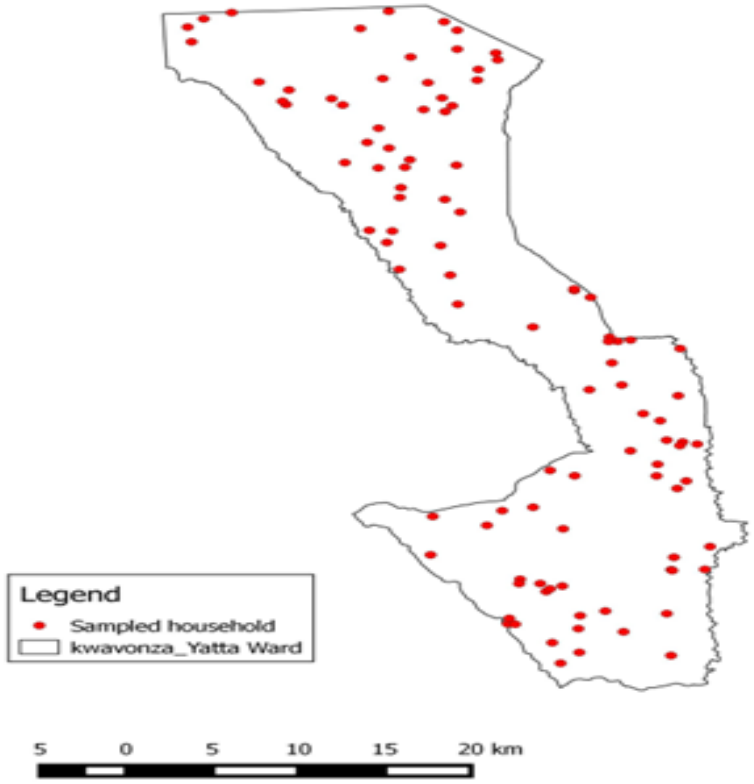


Figure 3.2: Map of Yatta/Kwavonza Ward showing randomized households

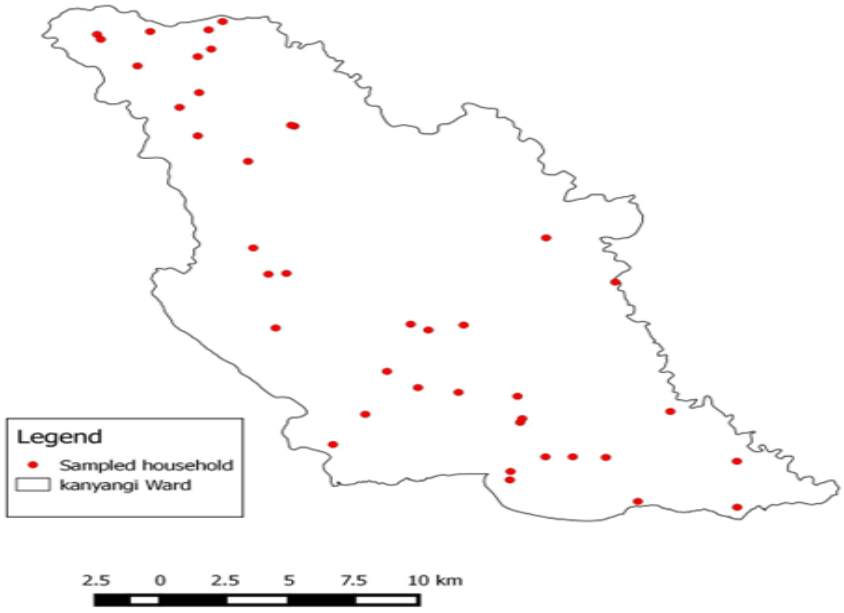


Figure 3.3: The Map of Kanyangi Ward showing and the randomized households.

3.2 Study Design

This was a cross sectional study conducted from July 2017 to January 2018.

3.3 Study Population

The study population was indigenous poultry and their farmers in Kanyangi and Yatta/Kwavonza wards in Kitui rural Sub-county.

3.4 Target Population

The study targeted households rearing indigenous poultry and their farmers in Kitui rural Sub-County.

3.4.1 Inclusion criteria for eligibility into the study

Households rearing indigenous poultry of more than six months old.

Indigenous poultry farmer ≥18 years of age, mentally sound and has lived in the study area for more than 6 months and consented to participate in the study.

3.4.2 Exclusion criteria

Sick indigenous poultry, indigenous poultry less than six months of age, households without poultry and households with persons <18 years of age.

3.5 Sample Size Determination

The sample size for both poultry and their farmers was calculated using a statistical package Epi Info version 7 (2012) statistical software based formula for comparing two sample size as shown below (Cochran, 1977).

$n = (Z_{\alpha/2}+Z_{\beta})^2 * (p_1(1-p_1)+p_2(1-p_2)) / (p_1-p_2)^2$

Where:

n= is the required sample size

$Z_{\alpha/2}$ = critical value of the normal distribution at α/2 (for a confidence level of 95%, α is 0.05 and the critical value is 1.96),

Z_{β} = critical value of the normal distribution at β (e.g. for a power of 80%, β is 0.2 and the critical value is 0.84)

p_1 = expected sample proportions of chicken.

p_2 = expected sample proportions of the humans.

$$n = \frac{(1.96 + 0.84)^2 * \{0.74(1 - 0.74) + 0.5(1 - 0.5)\}}{(0.74 - 0.5)^2} = 60$$

Sample size was multiplied by design effect of 2 due to multistage sampling technique used and 10% non-response rate. This gave a minimum sample size of 132 per population.

3.6 Sampling Procedure

Primary sampling unit were households keeping poultry. A two stage sampling method was applied in selection of study households in each Ward. In the first stage, all the Wards in Kitui rural Sub-county were listed and two wards randomly selected; Yatta/Kwavonza and Kanyangi and all the villages in the two selected Wards.

In the second stage, a list of households in the two Wards was obtained and the number of households to be sampled in each Ward calculated proportionate to size, based on the total households in the Wards; that is 88 households in Yatta/Kwavonza and 45 households in Kanyangi Wards as illustrated in figure 3.4.

Using geographic information system (GIS) Wards shape files were obtained, and geographical coordinates randomly generated, 88 in Yatta/Kwavonza and 45 in Kanyangi. These coordinates were given each a unique number representing households, downloaded and loaded into a Global Positioning System (GPS) device

which was then used to navigate the households corresponding to the number of households to be sampled. Once we got 500 meters of the GPS, we spinned a bottle to choose a direction and sampled the first household in that direction. Selected household which did not meet the inclusion criteria were replaced. Household with three (3) indigenous poultry, had all the poultry sampled while households with more than three (3) poultry, three (3) indigenous chicken were randomly selected. All poultry samples from one household were pooled together to form one sample.

For each household, one person above 18 years of age who takes care of poultry, (cleans the area where poultry sleep or collects eggs) was selected. In households with more than one person taking care of poultry, one person was randomly selected.

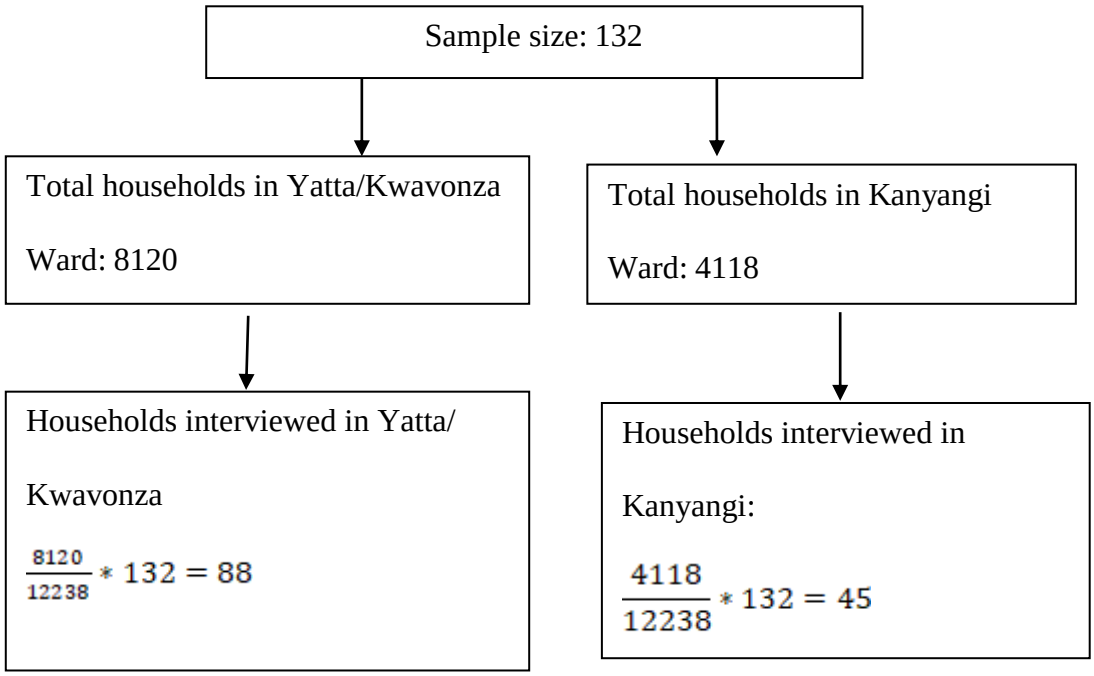


Figure 3.4: Sampling of households in Yatta/Kwavonza and Kanyangi Wards

3.7 Administration of Questionnaire

A semi-structured questionnaire was administered to the study respondents to obtain various variables, namely;

a. **Demographics**; Age, sex, marital status, education status, employment,
b. **Social-economic status** -House type, powered with electricity, sanitary facilities and healthy hygiene practices (source of drinking water), Education on hygiene.
c. **Poultry husbandry practices** - other type of animal kept, production system, person responsible for raising poultry, how poultry waste is used, where poultry sleep
d. **Antibiotic used to treat poultry in the last 3 months**- Drug source, type of drug, reason for using the drug, type of health care provider, when poultry should be given antibiotics, who should prescribe, where to obtain antibiotics, how long should the antibiotics be administered.
e. **Antibiotic use to treat human in the past 3 months** - Drug source, type of drug, reason for using the drug, type of health care provider, reasons for using antibiotics, when to use antibiotics, Knowledge on harmful effects of drugs, side effects encountered, who to prescribe antibiotics, where to obtain antibiotics, Knowledge on importance of dose completion, circumstances under which doctors prescribe antibiotics, if pharmacist, explain how to use antibiotics.

A trained Kamba speaking translator was used for ease of questionnaire administration. The translator was trained on questionnaire administration prior to embarking on data collection to minimize on time taken to administer the questionnaire.

3.8 Sample Collection

3.8.1 Cloacal sample collection from poultry

We used maize grains to lure and catch the poultry (chicken) at the household. The farmer was then requested to hold and restrain the selected poultry. Trained laboratory personnel cleaned their hands with soap and water and put on protective gear. A dry sterile swab was aseptically inserted into cloaca to ensure the collection of cloacal material from three chicken per household, and the sample pooled together to form one sample per household from the indigenous poultry which represented the poultry flock for that particular household. Each of the pooled sample was immediately inoculated into the Cary-Blair media, household number and GPS location labelled, stored in an insulated cool box (4^0 C – 8^0 C) and later in the day transferred into a refrigerator. The samples were transported to the National Public Health, Microbiology Laboratory, 48-72 hours after collection, in an insulated cool box between 4 ^{0}C -8^0 C and immediately processed.

3.8.2 Fecal sample collection from farmer

Poultry farmers, were provided with clean labelled sterile leak-proof containers for stool collection. Farmers were explained on how to collect the stool aseptically by spreading clean newspaper in the toilet and holding the stool ensuring that the sample did not touch inside the toilet, then using the provided sterile spatula, placed the sample into the clean plastic container and then closed and tighten the lid. Once the sample was collected, the laboratory personnel, obtained a swab and immediately placed it in a Cary-Blair transport medium. The sample was labelled using household number and GPS location and immediately kept in an insulated cool box (4^0 C -8^0 C). Later in the day, the sample were transferred in to a refrigerator. The samples were

transported to the National Public Health Microbiology Laboratory, 48-72 hours after collection, in an insulated cool box between 4^0C -8^0C and immediately processed.

3.9 Laboratory Analysis of Sample

Laboratory media which included MacConkey agar, Trypticase soy agar and Muller Hilton agar were prepared in the laboratory following the manufacture's instruction.

3.9.1 *E. coli* isolation and identification

E. coli isolation and identification was performed by standard bacteriological methods (Dalgleish *et al.*, 2007). The sample specimen was cultured on MacConkey agar (a selective medium) using the spread-plating technique and incubated at 37^oC overnight. Isolates suspected to be *E. coli* were picked the following day and re-streaked (sub-cultured) into Trypticase Soy Agar (TSA), non-selective media, to purify *E. coli* and incubated for 18-24 hours. Biochemical tests (Standard methods) for *E. coli* confirmation were done on Tryptophan medium for motility and indole production by addition of 1ml of Kovac's reagent to confirm *E. coli* (Dalgleish *et al.,* 2007).

3.9.2 Antibiotic Susceptibility testing of *E. Coli* isolates

One isolate of identical confirmed *E. coli* isolates per sample were emulsified in sterile water to conform to 0.5 MacFarland turbidity (prepared as 0.5 ml of 1.175% solution of Barium chloride dehydrate (BaCl2.2H2O) to 99.5ml of 0.36N (1%) and was then serially diluted ten times to give a density of approximately 10^6 CFU per ml which resulted in confluent growth (Dalgleish *et al.,* 2007). *Escherichia coli* isolates were spread evenly on Muller Hinton (MH) agar plate in three directions and then using sterile forceps 10 antibiotics disks placed on the MH. (Dalgleish *et al.,* 2007). *E. coli* isolates were subjected to sensitivity test for following antibiotics; Amoxicillin

(AMX10 µg), Ampicillin-cloxacillin (Ax 10µg), Ceftazidime (CAZ 30µg), Ciprofloxacin (CIP 5µg), Ceftriaxone (CTR 30µg), Kanamycin (K 30µg), Meropenem (Mr 10µg), Streptomycin (S 10µg), Sulphamethaxazole-trimethoprim (SXT 30µg) and Tetracycline (TET 30µg). This was carried out using disc diffusion method on antibiotic susceptibility testing.

These antibiotics were used because they are commonly used to treat bacterial diseases in humans and in animals. *E. coli* 25922 strain was used as control strain check for disk potency and to assess quality of the media.

The concentrations of the antimicrobial disks were selected based on the internationally recognized standards and guidelines on antimicrobial routine testing and reporting on *enterobacteriaceae* provided by Clinical and Laboratory Standards Institute (CLSI, 2010). Inoculated agar plates were incubated at 37°C for 24 hours. The susceptibility zones were measured and interpreted according to criteria set by the CLSI, (2010) (Appendix VIII).

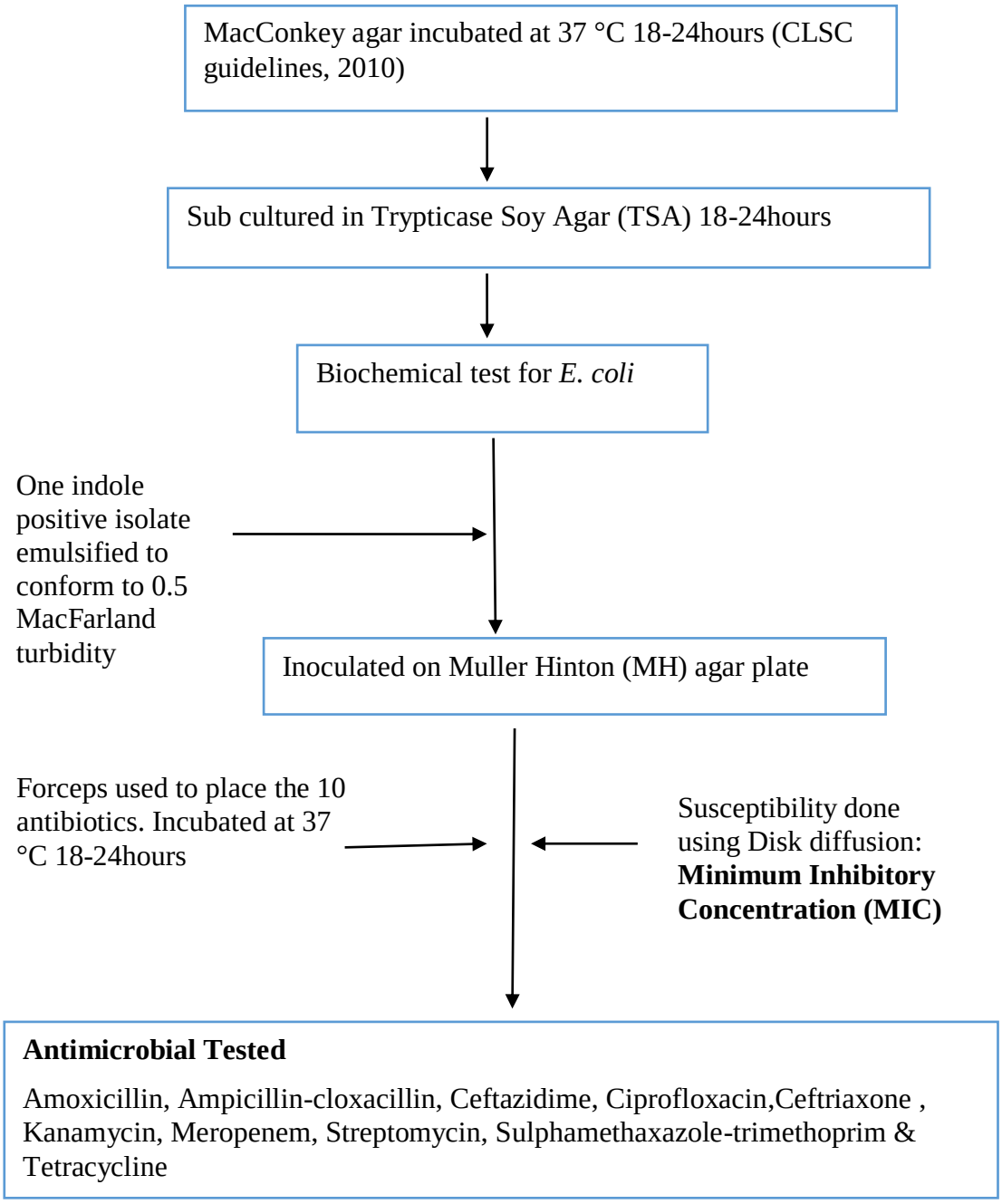


Figure 3 5: *E. coli* isolation, identification and antimicrobial susceptibility testing

3.10 Piloting of the Study

Piloting of the study was conducted before the intended study at Kisasi Ward in Kitui rural sub-County. Fourteen households (10% of the sample size) were interviewed and samples collected, 14 from farmers and 14 from poultry collected. This piloting helped in testing the feasibility of the study in terms of resources and to check for consistency and any ambiguities in the questionnaire after which, revisions were made

based on pre testing findings. Reviewers from Moi University also reviewed the proposal, item by item and gave guidance and corrections incorporated.

3.11 Data Management

3.11.1 Data collection

a) Questionnaire:-Data collection was conducted by trained interviewers. Training was done for one day on the background of the study, enrollment of participants, obtaining of consent from participants, how to avoid possible bias, use of GPS, and interviewing techniques. After establishing eligibility for enrollment, study purpose, benefits and risks were explained. A standard consent form was available in English and the trained interviewers explained to the participants in Kamba language. Data collection involved conducting face to face interviews with the indigenous poultry farmers using structured questionnaire (Appendix IX).

b) Camera – Digital camera Sony RX100V was used to capture photographs of the indigenous poultry and laboratory work showing clear zone of inhibition during susceptibility testing.

c) A ruler- For the laboratory results, the zones of inhibitions for different antibiotics were measured by qualified laboratory technician.

3.11.2 Data entry

a) Questionnaire: - Data from the questionnaires was entered in EPI info database on a personal laptop. The data was checked for errors and cleaned. The data for laboratory results were entered in excel sheet manually and later transferred to EPI info using the unique identifiers on the bar code labels.

b) Photographs - were transferred from the camera to a personal laptop, edited and label caption inserted (Appendix IV,V,VI)

c) Laboratory result- Data from Laboratory were written down on a paper, then entered in to excel sheet manually (Appendix VII) and later transferred to EPI info using the unique identifiers on the bar code labels.

3.11.3 Data storage

The electronic data were stored in a hard drive that was password restricted for backup.

3.12 Data Analysis

a) Questionnaire data: Analysis was carried out using EPI Info 7 (CDC, Atlanta, GA, USA) and Ms Excel 2007 (Microsoft, Seattle, WA, USA). Univariate analysis was done, proportions and 95% Confidence Interval (CI) for qualitative variables and measures of central tendency and measures of dispersion for quantitative data.

   Bivariate analysis was done for specific factors associated with antimicrobial resistance. In the bivariate analysis, resistance was defined as resistance to three antibiotic of the same class. Odds ratio and 95% CI were used and displayed in 2 x 2 tables to identify factors associated AMR *E. coli* in poultry and farmers.

b) Photographs: The photographs were inserted and label caption inserted.

c) Laboratory data: The data was calculated using Ms Excel 2007 (Microsoft, Seattle, WA, USA) to determine the prevalence of susceptible phenotype and interpreted as resistant, intermediate or sensitive.

Data was presented in prose, tables and graphs.

3.13 Ethical Issues

Ethical approval of this study was obtained from School of Public Health, Moi University, and the Institutional Research and Ethics Committee (IREC) approval number FAN: 1901 (appendix III). Prior to commencement of the study, permission was sought from the County Director of Medical Services and County Director of Veterinary Services of Kitui. The study was explained to the community through the area chief. A written informed consent was obtained from all the study participants before interview. The consent forms provided all information on risks, benefits to the study respondents and an assurance of confidentiality (appendix I).

3.14 Dissemination of Data

The finding of this study was shared with the County Departments of health and Agriculture, and livestock development of Kitui County. The results were also presented during the National Kenya Veterinary Association conference in Nyeri, April, 2018.

CHAPTER FOUR

RESULTS

A total of 134 households were interviewed; 91 (67.9%) from Yatta/Kwavonza and 43 (33.1%) from Kanyangi. For each ward, stool and pooled cloacal samples were collected from enrolled farmers and poultry.

4.1 Demographic Characteristic of Farmers

Age: The mean age of the poultry farmers was 43.5 years in Yatta/Kwavonza and 47.7 years in Kanyangi. Farmers ≥ 40 years were 18 (19.8%) in Yatta/Kwavonza and 13 (30.2%) in Kanyangi.

Gender: Majority 69 (75.8%) in Yatta/Kwavonza and Kanyangi 34 (79%) were females.

Marital Status: Seventy three (80.2%) and 41 (95.4%) of the farmers were married in Yatta/Kwavonza and Kanyangi respectively.

Education: Majority 49 (53.8%) of the farmers in Yatta/Kwavonza had education up to secondary level, 26 (28.6%) tertiary level, 13 (14.3%) primary, and 3 (3.3%) had no formal education. In Kanyangi, 29 (67.3%) had secondary education, 9 (20.9) primary education, 4 (9.3%) tertiary while 1 (2.3%) had no formal education.

Employment: As reported in Yatta/Kwavonza, 69 (75.8%) of the respondents were farmers, 12 (13.2%) business/self-employed, 8 (8.8%) worked as casuals while only 2 (2.2%) were employed by the government. The average income was reported as Ksh. 11,722 per month. In Kanyangi, most 31 (72.1%) of the respondents were farmers, 8 (18.6%) business/self-employed, 3 (4) as casuals, while 1 (2.3) were employed by the government. The average income was reported as Ksh.4000 per month.

Religion: Ninety nine percent (90) of the farmers in Yatta/Kwavonza and 43 (100%) of farmers in Kanyangi were Christians.

Table 4.1: Characteristics of households' respondents in Yatta/Kwavonza and Kanyangi Wards

Characteristics	**Yatta/Kwavonza**	**Kanyangi**
	Frequency n (%)	**Frequency n (%)**
Age of household head	Average 43.5 Median 40 (18-89)	Average 47.7 Median 47 (27-79)
Age group		
<40	18 (19.8)	13 (30.2)
41-60	47 (51.6)	16 (37.2)
>60	26 (28.6)	14 (32.6
Total	91 (100)	43 (100)
Gender		
Female	69 (75.8)	34 (79)
Male	22 (24.2)	9 (21)
Total	91 (100)	43 (100)
Marital Status		
Married	73 (80.2)	41 (95.4)
Single/Window	18 (19.8)	2 (4.7
Total	91 (100)	43 (100)
Education		
No formal education	3 (3.3)	1 (2.3)
Primary education	13 (14.3)	9 (20.9)
Secondary education	49 (53.8)	29 (67.4)
Tertiary Education	26 (28.6)	4 (9.3)
Total	91 (100)	43 (100)
Employment		
Farmer	69 (75.8)	31 (72.1)
Business/self	12 (13.2	8 (18.6)
Casual	8 (8.8)	3 (4)
Employed(Government)	2 (2.2	1 (2.3)
Total	91 (100)	43 (100)
Religion		
Christians	90 (98.9)	43(100)
No religion	1(1.1)	0 (0)
Total	91 (100)	43 (100)

4.2 Summary of *E. coli* Isolation in Farmers

A total of 134 fecal samples were collected from poultry farmers; 91 (67.8%) from Yatta/Kwavonza and 43 (32.2%) from Kanyangi. However, *E. coli* was isolated on 59 (64.8%) and 23 (53.5%) of the fecal swabs collected in Yatta/Kwavonza and Kanyangi respectively.

4.3 Summary of Resistant *E. coli* in Farmers

Resistance to at-least one drug was reported in 50 (84.7%) of the *E. coli* isolates in Yatta/Kwavonza and 18 (78.3%) of the *E. coli* isolates in Kanyangi. It was recorded that, 9 (15.3%) isolates from nine households in Yatta/Kwavonza and 5 (21.7%) isolates from five households in Kanyangi were susceptible to all the ten antibiotics tested.

4.4 Antimicrobial Resistant *E. coli* in Farmers

In Yatta/Kwavonza, resistant *E. coli* was found in tetracycline 25 (42.4%), Streptomycin 23 (39%), Sulphamethaxazole-trimethoprim 22 (37.3%), Amoxicillin 21 (35.6%), Ceftazidime 19 (32.2%) and ampicillin-cloxacillin 18 (30.5%) in that order. *E. coli* susceptibility was reported in Ceftriaxone 57 (96.6%), Meropenem 57 (96.6%), and Ciprofloxacin 56 (94.9%).

In Kanyangi, resistant *E. coli* was shown to be equal at 9 (39.1%) in Streptomycin, tetracycline and ampicillin-cloxacillin, 8 (34.5%) Amoxicillin and 7 (30.4%) Sulphamethaxazole-trimethoprim. Susceptibility of 23 (100%) in Meropenem, 22 (95.7%) Kanamycin, 22 (95.7%) Ceftriaxone and 21 (91.3%) Ciprofloxacin was noted. Intermediate resistance was highest in Ceftazidime at 17 (28.8%) and 6 (26.1%) in Yatta/Kwavonza and Kanyangi respectively as shown in table 4.2 below.

Table 4.2: Prevalence of resistant *E. coli* in farmers in Yatta/ Kwavonza and Kanyangi Wards

Yatta/Kwavonza (n=59)				**Kanyangi (n=23)**			
Poultry Farmer	**S** n (%)	**I** n (%)	**R** n (%)	**S** n (%)	**I** n (%)	**R** n (%)	*P*-value
CTR	57 (96.6)	2 (3.4)	0 (0.0)	22 (95.7)	0 (0)	1 (4.3)	0
SXT	37 (62.7)	0 (0.0)	22 (37.3)	14 (60.9)	2 (8.7)	7 (30.4)	0.492
MEM	57 (96.6)	1 (1.7)	1 (1.7)	23 (100)	0 (0)	0 (0)	0.529
AMX	26 (44.1)	12(20.3)	21 (35.6)	13 (56.5)	2 (8.7)	8 (34.8)	0.946
KAN	48 (81.4)	8 (13.6)	3 (5.1)	22 (95.7)	1 (4.3)	0 (0)	0.269
AX	36 (61.0)	5 (8.5)	18 (30.5)	10 (43.5)	4 (17.4)	9 (39.1)	0.457
CAZ	23 (39)	17(28.8)	19 (32.2)	15 (65.2)	6 (26.1)	2 (8.7)	0.030
TET	28 (47.5)	6 (10.2)	25 (42.4)	13 (56.5)	1 (4.3)	9 (39.1)	0.785
CIP	56 (94.9)	1 (1.7)	2 (3.4)	21 (91.3)	0 (0)	2 (8.7)	0.317
STR	29 (49.2)	7 (11.9)	23 (39)	12 (52.2)	2 (8.7)	9 (39.1)	0.993

Key: Ceftriaxone(CTR), Sulphamethaxazole-trimethoprim(SXT), Meropenem (MEM), Amoxicillin(AMX), Kanamycin(KAN), Ampicillin-cloxacillin (AX), Ceftazidime(CAZ), Tetracycline(TET), Ciprofloxacin (CIP), Streptomycin(S),
S-Susceptable **I**-Intermediate **R**-Resistant

4.5 Multidrug (MDR) resistant *E. Coli* in farmers

In Yatta/Kwavonza, 9 (15.3) of the isolates had resistance to two antibiotics, 11 (18.6) to three, 11 (18.6) to four, 5 (8.5%) to five, 3 (5.1%) to six and 1 (1.6%) to seven antibiotics. Thirty three (56%) *E. coli* isolates had resistant to two or more antibiotics, 24 (41%) to three or more antibiotics and 20 (34%) to four or more antibiotics. In Kanyangi, 1 (4.3%) *E. coli* isolate was resistant to two antibiotics, 4 (17.4%) to three, 5 (21.7%) to four and 1 (4.3%) to six.

Table 4.3: Multidrug (MDR) resistance *E. coli* in farmers, Yatta/Kwavonza and Kanyangi Wards

Number of antibiotics showing resistance to *E. coli* isolates	Yatta/Kwavonza n (%)	Kanyangi n (%)
0	9 (15.3)	5 (21.7)
1	17 (28.8)	7 (30.4)
2	9 (15.3)	1 (4.3)
3	11 (18.6)	4 (17.4)
4	4 (6.8)	5 (21.7)
5	5 (8.5)	0 (0)
6	3 (5.1)	1 (4.3)
7	1 (1.6)	0 (0)
	59 (100)	23 (100)

4.6 Multiple resistant *E. coli* profile in farmer *E. coli* isolates

Of the 50 isolates showing resistant *E. coli*, a total of 29 unique antibiotic resistant patterns were recorded in Yatta/Kwavonza among which, 23 (79.3%) of the patterns consisted of two or more antibiotic. It was noted that, most of the isolates with multiple resistance had three antibiotics as shown in 11 (45.8%) of the patterns with more than two antibiotics (Table 4.4). In Kanyangi, 13 different and unique patterns were recorded from the 18 resistant *E. coli* isolates. Most of the isolates with multiple resistance had four antibiotics as shown in 5 (55.5%) of the patterns with more than two antibiotics (Table 4.4).

Isolates with two or more resistance were 33 (56%) while three or more antibiotic resistance were 24 (40.7%) in Yatta/Kwavonza. In Kanyangi, resistance to two or more antibiotics was recorded in 11 (47.8%) of the isolates while 10 (43.5%) had resistance to three or more antibiotics. There was no isolates that were resistant to all the 10 antibiotics tested in indigenous poultry farmers in Yatta/kwavonza and

Kanyangi Wards. The broadest pattern had seven antibiotics in Yatta/Kwavonza and six antibiotics in Kanyangi as shown in (Table 4.4)

4.7 Summary of *E. coli* Isolation in Poultry

A total of 134 cloacal swabs were collected from indigenous poultry. *E. coli* was isolated in 90 (61.2%) of the poultry sample; 61 (67.8%) in Yatta/Kwavonza and 29 (32.2%) in Kanyangi.

4.8 Summary of Resistant *E. coli* in Poultry

Resistant *E. coli* isolates to at-least one antibiotic was observed in 57 (93.4%) Yatta/Kwavonza, and 26 (89.7%) in Kanyangi. It was recorded that, 4 (6.6%) isolates from four households in Yatta/Kwavonza and 3 (10.3%) isolates from three households in Kanyangi were susceptible to all the ten antibiotics tested.

Table 4.4: Multiple resistant *E. coli* profile in farmer *E.coli* isolates

No. of resistant antibiotic	Antimicrobial resistant pattern in farmers (n=59)	Frequency	Antimicrobial resistant pattern in farmers (n=23)	Frequency
		Yatta/ Kwavonza		Kanyangi
0	0	9	0	5
1	Ax; Caz; Amx; Tet; Sxt Cip	2;3;6;2;3; 1	Amx;Tet;Str	2;3;2;
2	Sxtcaz; TetStr; SxtAx; AxStr; CazStr; SxtTet; SxtAmx; AmxTet	1;1;2;1;1; 1;1;1	AxStr	1
3	KanTetStr;CazTetStr SxtAxStr;SxtAmxAx SxtTetStr;SxtKanTet	2;4;2;1;1; 1	SxtAmxAx CazTetStr SxtAxTet	2;1;1
4	AmxAxTetStr SxtAmxAxTet SxtMemCazStr AmxCazTetStr	1;1;1;1	CazTetCipStr SxtAxTetStr AmxAxTetStr CtrSxtAmxAx SxtAmxAxStr	1;1;1;1;1
5	AmxAxCazTetStr SxtAmxAxTetStr SxtAmxAxCazTet	2;1;2	-	
6	SxtAmxAxCazTetStr	3	SxtAmxAxTetCipStr	1
7	SxtAmxAxCazTetCipStr	1		

Key: Amoxicillin(Amx), Ampicillin-cloxacillin (Ax)), Ceftazidime(Caz), Ciprofloxacin (Cip), Ceftriaxone(Ctr), Kanamycin(K),Meropenem (Mr), Streptomycin(Str), Sulphamethaxazole-trimethoprim(Sxt), Tetracycline(Tet)

4.9 Antimicrobial drug resistant *E. coli* from poultry in Yatta/Kwavonza and Kanyangi Wards

Antimicrobial resistant *E. coli* in poultry was conducted in 90 (87.4%) of the *E. coli* of which 61 (68%) and 29 (32%) isolates were from Yatta/Kwavonza and Kanyangi Ward respectively. In Yatta/Kwavonza, amoxicillin 29 (47.5%) was reported as the most resistant antibiotic followed by tetracycline 28 (45.9%), sulphamethaxazole-trimethoprim 24 (39.3%), amoxicillin-cloxacillin 21 (34.4%), Ceftazidime 21 (34.4%), and streptomycin 20 (32.8%) in that order.

In Kanyangi, highest resistance was evident in sulphamethaxazole-trimethoprim and streptomycin at 15 (51.7%) each, tetracycline 14 (48.3%), amoxicillin-cloxacillin, 13 (44.8%), amoxicillin 12 (41.4) and Ceftazidime 11 (37.9%). Antibiotic susceptibility was exhibited by Ceftriaxone 59 (96.7%), Ciprofloxacin 58 (95.1%) and Meropenem 56 (91.8%) in Yatta/Kwavonza while in Kanyangi, antibiotic susceptibility was evident on Ceftriaxone 29 (100%), Ciprofloxacin 28 (96.6%) and Meropenem 27 (93.1). (Table 4.5).

Table 4.5: Prevalence of resistant *E. coli* from poultry in Yatta/Kwavonza and Kanyangi Wards.

Yatta/Kwavonza (N=61) n (%)				Kanyangi (N=29) n (%)			*p*-value
Poultry	**S**	**I**	**R**	**S**	**I**	**R**	
CTR	59 (96.7)	0 (0)	2 (3.3)	29 (100)	0 (0)	0 (0)	0.323
SXT	36 (59)	1 (1.64)	24 (39.3)	12 (41.4)	2 (6.9)	15 (51.7)	0.2672
MEM	56 (91.8)	3 (4.92)	2 (3.3)	27 (93.1)	0 (0)	2 (6.9)	0.4394
AMX	22 (36.1)	10 (16.39)	29 (47.5)	13 (44.8)	4 (13.7)	12 (41.4)	0.587
KAN	51 (83.6)	4 (6.56)	6 (9.8)	23 (79.3)	4 (13.8)	2 (6.9)	0.3415
AX	32 (52.5)	8 (13.11)	21 (34.4)	13 (44.8)	3 (10.3)	13 (44.8)	0.3415
CAZ	26 (42.6)	14 (22.9)	21 (34.4)	15 (51.7)	3 (10.3)	11 (37.9)	0.7458
TET	29 (47.5)	4 (6.6)	28 (45.9)	13 (44.8)	2 (6.9)	14 (48.3)	0.8311
CIP	58 (95.1)	1 (1.6)	2 (3.3)	28 (96.6)	0 (0)	1 (3.4)	0.09803
STR	32 (52.5)	9 (14.8)	20 (32.8)	12 (41.4)	2 (6.9)	15 (51.7)	0.0856

Key: Ceftriaxone(CTR), Sulphamethaxazole-trimethoprim(SXT), Meropenem (MEM), Amoxicillin(AMX), Kanamycin(KAN), Ampicillin-cloxacillin (AX), Ceftazidime(CAZ), Tetracycline(TET), Ciprofloxacin (CIP), Streptomycin(S),

Some drugs demonstrated high intermediate resistance such as ceftazidime 14 (22.9%), amoxicillin 10 (16.39%), streptomycin 9 (14.8%) and 8 (13.11%) in Yatta/Kwavonza. Similarly, Kanyangi had the same trade as shown in amoxicillin 4 (13.7%), amoxicillin-cloxacillin and ceftazidime both at 3 (10.3%).

4.10 Multidrug Resistant *E. coli* in Poultry

In Yatta/Kwavonza, 14 (23%) of the 59 isolates were resistant to two antibiotics, 12 (19.7%) to three, 7 (11.5%) to four, 3 (4.9%) to five and 1 (1.6%). In Kanyangi, 4 (13.8%) were resistant to two antibiotics, 5 (17.2%) to three, 7 (24.1%) to four, 2 (6.9%) to five and six respectively and 1 (3.4%) seven antibiotics as shown in table 4.6 below.

Table 4.6: Multidrug resistant in poultry in Yatta/Kwavonza and Kanyangi Wards

Number of antibiotics showing resistance to *E. coli* isolates	Yatta/Kwavonza n (%)	Kanyangi n (%)
0	4 (6.6)	3 (10.3)
1	16 (26.2)	5 (17.2)
2	14 (23)	4 (13.8)
3	12 (19.7)	5 (17.2)
4	7 (11.5)	7 (24.1)
5	3 (4.9)	2 (6.9)
6	4 (6.5)	2 (6.9)
7	0 (0)	1 (3.4)
8	1 (1.6)	0 (0)
Total	**61 (100)**	**29 (100)**

4.11 Multiple Resistance Profile in Farmer *E .coli* Isolates

In Yatta/Kwavonza, a total of 39 unique antibiotic resistant patterns were recorded from 61 *E. coli* isolates. Thirty four (57.2%) of the *E. coli* isolates consisted of two or more antibiotic and 23 (37.7%) with three or more antibiotic resistance. It was noted that, most occurring pattern consisted of three antibiotics as shown in the table (Table 4.7).

In Kanyangi, 26 different and unique patterns were recorded from the 29 resistant *E. coli* isolates of which 21 (72.4%) had two or more antibiotics (Table 4.7). Resistance to three or more antibiotics was recorded in 16 (55.2%). Most frequent pattern had four antibiotics as shown in table 4.7 below.

There was no isolates that were resistant to all the 10 antibiotics tested in indigenous poultry in Yatta/Kwavonza and Kanyangi Wards. The broadest pattern had eight antibiotics in Yatta/Kwavonza and seven antibiotics in Kanyangi as shown in table 4.7.

4.12 Linking Antimicrobial Resistant *E. coli* in Farmers and Poultry at Household Level

Among the household enrolled in the study, 77 (57%) had antibiotic resistance interrelated in poultry and farmers across different antibiotics tested. Amoxicilin 30 (38.8%), ceftazidime 29 (37.6%), Tetracycline 25 (32.5%) and Ampicillin-cloxacillin 22 (28.5) reported resistance in both poultry and farmers in that order in the same households. However, ceftriaxone, meropenem and ciprofloxacin did not have linked resistance in poultry and farmers.

Table 4.7: Multidrug (MDR) resistance profile in poultry in Yatta/Kwavonza and Kanyangi Wards

No. of resistant antibiotic	Antimicrobial resistant pattern in Poultry(n=61)	Frequency Yatta/Kwavonza	Antimicrobial resistant pattern in farmers (n=29)	Frequency Kanyangi
0	-	4	-	3
1	Sxt;Tet;Amx;Caz;Ax	6;2;6;1;1	Amx;Sxt;Tet;Str	2;1;1;1
2	CazStr;SxtCaz;AxStr AmxAx;SxtTet,CtrSxt; TetStr,CazTet;KanCaz Amxcaz;Amxtet	2;1;1;1;1;1;2; 1;1;2;1	SxtCaz;TetStr SxtAx;SxtAmx	1;1;1;1
3	KanCazStr AmxCazTet;AmxTetStr SxtAxStr;Amxaxtet Sxtamxax;Caztetstr Sxtamxtet;Sxtaxtet	2;1;1;3;1;1;1; 1;1	SxtCazTet CazTetStr AmxAxStr SxtAxStr SxtAmxStr	1;1;1;1;1
4	SxtAmxCazTet CazTetCipStr AmxAxTetStr MemAmxTetStr SxtAmxAxTet AmxAxCazTet	1;1;1;1;1;2	AmxCazTetStr SxtAxCazStr SxtAxTetStr SxtAxCazTet SxtAmxAxStr	2;1;2;1;1
5	SxtAmxAxCazTet AmxAxCazTetStr SxtAmxAxTetStr	1;1;1	AmxCazTetStr SxtAmxAxTetStr	1;1
6	SxtAmxAxCazTetStr SxtAmxKanAxCazTet SxtMemAmxKanAxTet CtrAmxAxTetCipStr	1;1;1;1	MemAmxKanAxCazTet SxtAxCazTetCipStr	1;1
7	-	-	SxtMemAmxKanAxCazTet	1
8	Ctrsxtamxkanaxcaztetstr	1	-	-

Key: Amoxicillin(Amx), Ampicillin-cloxacillin (Ax), Ceftazidime(Caz), Ciprofloxacin (Cip), Ceftriaxone(Ctr), Kanamycin(K),Meropenem (Mr), Streptomycin(Str), Sulphamethaxazole-trimethoprim(Sxt), Tetracycline(Tet)

4.13 Description of Poultry Management, Husbandry Practices and Antibiotic Use Production System

Free range was the main production system at 84 (92.3%) in Yatta/Kwavonza and 41 (95.3%) in Kanyangi, while only 7 (7.7%) and 2 (4.7%) in Yatta/Kwavonza and Kanyangi practiced semi-intensive farming.

Number of poultry owned: Fifty one (56%) of the farmers in Yatta/Kwavonza owned ≤ 10 poultry, while 40 (44%) had >10. In Kanyangi, 30 (69.7%) owned ≤ 10 and 13 (30.3%) less than 10 poultry.

Where the poultry sleeps: Most of the poultry had sleeping poultry pens as recorded in 72 (79.1%) and 37 (86%) in Yatta/Kwavonza and Kanyangi respectively. Only 19 (20.9%) in Yatta/Kwavonza and 6 (14%) in Kanyangi slept in the kitchen or main house.

Owning other livestock: In Yatta/Kwavonza, 89 (97.8%) of the households had other livestock other than poultry. Similarly, all the 43 (100%) households in Kanyangi had other livestock.

Household antibiotic use: Thirty two (35%) households in Yatta/Kwavonza, and 25 (58%) in Kanyangi had used antibiotics on poultry for the last three months, while 59 (64.8%) in Yatta/Kwavonza and 18 (42%) in Kanyangi reported not having used antibiotics in the last three months however, their drug of choice was herbs.

Source of poultry drugs: Among farmers using antibiotics 30 (93.8%) Yatta/Kwavonza bought from agro-vets shops, 1 (3.3%) government officers and 1 (3.3%) from neighbor. In Kanyangi, 24 (96%) bought from agro-vets shops while 1 (4%) from government officers.

Who treats your poultry: Female household members were responsible for administering drugs to the poultry as reported in 88 (96.7%) and 42 (97.7%) in Yatta/Kwavonza and Kanyangi respectively. The veterinary officer was responsible for only 3 (3.2%) in Yatta/Kwavonza and 1 (2.3%) in Kanyangi.

Drug administration: The drug/herbs medicines were given to the flock in water in 90 (98.9%) Yatta/Kwavonza and 39 (90.7%) in Kanyangi.

Use of poultry waste: Poultry waste was used as manure in the farm 88 (96.7%) and 42 (97.7%) in Yatta/Kwavonza and Kanyangi respectively while 3 (3.3%) and 1 (2.3%) put the waste in composite pit in Yatta/Kwavonza and Kanyangi respectively as shown in table.

Table 4.8: Descriptive characteristic of poultry management, husbandry practices and antibiotic use in Yatta/Kwavonza and Kanyangi Ward

Variables	Yatta/Kwavonza Frequency (%)	Kanyangi Frequency (%)
Production System		
Free range	84 (92.3)	41 (95.3)
Semi-intensive	7 (7.7)	2 (4.7)
Total	91 (100)	43 (100)
Number of poultry		
≤10	51 (56)	30 (69.7)
>10	40 (44)	13 (30.3)
Total	91 (100)	43 (100)
Where poultry sleeps		
Pen	72 (79.1)	37 (86)
Main house/Kitchen	19 (20.9)	6 (14)
Total	91 (100)	43 (100)
Owning other livestock		
Yes	89 (97.8)	43(100)
No	2 (2.2)	0 (0)
Total	91 (100)	43 (100)
Antibiotic use in poultry		
Yes	32 (35.2)	25 (58.1)
No (Herbal)	59 (64.8)	18 (41.9)
Total	91 (100)	43 (100)
Source of poultry drug (n=57)		
Agro-vet shop	30 (93.8	24 (96)
Government officer	1 (3.13)	1 (4)
Neighbor	1 (3.13)	0 (0)
Total	32 (100)	25 (100)
Who treats Poultry		
Mother/Wife	88 (96.7)	42 (97.7)
Veterinary officer	3 (3.2)	1 (2.3)
Total	91 (100)	43 (100)
Drug administration		
Flock in water	90 (98.9)	39 (90.7)
Treat sick only	1 (1.1)	4 (9.3)
Total	91 (100)	43 (100)
Use of poultry waste		
Composite pit	3 (3.3)	1 (2.3)
Farm	88 (96.7)	42 (97.7)
Total	91 (100)	43 (100)

4.14 Characteristics of Poultry Farmer Practices and Antibiotic Use in Yatta/Kwavonza And Kanyangi

Source of drinking water: Sixty seven (73.6%) households in Yatta/Kwavonza got water for domestic use from rivers/wells while 22 (24.1) got from dams. In Kanyangi, 35 (81.4%) households got water from boreholes, 6 (14%) from rivers/wells and 2 (4.6%) from dams.

Human waste disposal: Majority of the households in Yatta/Kwavonza 90 (98.9%) and Kanyangi, 42 (97.7%) had latrines for human waste disposal

Farmers taken antibiotics: In the past three months, 67 (73.6%) and 34 (79%) had taken antibiotics in Yatta/Kwavonza and Kanyangi respectively.

Reasons for taking antibiotics: Among the farmers who took antibiotics in Yatta/Kwavonza, 46 (50.5%) took because they felt sick, 17 (18.7%) had common cold and 4 (4.4%) had fever. In Kanyangi, 23, (53.5%) took antibiotics because they felt being sick, 9 (20.9%) had colds and 2 (4.7%) had fever.

Source of antibiotics: In Yatta/Kwavonza, 14 (13.9%) got antibiotics direct from the pharmacy, while 53 (79.1%) obtained the drugs with physician prescription. All the farmers 34 (100%) reporting having used antibiotics in Kanyangi obtained the drugs with physician prescription.

Keep antibiotics at home for emergency: Of all the households in Yatta/Kwavonza and Kanyangi, only 8 (8.8%) and 5 (11.6) kept antibiotics home for emergency in the two wards respectively while 83 (91.2%) in Yatta/Kwavonza and 38 (88.4%) in Kanyangi reported not having antibiotics at home for emergency as shown in the table 4.9.

Table 4.9 Characteristics of poultry farmer practices and antibiotic use in Yatta/Kwavonza and Kanyangi Wards

Variable	Yatta/Kwavonza n (%)	Kanyangi n (%)
Source of drinking water		
River/well	67 (73.6)	6 (14)
Borehole	0 (0)	35 (81.4
Dam	22 (24.1)	2 (4.6)
Total	91 (100)	43 (100)
Human waste disposal		
Pit latrine	90 (98.9)	42 (97.7)
Open defecation	1 (1.1)	1 (2.3)
Total	91 (100	43 (100)
Taken antibiotics		
Yes	67 (73.6)	34 (79)
No	24 (26.4)	9 (21)
Total	91 (100)	43 (100)
Why take antibiotics (n=101)		
Fever	4 (4.4)	2 (4.7)
Common cold	17 (18.7)	9 (20.9)
When you feel sick	46 (50.5)	23 (53.5)
Total	67 (100)	34 (100)
Source of antibiotics (n=101)		
Direct from Chemistry	14 (13.9)	0 (0)
Pharmacy by physician	53 (79.1)	34 (100)
Total	67 (100)	34 (100)
Antibiotics for emergency (n=134)		
Yes	8 (8.8	5 (11.6)
No	83 (91.2)	38 (88.4)
Total	91 (100)	43 (100)

4.15 Factors Associated with Antimicrobial Resistant *E. coli* in Poultry at the Household Level in Yatta/Kwavonza And Kanyangi Wards

Bivariate analysis, was not statistically significant for poultry isolates regarding poultry production system, owning other livestock, use of antibiotics in poultry, mode of drug administration or the number of poultry owned in the household as shown in table 4.10.

Table 4.10: Factors associated with antimicrobial resistant *E. coli* in poultry at household level in Yatta/Kwavonza and Kanyangi Wards

Variable		Resistant	Not resistant	OR (95% CI)	P value
Owning other livestock		65(64.36)	36(35.64)	2.7(0.0-9.9)	0.54
No other livestock		2(100)	0(0)		
Production system					
Free range		64(66.7)	32(33.3)	2.6(0.412-	0.24
Semi-intensive		3(42.86)	4(57.14)	19.1)	
Antibiotic use	Yes	35(72.9)	13(27.08)	1.94(0.84-	0.17
	No	32(58.2)	23(41.8)	4.4)	
Mode of administration					
Flock in water		64(64.65)	35(35.35)	0.6(0.06-	0.9
Treat sick only		3(75)	1(25)	6.08)	
Poultry owned	≤ 10	35(64.8)	19(35.9)	0.837(0.32-	0.89
	>10	22(68.75	10(31.25)	2.12)	

4.16 Factors Associated With AMR *E. coli* at the Household Level in Farmers at Household Level in Yatta/Kwavonza and Kanyangi Wards

Bivariate analysis had no statistically significant difference between antimicrobial resistant *E. coli* observed in indigenous poultry farmers and demographic factors, keeping antibiotics at home, using of antibiotics, and source of water or owning other livestock species as shown in table 4.11.

Table 4.11: Factors associated with antimicrobial resistant *E. coli* in poultry farmer at household level, Yatta/Kwavonza and Kanyangi Wards

Variable		Resistant	Not-resistant	OR (95% CI)	P value
Gender	Male	8 (42)	11 (57.89)	0.3 (0.14-1.1)	0.13
	Female	44 (64.7)	24 (35.29)		
Source of drinking water					
Borehole		18 (51.4)	17 (48.5)	0.72 (0.2-1.3)	0.28
River/well		34 (65.38)	18 (34.62)		
Number of poultry					
≤ 10		30 (55.56)	24 (44.4)	0.6 (0.25-1.5)	0.4
>10		22 (66.67)	11 (33.3)		
Keep drugs at home					
Yes		6 (60)	4 (40)	1 (0.21-5.2)	1.0
No		46 (59.7)	31 (40.26)		
Use of antibiotics					
Yes		40 (60.6)	26 (39.39)	1.15 (0.43-3.1)	0.97
No		12 (100.0)	9 (42.86)	1.15 (0.43-3.1)	0.97
Disposal of poultry waste					
Composite pit		0 (0)	3 (100)	0.0 (0.0-1.5)	0.06
Farm		52 (61.9)	32 (38.10)		

CHAPTER FIVE

DISCUSSION

This study determined prevalence of AMR *E. coli* in poultry and their farmers living in the same households and assessed factors associated with antimicrobial resistant *E. coli* at the animal–human interface. The study found that, three quarters of the farmers rearing indigenous poultry in Yatta/Kwavonza and Kanyangi Wards were females. This could be due to the belief that poultry is considered as an enterprise carried out by females and that they can easily be disposed for subsistence purposes while males are less interested due to its low value (Kyule *et al.*, 2015). These findings were similar to findings by Murangiri (2016) who found that 54% and 67% of the respondents rearing poultry were females in rural and urban setting in Kitui respectively (Murangiri *et al.,* 2016). Similarly, Kyule reviewed indigenous poultry performance in Mau-Narok (Kyule *et al.,* 2015) and reported that 55% of indigenous chicken farmers were women.

The majority of the farmers were above the age of 40 years in the two Wards. This showed that youth were not involved in indigenous poultry farming. This could be attributed to rural-urban migration in search of white collar jobs. This agrees with a study by Kyule in Mau-Narok where mean age of indigenous poultry farmer was 47 years (Kyule *et al.*, 2015). Almost every household in the study area owned other livestock species similar to a study in Rwanda where most of the households owned other livestock species (Manishimwe, Buhire, Uyisunze, Turikumwenayo, & Tukei, 2017).

Objective 1: Prevalence of antimicrobial resistant *E. coli* in poultry farmer

For every ten isolates from farmers, eight isolates in Yatta/Kwavonza and seven in Kanyangi showed resistance to at-least one antibiotic while for every five isolates in Yatta/Kwavonza and Kanyangi Wards, two had resistance to three or more antibiotics. This was similar to a study in India where 92% antimicrobial resistance *E. coli* was recorded with 24% resistance to three antibiotics from healthy children. (Sahoo *et al.*, 2012). Similarly in Korea, a study on *E. coli* isolated from healthy poultry and swine farm workers using antibiotics, high multidrug resistance was observed with tetracycline's and ampicillins (Cho *et al*, 2012) and 78% of the control group (restaurant workers).

This study recorded high resistance from ranging 30.5% to 42.4% amoxicillin, sulfamethoxazole-trimethoprim, ampicillin-cloxacillin, streptomycin and tetracycline in that order (lowest to highest) in Yatta/Kwavonza and Kanyangi Wards. These drugs are most commonly used antibiotics in human. Low resistance was found in ceftriaxone, meropenem and ciprofloxacin in the two wards Wards similar to a study to determine prevalence of *E. coli* in health persons in urban (Kenya, Mexico, Peru and Philippines) and rural (Venezuela, Ghana and Zimbabwe) areas where older drugs such as oxytetracycline, ampicillin, trimethoprim had high resistance and resistance was emerging in gentamycin, cefazolin and ciprofloxacin.

Although it was difficult to pinpoint the source of drug resistance that we observed, it was assumed that, resistant bacteria may have been readily transferred from poultry to the poultry farmers given that, resistance patterns were phenotypically similar. It could be that the farmers had exposed to self-medication or high frequency of antibiotic use or sub-therapeutic. Other source of resistance to farmers is the

environment. Antibiotic resistant bacteria has been documented in soil and aquatic environments (Sahoo *et al.*, 2012) and human might be exposed to resistant bacteria in the environment.

Objective 2: Prevalence of antimicrobial resistant *E. coli* in indigenous poultry

For every ten samples tested, nine in Yatta/Kwavonza and eight in Kanyangi were resistant to at-least one antibiotic. Again, two isolates for every five in Yatta/Kwavonza and Kanyangi Wards were resistant to three or more antibiotics. Given that free range poultry feed freely on the environment, there are number of probable reasons for the high prevalence. First, samples were collected from birds aged more than six months in the households and this could have increased the chances of resistant isolates similar to a study in Kericho, Kenya demonstrated that, resistance in poultry increased with age of the poultry (NALIAKA, 2011). Secondly, the fact that the majority of antibiotics have been produced from the soil for many years, soil could be the major reservoir for the resistance (Woolhouse *et al*., 2015b; Manishimwe *et al.,* 2017) therefore *E. coli* resistant reservoir/ populations naturally occurring in the environment could be responsible for resistant bacteria and resistance evident in this study.

Compared to other antibiotics used in the study, poultry isolates were resistant to amoxicillin, tetracycline, trimethoprim/sulfamethoxazole, ampicillin-cloxacillin, streptomycin, and ceftazidime in that order with a range of between 32.8% to 51.7% in Yatta/Kwavonza and Kanyangi Wards. This could be because these drugs are easily available and affordable and have been reported to be used more frequent in African countries (Rugumisa *et al.,* 2016). This was similar to a study by Campbell, *et al.* (2015) on small scale chicken farms in Vietnum, where high resistance was shown

to commonly used antibiotics such as tetracycline 93.4%, ampicillin 86%, trimethoprim/ sulfamethoxazole 69.7% and ampicillin 47.9%. A cross sectional study conducted in Arusha Tanzania, on meat chicken showed that antimicrobial resistant *E. coli* in local chicken was high and ranged from 20.8% to 48.3% in sulfamethoxazole, 22.5% to 44.2% in trimethoprim, 15% to 30.8% in streptomycin and 3.3% to 44.2% in tetracycline across five different Wards (Rugumisa *et al.*, 2016). In Rwanda, a cross-sectional study isolating *E. coli* from free range chicken reported resistant of 79.4% and 75.8% in streptomycin and doxycline respectively (Manishimwe *et al.*, 2017). In Kenya *E. coli* from poultry isolates has demonstrated resistance to commonly used antibiotics, ampicillin (76%), tetracycline (71.1%), co-trimoxazole (65%), sulphamethaxazole (69.5%), and gentamycin (8.3%) (Ayul & Ajak, 2017). Our findings reported high resistance to ceftazidime unlike in Tanzania where no resistance was detected in local poultry (Rugumisa *et al.*, 2016).

Low levels of resistance was observed in ciprofloxacin, ceftriaxone and meropenem in Yatta/Kwavonza and Kanyangi Wards. These drugs are used for treatment of human infections and they are expensive and therefore most people may not afford them. Ciprofloxacin is a fluoroquinolone, resistance is mediated by chromosomal point mutation and it exists in the environment for long time hence can be transmitted to different species (Manishimwe et al., 2017). Similarly, a study in Tanzania showed low resistance to ciprofloxacin in local poultry (Rugumisa *et al.*, 2016). Meropenem was reported to be susceptible in broilers in European countries (European Food Safety Authority, & Centre, 2016) while in Vietnam a study done in small scale poultry had no resistance to meropenem (Campbell, *et al.*, 2015). In Jamica, a study on broiler chicken and humans recorded 8.8% ciprofloxacin resistance on chicken isolates (Tricia D Miles, 2006).

More than half of the poultry isolates were resistant to ceftazidime, a third generation cephalosporin antibiotic, which contrasted to studies in Vietnam and Thailand (Campbell *et al.,* 2015; Ström, *et al.,* 2017) were they reported low resistance of 11.1% and 3% respectively. However, ceftriaxone which is in the same class with ceftazidime had < 5% prevalence similar to findings in Vietnam (Campbell *et al.,* 2015). Our finding showed low resistance prevalence of < 10% to ciprofloxacin. However in Vietnam prevalence of 24.2% was reported while five European countries reported high prevalence resistance of ciprofloxacin of (57.6%). This difference in resistances could be associated with different study sampling procedures, populations and settings given that this are middle income countries.

Varying AMR *E. coli* profiles for both poultry and farmers were evident. AmxTet profile was recorded in half of poultry farmer isolates and two fifth of the poultry isolates in Yatta/Kwavonza and Kanyangi Wards. A fifth of the isolates in both poultry and farmers contained Amoxicilin, Amoxicillin-cloxacillin and Ceftazime. This shows that AMR *E. coli* shared same profile pattern in poultry and farmers. The resistance pattern difference could have been due to different household characteristics such as the antimicrobials used, the mode of administration and period of administration. Study done in Korea on healthy swine and poultry workers, tetracycline showed *E. coli* resistance of 97% in both groups while ampicillin had resistance of 93% in poultry workers and 87% in swine workers (Cho *et al.*, 2012). This study revealed that, resistance *E. coli* in food animals may colonize the human population through contact or occupational exposure or environment contaminated by animal waste (Cho *et al.,* 2012). Similarly, a study in Netherlands documented resistance patterns in turkey, turkey farmers broilers and broiler farmers. For instance, amoxicillin had 87% resistance in turkey, 66% in turkey farmers, 82% in broilers, and

57% in broiler farmers. This was believed to be due to dissemination of resistance bacteria from the poultry to their respective farmers (London *et al*., 2001).

Objective 3: Factors associated with antimicrobial *E. coli* at household level

Description of poultry management, husbandry practices and antibiotic use

For every ten households in Yatta/Kwavonza and Kanyangi, nine kept their poultry in free range production systems. This could be due to the minimal cost involved and limited labor given that the poultry scavenge the whole day. Similar findings was reported in Katulani district in Kitui (Murangiri *et al.*, 2016) and Mau-Narok, Kenya, Kyule 2016. The average poultry per household was two times lower than that reported in rural Katulani, Kitui (Murangiri *et al.*, 2016), However, in Mau-Narok, farmers kept less than 20 birds per household (Kyule *et al*,. 2015). The low number of birds could be attributed to challenges such as poultry diseases and parasites, poor housing and predators. In the two Wards, it was reported that three quarters of the households had a pen for the poultry and all the households owned other livestock species such as cattle, sheep, goats and donkey similar to study in Katulani, Kitui (Murangiri *et al.*, 2016; Kivunzya *et al.*, 2018) and a study by the South Eastern Kenya University in Kitui and Makueni Counties (Kanui *et al.*, 2016). Mostly, female household member, administered drugs to the poultry, in water and also used the poultry waste as manure in the farms similar to a study in Bangladesh were lay people such as farmers treated poultry, and was used poultry waste as farm manure (Roess *et al.*, 2015) . Use manure as fertilizer has been associated with development of antimicrobial resistance in humans.

Characteristics of farmer practices and antibiotic use

Farmers in Kanyangi reported using water from borehole while in Yatta/Kwavonza, most farmers used water from wells. This was different from a study in Bangladesh where people and animals would swim in the same water used for other household purposes (Roess *et al.,* 2015). Two thirds of the farmers had taken antibiotics in the past three months, majority with prescription from physician in both Yatta/Kwavonza and Kanyangi Wards. However, most of them did not have antibiotics at home for emergency. Antibiotic use has been associated with antimicrobial resistance in human (Omulo *et al.,* 2015).

Factors associated with antimicrobial resistant *E. coli* in poultry at household level in Yatta/Kwavonza and Kanyangi Ward.

This study found that, poultry production systems, owning other livestock, use of antibiotics in poultry, mode of drug administration or the number of poultry owned in the household were not associated with antimicrobial resistant *E. coli*. Similarly, in Rwanda, no statically significant findings were observed regarding isolate resistance against antibiotic according to poultry husbandry (Manishimwe *et al.,* 2017). However, antimicrobial use in poultry has been stated the main factor contributing to antimicrobial resistance (Graham *et al.,* 2016). Resistance develops and spreads through genetic elements; which involves movement of one bacteria cell to another or movement of one genetic location to another within the same cell (Bennett, 2008).

Factors associated with antimicrobial resistant *E. coli* in farmers at household level in Yatta/Kwavonza and Kanyangi Ward.

Demographic characteristics, keeping antibiotics at home, using of antibiotics, and source of water or owning other livestock species had no statistically significant

differences between antimicrobial resistant *E. coli* observed in poultry and farmers. This was attributed to of high antimicrobial resistant *E. coli* evident in the two groups. This was similar to a study in Bangladesh were no statistically significant as association between social-economic and demographic and use of antibiotics in poultry (Roess *et al.,* 2015). However, studies have stated that antimicrobial use, crowding and poor sanitation (London *et al.,* 2001) in human populations as factors associated with AMR at household level. Other factors described includes close proximity of poultry to human, sharing of water bodies for human and animals, use of poultry waste as fertilizer or when the female/women are the poultry care takers (Roess *et al.,* 2015). According to Jay *et al,* (2016), AMR can spill from small scale farming practices to human resulting to carriage AMR bacteria which results to further transmission of AMR (Graham *et al.,* 2016).

Limitation

This study had one major study limitation. Only households with poultry were included in the study hence it was not possible to generalize the findings to households with no poultry.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Prevalence of resistant *E. coli* in poultry farmers: High resistance of 78% - 84% of the *E. coli* isolate in farmers were resistant with the highest resistance to Tetracycline (42.4%) and Streptomycin (39%). Low resistance of < 5% was recorded to meropenum, ceftriaxone and kanamycin in farmers in the two Wards.

Prevalence of resistant *E. coli* in poultry: High proportion of resistance of 89.7% - 93.4% of the *E. coli* isolate in poultry were resistant with highest proportion of >40% resistance in amoxicillin and tetracycline compared to other antibiotics. Low resistance to ceftriaxone, ciprofloxacin and meropenem of <5% was recorded.

Factors associated with antimicrobial *E. coli* at household level: There were no statistically significant factors associated with antimicrobial-resistant *E. coli* in both poultry and poultry farmers at household level.

6.2 Recommendations

Prevalence of resistant *E. coli* in poultry farmers: It would be necessary to conduct further research such as genotyping to confirm transmission chains of the *E. coli* isolated in poultry farmers in this study. There is also need for more effective public health policies and infection control measures than those currently being implemented.

Prevalence of resistant *E. coli* in poultry: Genotyping should be done on the *E. coli* isolates to identify *E. coli* in poultry. Further studies should also be conducted in other areas in Kitui and other Counties in the Country to ascertain the results from this study. There is need for more effective veterinary policies and disease control

measures /programmes ensure susceptible antimicrobial remain sensitive in the near future.

Factors associated with antimicrobial *E. coli* at household level: A research including all sample collection on the environment, in all households, both poultry owning and non-poultry owning households should be conducted with the aim of identifying factors associated with resistant *E. coli.*

REFERENCES

Alekshun, M. N., & Levy, S. B. (2007). Molecular Mechanisms of Antibacterial Multidrug Resistance. *Cell*, *128*(6), 1037–1050. https://doi.org/10.1016/j.cell.2007.03.004.

Amaechi, N., Abbey, S. D., & Achi, O. K. (2015). Original Research Article Plasmid Profile and Antimicrobial Resistance Ratings of Escherichia coli Isolates from Pigs and Poultry Birds in Abia State , Nigeria. *Int.J.Curr.Microbiol.App.Sci*, *4*(2), 834–842.

Amy R. Sapkota, R. Michael Hulet, Guangyu Zhang, Patrick McDermott, Erinna L. Kinney, 1 Kellogg J. Schwab, & S. W. J. (2011). Poultry farms that transitioned to organic practices. *Environmental Health Perspectives*, *119*(11), 1622–1629. https://doi.org/10.1289/ehp.1003350.

Ayul, T., & Ajak, D. (2017). Status of Antimicrobial Resistance in bacteria isolated from Kenyan Chicken. *Thesis Submitted in Partial Fulfillment of Requirements for the Master of Science Degree of University of Nairobi in Applied Microbiology (Bacteriology Option)*.

Bennett, P. M. (2008). Plasmid encoded antibiotic resistance : acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*, (July 2007), 347–357. https://doi.org/10.1038/sj.bjp.0707607.

Bergevoet, R., & Engelen, A. Van. (2014). *The Kenyan meat sector Opportunities for Dutch agribusiness; . Wageningen, LEI Wageningen UR (University & Research centre), LEI Report 2014-032. 64 pp.; 4 fig.; 9 tab.; 30 ref. Key*.

Call, D. R., Matthews, L., Subbiah, M., & Liu, J. (2013). Do antibiotic residues in soils play a role in amplification and transmission of antibiotic resistant bacteria in cattle populations? *Frontiers in Microbiology*, *4*(JUL), 1–8. https://doi. org/10. 3389/fmicb.2013.00193.

Campbell, J. I., Nhung, N. T., Nhung, H. N., Minh, P. Van, & Wagenaar, J. A. (2015). Prevalence and risk factors for carriage of antimicrobial-resistant Escherichia coli on household and small-scale chicken farms in the Mekong Delta of Vietnam, (March), 2144–2152. https://doi.org/10.1093/jac/dkv053.

Census. (2010a). The 2009 Kenya Population and Housing Census. *Kenya Population and Housing Census*, *I*, 82–85.

Census, 2009. (2010b). The 2009 Kenya Population and Housing Census : Volume II Livestock Population by Type and District Cattle Ind Ch. *Kenya Population and Housing Census*, *II*, 1–4.

Centre for Disease Control and Prevention(CDC). (2013). Antibiotic Resistance Threats, *United States,Department of Health and Human Services,*1-114.

Cho, S., Lim, Y., & Kang, Y. (2012). Comparison of Antimicrobial Resistance in Escherichia coli Strains Isolated From Healthy Poultry and Swine Farm Workers Using Antibiotics in Korea. *Osong Public Health and Research Perspectives*, *3*(3), 151–155. https://doi.org/10.1016/j.phrp.2012.07.002.

Clinical and Laboratory Standards Institute. (2010). Consensus Statement on the Adherence to Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing Guidelines (CLSI-2010 and CLSI-2010-update) for Enterobacteriaceae in Clinical Microbiology Laboratories in Taiwan. *Journal of Microbiology, Immunology and Infection*, *43*(5), 452–455. https://doi. org/ 10. 1016/S1684-1182(10)60070-9.

Cox, G., & Wright, G. D. (2013). Intrinsic antibiotic resistance: Mechanisms, origins, challenges and solutions. *International Journal of Medical Microbiology*, *303*(6–7), 287–292. https://doi.org/10.1016/j.ijmm.2013.02.009.

Dalgleish, T., Williams, J. M. G. ., Golden, A.-M. J., Perkins, N., Barrett, L. F., Barnard, P. J., … Watkins, E. (2007). *Manual of Antimicrobial Susceptability Testing*. *Journal of Experimental Psychology: General* (Vol. 136).

Daniel A. Tadesse, Shaohua Zhao, Emily Tong, Sherry Ayers, Aparna Singh, Mary J. Bartholomew, and P. F. M., & We. (2012). Antimicrobial Drug Resistance in Escherichia coli from Humans. *Emerging Infectious Diseases*, *18*(5), 741–749.

Economou, V., & Gousia, P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and Drug Resistance*, *8*, 49–61. https://doi.org/10.2147/IDR.S55778.

European Food Safety Authority European Centre for Disease Prevention and Control Abstract. (2016). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans , animals and food in 2014 European Food Safety Authority European Centre for Disease Prevention and Control. *EFSA*, *14*(March). https://doi.org/10.2903/j.efsa.2016.4380.

Global Antibiotic Resistance Partnership—Kenya Working Group. (2011). Situatinal Analysis and Recommendatios: Antibiotic Use and Resistance in Kenya. *Washington, DC and New Delhi: Center for Disease Dynamics, Economics & Policy*, (August).

Graham, J. P., Eisenberg, J. N. S., Trueba, G., Zhang, L., & Johnson, T. J. (2016). Small-Scale Food Animal Production and Antimicrobial Resistance : Mountain , Molehill , or Something in-between ? *Environmental Health Perspectives*, 1–5.

Hammerum, A. M., & Heuer, O. E. (2009). Human Health Hazards from Antimicrobial-Resistant Escherichia coli of Animal Origin. *Clinical Infectious Diseases*, *48*, 916–921. https://doi.org/10.1086/597292.

Hedberg, S. T., Fredlund, H., Nicolas, P., Caugant, D. A., Olcén, P., & Unemo, M. (2009). Antibiotic susceptibility and characteristics of neisseria meningitidis isolates from the African Meningitis belt, 2000 to 2006: Phenotypic and genotypic perspectives. *Antimicrobial Agents and Chemotherapy*, *53*(4), 1561–1566. https://doi.org/10.1128/AAC.00994-08.

Howard, D., Cordell, R., Jr, J. E. M., Packard, R. M., Ii, R. D. S., & Solomon, S. L. (2018). Measuring the Economic Costs of Antimicrobial Resistance in Hospital Settings : Summary of the Centers for Disease Control and Prevention – *Emory Workshop*, *33*(May), 1573–1578.

Hummel R, Tschäpe H, W. W. (1986). Spread of plasmid - mediated nourseothricin resistance due to antibiotic use in animal. *J Basic Microbiol.*, *26*(8)), 461-6.

Kariuki, S., Gilks, C., Kimari, J., Obanda, A., Muyodi, J., Waiyaki, P., & Hart, C. A. (1999). Genotype analysis of Escherichia coli strains isolated from children and chickens living in close contact. *Applied and Environmental Microbiology*, *65*(2), 472–476.

Kariuki, Samuel, Revathi, G., Kariuki, N., Kiiru, J., Mwituria, J., & Hart, C. A. (2006). hospital in Nairobi , Kenya. *BMC Microbiology ·*, (December 2006). https://doi.org/10.1186/1471-2180-6-101.

Kikuvi, G. M1*, Ole-Mapenay I. M2, Mitema, E. S2 and Ombui J. N.2Soediono, B. (2013). Veterinary medicine. *Journal of Chemical Information and Modeling*, *53*, 160. https://doi.org/10.1017/CBO9781107415324.004.

Kimang'a, A. (2012). Review article a situational analysis of antimicrobial drug resistance in africa : Are we losing the battle ? *Ethiopia Journal of Health Sciences*, *22*(2), 135–143.

Kivunzya, A. Ndungwa, Kanui, I. Titus, Amwata, A. D. (2018). The Status of Livestock Production Systems in Semi-Arid Farming and Arid Pastoral Agro-Ecological Zones in Kitui County , Kenya. *International Journal of Education and Research*, *6*(2), 141–148.

Kyule N. Miriam, (2015). Performance and Constraints of Indigenous Chicken Rearing among Small Scale Farmers in Mau-Narok Ward, Njoro Sub County , Nakuru County , Kenya. *International Journal of Advanced Research*, *3*(3), 283–289.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A., Wertheim, H., Sumpradit, N., Vlieghe, E., Hara. G, & Goossens, H. (2013). Antibiotic resistance-the need for global solutions. *The Lancet Infectious Diseases*, *13*(12), 1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9.

London, N., Driessen, C., & Stobberingh, E. E. (2001). JAC Antibiotic resistance of faecal Escherichia coli in poultry , poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy*, (47), 763–771. Retrieved from http://jac.oxfordjournals.org/

Lucas Ampaire1 Abraham Muhindo1 Patrick Orikiriza2, 3 Juliet Mwanga-Amumpaire2 Lisa Bebell4 Yap Boum2. (2012). A review of antimicrobial resistance in East Africa. *African Journal of Laboratory Medicine*, (SSN: (Online) 2225-2010, (Print) 2225-2002 Page), 1–6.

Manishimwe, R., Buhire, M., Uyisunze, A., Turikumwenayo, J. B., & Tukei, M. (2017). Characterization of antibiotic resistant Escherichia coli in different poultry farming systems in the Eastern Province and Kigali City of Rwanda. *Rev. Elev. Med. Vet. Pays Trop*, *70*(1), 13. https://doi.org/10.19182/remvt.31392.

Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: Impacts on human health. *Clinical Microbiology Reviews*, *24*(4), 718–733. https:// doi.org /10.1128/CMR.00002-11.

Marshall, B. M., Ochieng, D. J., & Levy, S. B. (2009). Commensals: Underappreciated Reservoir of Antibiotic Resistance. *Microbe*, *4*(5), 231–238.

Mason, P. R., Gwanzura, L., Latif, A. S., Marowa, E., Ray, S., & Katzenstein, D. A. (1998). Antimicrobial resistance in gonococci isolated from patients and from commercial sex workers in Harare, Zimbabwe. *International Journal of Antimicrobial Agents*, *9*(3), 175–179. https://doi.org/10.1016/S0924-8579(97)00052-6.

Michael A. Kohanski, M. A. D. & J. J. C. (2010). Sub-lethal antibiotic treatment leads to multidrug resistance via radical induced mutagenesis. *Molecular Cell*, *37*(3), 311–320. https://doi.org/10.1016/j.molcel.2010.01.003.Sub-lethal.

Momtaz, H., Rahimi, E., & Moshkelani, S. (2012). Molecular detection of antimicrobial resistance genes in *E . coli* isolated from slaughtered commercial chickens in Iran. *Veterinarni Medicina*, *57*(4), 193–197.

Moyo, S. J., Gro, N., Matee, M. I., Kitundu, J., Myrmel, H., Mylvaganam, H., … Langeland, N. (2011). Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam , Tanzania, *BMC Pediatrics* 2–7.

Murangiri, M. R., Ikusya, K. T., Akinyi, A. D., Susan, N. A., County, K., & Sciences, V. (2016). Comparing use of ethno veterinary products among rural and periurban chicken farmers in Katulani District, Kenya. *International Journal of Advanced Research*, *4*(1), 550–558.

Naliaka, M. A. (2011). A Comparative Study of Antibiotic Resistance Profiles among Enteric Bacteria in Broilers and Traditional Chicken from Selected Farms in Kericho, Kenya. *A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in the School of Pure and Applied Sciences of Kenyatta University*, (July).

Nelson N., Joshi M., K. R. 2009. (2009). Antimicrobial Resistance: The Need for Action in the East, Central and Southern Africa Region. *Submitted to the U.S. Agency for International Development by the Strengthening Pharmaceutical Systems (SPS) Program. Arlington, VA: Management Sciences for Health. Key*.

O'Neill. (2014). Antimicrobial Resistance : Tackling a crisis for the health and wealth of nations. *The Review on Antimicrobial Resistance*, (December), 1–20.

Omulo, S., Thumbi, S. M., Njenga, M. K., & Call, D. R. (2015). A review of 40 years of enteric antimicrobial resistance research in Eastern Africa : what can be done better ? *Antimicrobial Resistance and Infection Control*, *4*(1), 1–13. https://doi.org/10.1186/s13756-014-0041-4.

Roess, A. A., Winch, P. J., Akhter, A., Afroz, D., Ali, N. A., Shah, R., … Baqui, A. H. (2015). Household Animal and Human Medicine Use and Animal Husbandry Practices in Rural Bangladesh: Risk Factors for Emerging Zoonotic Disease and Antibiotic Resistance. *Zoonoses and Public Health*, *62*(7), 569–578. https://doi.org/10.1111/zph.12186.

Rugumisa, B. T., Call, D. R., Mwanyika, G. O., Mrutu, R. I., Luanda, C. M., Lyimo, B. M., … Buza, J. J. (2016). Prevalence of Antibiotic-Resistant Fecal Escherichia coli Isolates from Penned Broiler and Scavenging Local Chickens in Arusha , Tanzania, *79*(8), 1424–1429. https://doi.org/10.4315/0362-028X.JFP-15-584.

Sahoo, K. C., Tamhankar, A. J., & Sahoo, S. (2012). Geographical Variation in Antibiotic-Resistant Escherichia coli Isolates from Stool , Cow-Dung and Drinking Water. *Int. J. Environ. Res. Public Health*, (Mic), 746–759. https://doi.org/10.3390/ijerph9030746.

Sandra Mudhune and Maranga Wamae. (2009). Europe PMC Funders Group Report on Invasive Disease and Meningitis due to Haemophilus influenzae and Streptococcus pneumonia from The Network for Surveillance of Pneumococcal diseases in the East African Region (netSPEAR). *Clin Infect Dis.*, *48*(Suppl 2), 1–12. https://doi.org/10.1086/596494.Report.

Sitati, S. K. (2017). Genetic Diversity of Indigenous Chicken(Gallus domesticus) Population in Kenya. *A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Animal Genetics and Breeding, University of Nairobi.*

Ström .G . Halje.M Karlsson. D, Jiwakanon. J, Pringle.M, F. . and M. . (2017). Antimicrobial use and antimicrobial susceptibility in Escherichia coli on small - and medium - scale pig farms in north - eastern Thailand. *Antimicrobial Resistance & Infection Control*, *20176:75 h*(March).

Swedres-Svarm. (2013). Use of antimicrobials and occurance of antimicrobial resistance in Sweden. *Use of Antimicrobials and Occurrence of Antimicrobial Resistance in Sweden. Solna, Solna/ Upp*, 1650–6332.

Sykes, R. (2010). The 2009 Garrod Lecture: The evolution of antimicrobial resistance: A Darwinian perspective. *Journal of Antimicrobial Chemotherapy*, *65*(9), 1842–1852. https://doi.org/10.1093/jac/dkq217.

Szmolka, A., & Nagy, B. (2013). Multidrug resistant commensal Escherichia coli in animals and its impact for public health. *Frontiers in Microbiology*, *4*(SEP), 1–13. https://doi.org/10.3389/fmicb.2013.00258.

Tricia D Miles, W. M. & P. D. B. (2006). Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans. *BioMed Central Research Veterinary Research*, *9*, 2:7. https://doi.org/10.1186/1746-6148-2-7.

Uganda National Academy of Sciences (UNAS). (2016). Antimicrobial resistance National action plans. *Government of Uganda*, *2015*(March), 1–16. Retrieved from http://www.who.int/antimicrobial-resistance/national-action-plans/en/

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. a, Robinson, T. P., … Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America*, (16), 1–6. https://doi.org/10.1073/pnas.1503141112.

Van den Bogaard AE1, S. E. (2000). Epidemiology of resistance to antibiotics . Links between animals and humans . *Int J Antimicrob Agents*, *14*(4), 327–335.

Vuuren, M. Van. (2001). Antibiotic Resistance with Special Reference to Poultry Production. *Conf. OIE*, 135–146.

Wambugu, P., Habtu, M., Impwi, P., Matiru, V., & Kiiru, J. (2015). Antimicrobial Susceptibility Profiles among <i>Escherichia coli</i> Strains Isolated from Athi River Water in Machakos County, Kenya. *Advances in Microbiology*, *05*(10), 711–719. https://doi.org/10.4236/aim.2015.510074.

Wanjiru, M. M. (2014). Analysis of Demand for Antibiotics in Poultry Production in Kiambu County, Kenya. *A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of Master of Science Degree in Agricultural and Applied Economics,University of Nairobi*.

Wegener, H. C. (2012). Improving Food Safety through a One Health Approach. *Improving Food Safety Through a One Health Approach: Workshop Summary*, 1–418.

Wellington E.M, Boxall A.B, Cross P, Feil E.J, Gaze W.H, Hawkey P.M, Johnson-Rollings A.S, Jones D.L, Lee N.M, Otten .W, Thomas C.M, W. A. A. (2013). The role of the natural environment in the emergence of antibiotic resistance in gram - negative bacteria . *Lancet Infect Dis*, *13*(2), 155–165. https://doi. org/10 .1016/S1473-3099(12)70317-1.

Woolhouse, M., Ward, M., Bunnik, B. Van, Farrar, J., Ward, M., & Woolhouse, M. (2015a). Antimicrobial resistance in humans , livestock and the wider environment. *Phil. Trans. R. Soc. B 370:20140083. Http://Dx.Doi. Org /10.1098/Rstb.2014.0083*.

World Health Organisation. (2001). Original : English Who Global Strategy for Containment of Antimicrobial Strategy for Containment of Antimicrobial Resistance. *World Health*, *WHO/CDS/CS*, 105.

World Health Organisation. (2010). World Health Organisation. Retrieved from www.who.int.

World Bank. (2017). "Drug-Resistant Infections: A Threat to Our Economic Future." Washington, DC: *World Bank. License: Creative Commons Attributi*on CC BY 3.0 IGO.

Zahraei Salehi, T., & Farashi Bonab, S. (2006). Antibiotics susceptibility pattern of *Escherichia coli* strains isolated from chickens with colisepticemia in Tabriz province, Iran. *International Journal of Poultry Science*, *5*(7), 677–684. https://doi.org/10.3923/ijps.2006.677.684.

APPENDICES

Appendix I: Informed Consent form

Title of study: Prevalence and Factors Associated with Antimicrobial Resistant *Escherichia coli* among Poultry and Farmers in Kitui County-Kenya

Introduction:

Dear Respondent... My name is Augusta Ndungwa Kivunzya, MSc FELTP student at Moi University, School of Public Health. Am currently working on my research project and I have selected this area for my study. I am trying to learn more about antimicrobial resistance (AMR) in both poultry and poultry farmers in this county. Antimicrobial resistance is the ability of microorganism to overcome the effect of antimicrobial drug that was initially effective to treat/kill and this threatens public health achievements of many years. It comes about due to inappropriate use of antibiotics in both human and livestock, social economic status and livestock husbandry practices. As a member of this community your participation in this study will highly be appreciated. If you agree to participate, the interview will take about twenty five (25) minutes of your time. Your participation will help us determine prevalence of AMR hence benefiting the entire community, although you will not receive any immediate benefit as of now. We understand that some of the questions regarding the topic may be sensitive to you, however, your response will be handled confidentially. Conversely, you do not have to answer any question that you are not comfortable with. There are minimal if any risks when participating in this study and you can choose to withdraw at any time if necessary. Feel free to ask any questions that you might have regarding this study.

Name of respondent…….Signature……

Date……..Contact….....

Purpose of study:

Due to the great public health importance of antimicrobial resistance, I am requesting for your participation in this study whose main objective is to find out how many of your poultries and persons keeping the poultry in Kitui County are exposed to this major problem; what are the factors associated with transmission or acquisition of this problem by the poultry and the persons keeping the poultry; also gauge the knowledge, and practices of poultry farmers on the ground. This is important for the relevant authorities to find ways of dealing with this problem in this county. You are being asked to join this study because your household was picked by chance among other households in this area.

Expectations of the study:

If you agree to participate in the study, we wish to test some of your poultry and a household member to determine whether they could have been exposed to antimicrobial resistance. If you agree to take part in the study, a trained laboratory technologist will collect fecal material from the cloaca of few selected poultry using sterile swab. We will also request the person in the household who takes care of poultry to collect his/her stool into a clear leak-proof container. The samples will be transported to National Public Health Microbiology Laboratory, where we will test for antimicrobial resistance.

We shall then ask you some questions which are written on a paper on antibiotic use and your knowledge and practices regarding antimicrobial resistance. The test results shall be availed as soon as possible to Veterinary Officer and Medical Officer of this sub-County who shall forward them to you and advice on any necessary control measures if need be.

Risks:

There are no envisaged risks from participating in this study.

Benefits:

The results of this study will be communicated and disseminated to the county government, department of health and veterinary services and people concerned for them to take action on the recommendations that will come out from the study results. This will include necessary control measures if need be.

Confidentiality:

Any information obtained from you will be kept confidential and used solely for purposes of this research only. The results of this research may be published in scientific journals or presented at medical or veterinary meetings, conferences, and workshops but your identity will not be disclosed.

Compensation:

If you accept to take part in this study, there will be no payment for participation.

Volunteerism:

You have a choice to agree or not to agree to participate in this study. If you agree to participate in study you are allowed to withdraw from the study at any time if you so wish without any consequences whatsoever.

Approval of the study:

This study will be approved by:

Institutional Research and Ethical Committee (IREC)

Moi University,

College of Health Sciences, School of Public Health

P.O Box 3900-30100 Eldoret

Email: info@mu.ac.ke

In case of any further questions or concerns, you are encouraged to address them to the directors of the above institutions.

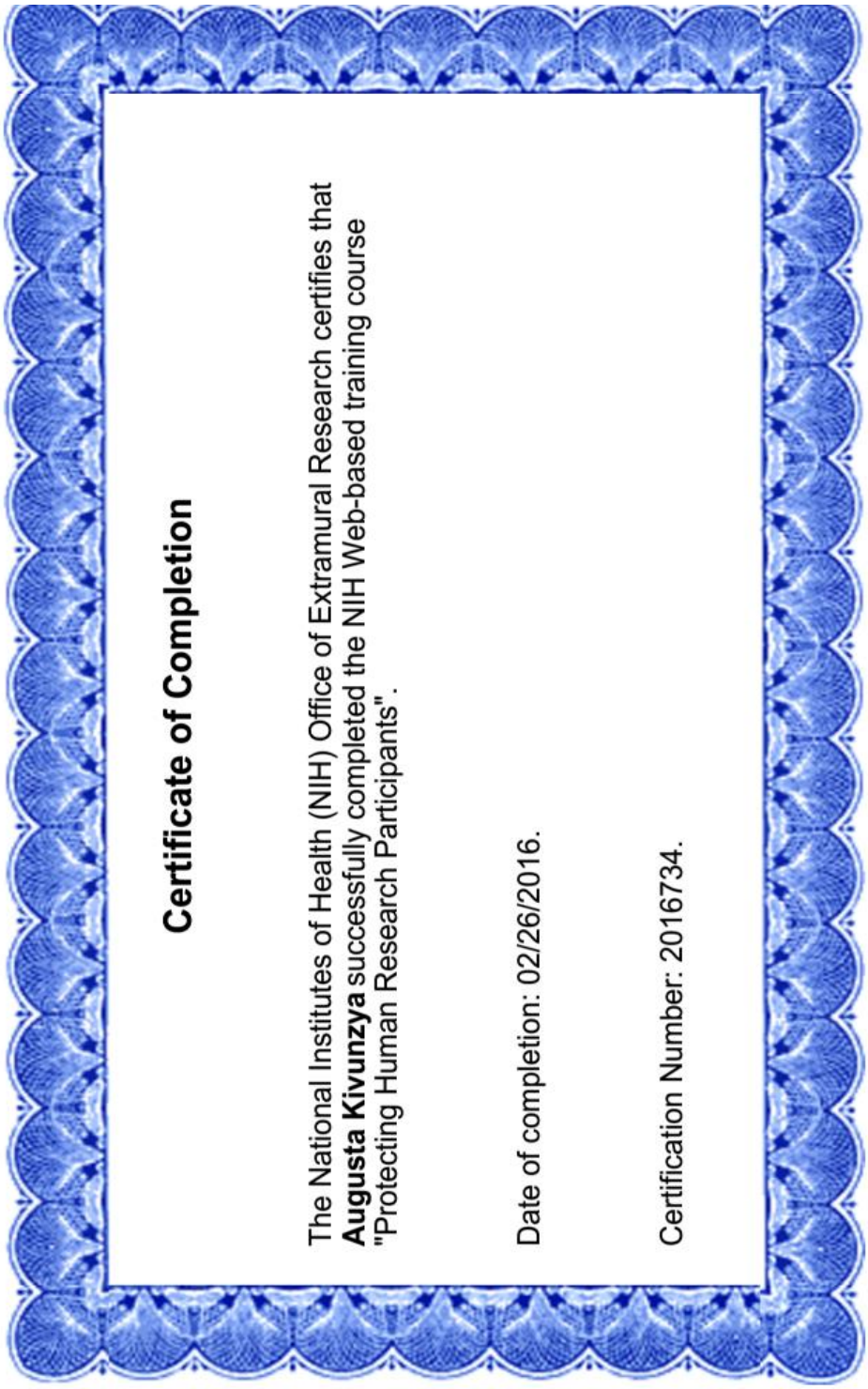
Consent:

I have been fully informed about the study, the risks and benefits of this study. I have the opportunity to ask questions which were satisfactorily answered. I, therefore, consent to voluntarily participate in the study.

Name of participant……Signature……Date……..

Name of researcher……Signature…….……Date ……..

Appendix II: Online ethics certificate



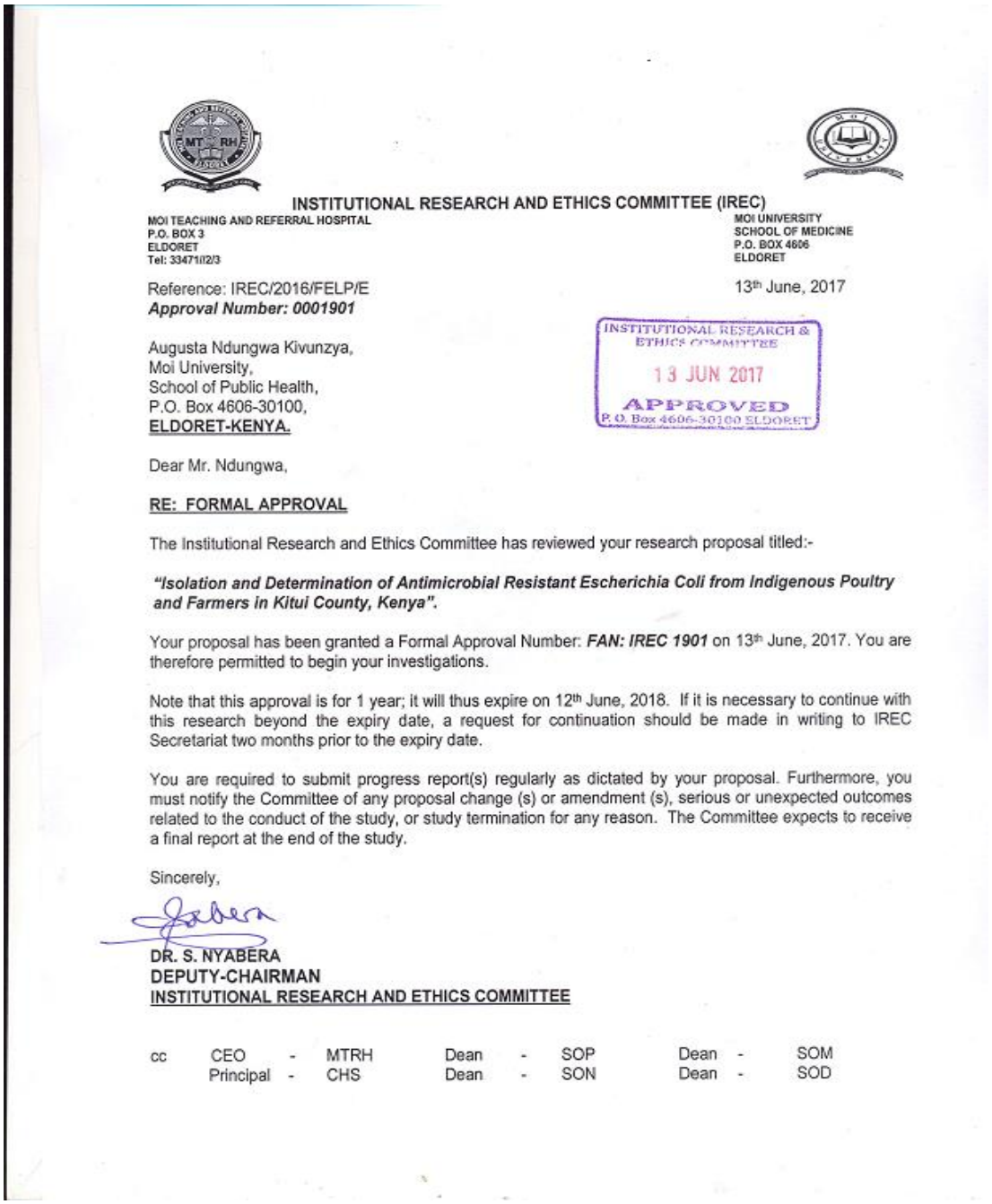
Certificate of Completion

The National Institutes of Health (NIH) Office of Extramural Research certifies that **Augusta Kivunzya** successfully completed the NIH Web-based training course "Protecting Human Research Participants".

Date of completion: 02/26/2016.

Certification Number: 2016734.

Appendix III: IREC study approval



INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/FELP/E
Approval Number: 0001901

13th June, 2017

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
13 JUN 2017
APPROVED
P.O. Box 4606-30100 ELDORET

Augusta Ndungwa Kivunzya,
Moi University,
School of Public Health,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Mr. Ndungwa,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Isolation and Determination of Antimicrobial Resistant Escherichia Coli from Indigenous Poultry and Farmers in Kitui County, Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1901*** on 13th June, 2017. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 12th June, 2018. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

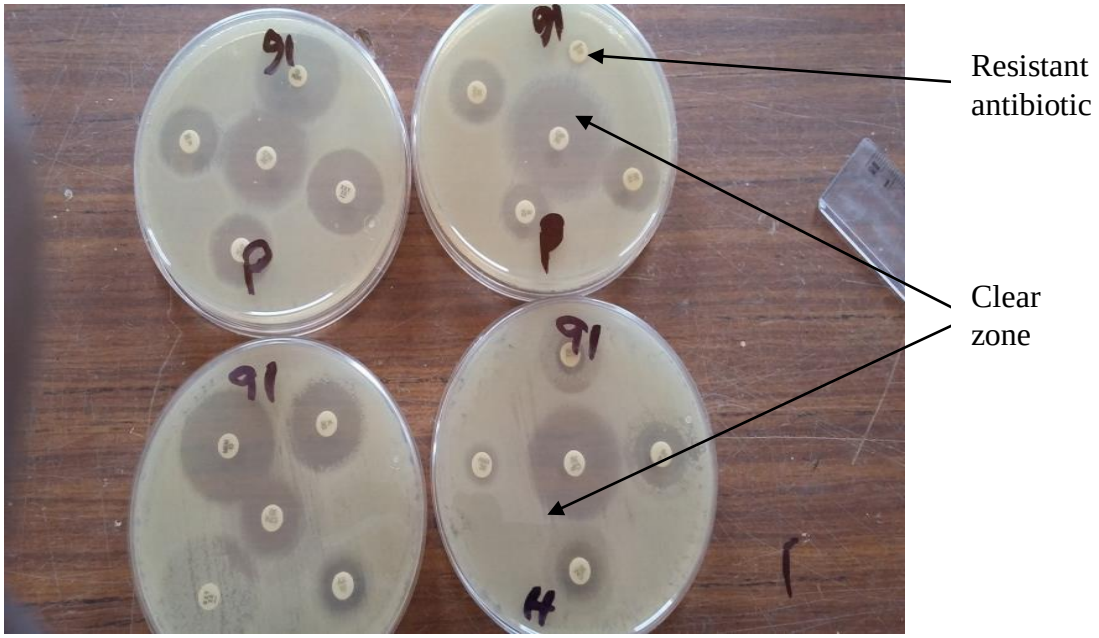
Sincerely,

DR. S. NYABERA
DEPUTY-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

Appendix IV: A photograph of a student in laboratory measuring sample susceptibility zones using a ruler

Appendix V: Antimicrobial susceptibility test profile (Clear zones)/Zones of inhibition photograph for one *E. coli* isolate from the same household



NB: Clear zones indicates susceptibility (No growth)

Key: 91-Sample number, **P**- poultry isolate and **H**- farmer isolates

Appendix VI: Example of indigenous poultry in a household compound in Kanyangi Ward Kitui-rural sub-county, Kitui County

Appendix VII: Results of laboratory antimicrobial susceptibility testing

Sample Number	CTR	SXT	MER	AX	KAN	AMX	CAZ	TET	CIP	S
012 P	S	R	S	R	R	R	R	R	S	S
019 H	S	S	S	S	S	S	S	R	S	R
019 P	S	S	S	S	S	S	R	R	R	R
024 P	S	S	S	S	S	S	S	R	S	R
028 P	S	R	R	R	R	R	S	R	S	S
031 H	S	R	S	S	S	S	S	R	S	S
031 P	S	R	S	S	S	S	S	S	R	S
044 P	S	S	S	S	S	R	S	I	S	S
058 P	S	R	S	R	S	R	I	R	S	S
058 P	I	R	R	S	S	I	R	S	S	R
065 P	S	R	S	R	S	S	S	R	S	S
075 H	S	R	S	S	R	S	S	R	S	S
075 P	S	S	I	S	R	I	R	S	S	S
101 H	S	I	S	R	S	I	S	S	S	S
101 P	S	S	S	R	S	I	S	S	S	S
103 H	S	R	S	R	S	R	S	S	S	S
103 P	S	R	S	R	S	S	R	S	S	S
105 P	S	I	R	S	R	R	R	R	S	S
108 H	S	R	S	R	S	R	S	S	S	R
108 P	R	R	S	R	S	R	S	S	S	S
109 P	S	S	S	S	S	R	R	R	S	R
111 H	S	R	S	R	S	R	S	R	R	R
111 P	S	R	S	R	S	R	R	R	R	R
112 H	S	S	S	S	S	S	S	S	S	S
112 P	S	S	S	S	S	S	S	S	S	S
113 H	S	S	S	S	S	S	S	S	S	S
113 P	S	R	S	S	S	R	S	R	S	R
113 P	S	S	S	S	S	S	S	S	S	S
114 H	S	R	S	S	S	S	S	S	S	S
114 P	S	R	S	R	S	S	S	R	S	S
116 H	S	R	S	R	S	R	S	S	S	R
116 P	R	R	S	R	S	R	S	S	S	S
117 H	S	R	S	I	S	R	S	R	S	S
119 H	S	S	S	S	S	S	S	S	S	S
119 H	S	R	S	S	S	S	S	S	S	R
119 P	S	I	R	S	R	R	R	R	S	S
120 H	S	S	S	S	S	S	R	R	R	R
120 P	S	R	R	R	R	R	R	R	S	S
127 H	S	R	S	R	S	R	R	R	R	R

127 P	S	R	S	S	S	R	S	R	S	R
13 H	S	S	S	S	S	S	S	R	S	R
13 P	S	S	S	S	S	S	S	S	S	S
130 H	S	I	S	R	S	I	S	S	S	S
130 P	S	R	S	R	S	S	S	I	S	S
131 H	R	R	S	R	S	R	S	S	S	S
137 H	S	S	S	S	S	S	S	R	S	S
137 P	S	R	S	R	S	R	S	S	S	R
139 P	S	S	S	R	S	I	S	S	S	S
140 P	S	S	S	S	S	R	S	I	S	R
141 H	S	S	S	S	S	S	S	S	S	R
141 H	S	R	S	S	S	R	S	S	S	R
141 P	S	R	S	I	S	S	S	S	S	R
141 P	S	R	S	S	S	R	S	S	S	R
143 H	S	R	S	R	S	R	S	S	S	R
143 P	S	S	S	R	S	S	S	I	S	S
15 H	S	R	S	R	S	S	R	R	S	S
15 P	S	R	S	S	S	S	R	S	S	S
16 H	S	R	S	S	S	S	S	S	S	S
16 P	S	R	S	S	S	S	S	S	S	S
21 H	S	S	S	S	S	R	S	I	S	S
21 P	S	S	S	S	S	R	S	I	S	R
2H	S	R	S	I	S	S	S	S	S	S
2P	S	R	S	I	S	S	S	S	S	S
31 H	S	R	S	S	S	S	S	S	S	S
31 P	S	R	S	I	S	S	S	S	S	S
32 P	S	R	S	S	S	R	S	S	S	R
32H	S	S	S	S	S	R	S	S	S	R
33H	S	R	S	R	S	R	S	S	S	S
33P	S	R	S	S	S	R	S	S	S	R
35 H	S	R	S	R	S	R	R	R	R	R
35 P	S	R	S	R	S	R	S	R	R	R
36 H	S	S	S	S	S	S	S	S	S	S
36 P	R	R	S	S	S	S	S	S	S	S
37 H	S	S	S	R	S	I	S	S	S	S
37 P	S	I	S	R	S	I	S	S	S	S
3H	S	S	S	S	R	S	S	R	S	R
3P	S	S	I	S	R	I	R	S	S	R
52 H	S	S	S	S	S	S	S	S	S	S
52 P	S	P	S	S	S	S	S	S	S	S
53 H	S	R	S	S	S	R	S	S	S	R
53 P	S	S	S	S	S	R	S	S	S	R
55 H	S	S	S	S	S	S	R	S	S	R

55 P	S	S	S	S	S	S	R	S	S	R
57 H	S	S	S	S	R	S	S	R	S	R
57 P	S	S	I	S	R	I	R	S	S	R
59 H	S	R	S	S	S	R	S	S	S	R
59 P	S	R	S	R	S	R	S	S	S	S
5H	S	S	S	S	S	R	S	S	S	S
5P	S	S	S	S	S	S	S	S	S	S
60 P	S	R	S	R	S	R	R	I	S	R
64 H	S	R	S	R	S	S	S	R	S	S
64 P	S	R	S	S	S	S	S	S	S	S
67 H	S	R	S	R	S	S	S	R	S	S
67 P	S	S	S	R	S	S	S	I	S	S
69 H	S	R	R	R	R	R	R	R	S	S
69 P	S	R	R	R	R	R	S	R	S	S
6H	S	R	S	R	S	R	S	R	S	R
6P	S	R	R	I	S	S	S	S	S	S
7 H	S	R	S	I	S	R	S	R	S	S
74 P	S	R	S	R	S	R	S	S	S	R
75 H	S	R	S	S	S	S	S	S	R	S
75 P	S	R	S	S	S	S	S	R	S	S
7P	S	R	S	R	S	S	S	I	S	S
9H	S	S	S	S	S	S	S	R	S	R
9P	S	S	S	S	S	S	S	R	S	S

Key:Amoxicillin(AMX), Ampicillin-cloxacillin (AX)), Ceftazidime(CAZ), Ciprofloxacin (CIP), Ceftriaxone(CTR), Kanamycin(K),Meropenem (Mr), Streptomycin(S), Sulphamethaxazole-trimethoprim(SXT), Tetracycline(TE)

S-Susceptibility; **I**-Intermediate ; **R**-Resistant

Appendix VIII: Interpretive zone inhibition for disk diffusion susceptibility testing for the 10 antibiotics by clinical and laboratory standards institute

Interpretive categories and zone diameter breakpoints (nearest mm)			
Name of the Antibiotics	Susceptible	Intermediate	Resistant
Amoxicillin 10 µg (AMX)	≥17	14-16	≤13
Ampicillin-cloxacillin (AX)	≥15	12-14	≤11
Ceftazidime µg 30 µg` (CAZ)	≥21	18-20	≤17
Ciprofloxacin 5µg (CIP)	≥23	16-20	≤15
Ceftriaxone 30 µg (CTR)	≥18	14-17	≤13
Kanamycin 30 µg (K),	≥18	14-17	≤13
Meropenem 10 µg (Mr),	≥23	20-22	≤19
Streptomycin 10 µg (S)	≥15	12-14	≤11
`Trimethoprim/Sulphamethaxazole 1.25/23.75 µg (SXT)	≥16	11-15	≤10
Tetracycline µg 30 µg (TE)	≥15	12-14	≤ 11

Appendix IX: Qquestionnaire for poultry farmers on

Isolation and determination of Antimicrobial resistance of *Escherichia coli* from Indigenous Poultry and Farmers in Kitui County, Kenya

PART A: HOUSEHOLD IDENTIFICATION

Questionnaire No……..Household No……..

Sub- location…… Village……GPS coordinates……...

PART B: DEMOGRAPHIC CHARACTERISTICS

1. Gender of respondent 1 Male ☐ 2Female ☐

2. Age of respondent (years)……

3. What is your Religion?

1 Christian ☐ 2 Muslim ☐ 3 No religion ☐ 4. Animist

4. Marital Status of respondent

1 Single ☐ 2 Divorced ☐ 3 Married ☐

4 Widower/widow ☐

5. Education levels of respondent

1None ☐ 2 Primary ☐ 3 Secondary ☐ 4 Collage ☐

5 University ☐

PART B:SOCIAL ECONOMIC CHARACTERISTICS

1. Are you employed?

Yes ☐ 2. No ☐

2. If Yes above, what type of employment do you have?

1 Self eg motorcycle rider ☐ 2 Casual ☐ 3 Business ☐

4 Government employee ☐

3. What is your average monthly income?

1. Less than Ksh.3000 ☐

2. 3000 to 5999 ☐

3. 6000 to 8999 ☐

4. 9000 to 11,999 ☐

5. 12000 to 14999 ☐

6. 15000 to 17999 ☐

7. 18000 to 20999 ☐

8. 21000 and above ☐

4. Do you own any livestock?

1 Yes ☐ 2 No ☐

5. If Yes above, name the number of each group.

1. Cattle ☐ 2. Sheep ☐ 3. Goats ☐ 4.Poultry ☐

6. Number of Poultry kept in the household?

1. Indigenous poultry ☐ 2. Layers/Broilers ☐ 3 Ducks 4 Geese ☐

7. What are the common disease experienced by poultry in your household? ☐

1New castle ☐ 2 Fowl typhoid ☐ 3 Fowlpox ☐

8. What is the source of drinking water for your household?

1 Dam ☐ 2 River ☐ 3 Well ☐

4 Borehole ☐ 5 piped water ☐

9. What is your source of water for your poultry/animals?

1 Dam ☐ 2 River ☐ 3 Well ☐ 4 Borehole ☐

5 piped water ☐

10. What kind of sanitary facilities do you use in disposing human waste?

1 Modern/Flush toilet ☐ 2 Un-plastered pit latrine ☐

3 open defecation (bush) ☐

11. What is your house type?

1. Grass thatched mud house ☐

2. Iron sheet roof, mud house ☐

3. Iron sheet roof semi-permanent house ☐

4. Permanent dressed house stone ☐

PART C: POULTRY MANAGEMENT AND HUSBANDRY PRACTICES

1. Production system

1 Free range ☐ 2Semi intensive ☐ 3 Intensive ☐

2. Who takes care of poultry?

1 Household head ☐ 2 Wife ☐ 3 Children ☐

4 Worker ☐

3. Where does your Poultry sleep?

1Kitchen ☐ 2 Main house ☐ 3 Pen ☐

4. How do you dispose Poultry waste/faeces?

1 Compound ☐ 2 Composite pit ☐ 3 Farm ☐

4 Kitchen garden ☐

PART D: ANTIBIOTIC USE FOR POULTRY

1. Have you ever used antibiotics to treat Poultry in the last 3 months?

1 Yes ☐ 2 No ☐

2. What type of drug have you use?

1. Penicillin ☐ 2Tetracycline ☐ 3. Multivitamin ☐ 4 Any other ☐

3. Where do you get the drugs supply from?

1 Agrovet/Drug dealer ☐ 2 Government ☐

3 Neighbour ☐

4. Who treats your poultry?

1. Household head ☐ 2 Mother ☐

3. Vet/Livestock Officer ☐

5. How many days do you administer the drug?

1.1 day 2. 2 days 3. 3days 4. 4days 5. 5days

6. 6 days 7. 7 days 8. More than 8 days

On average, how much money do you use to buy poultry drugs per month Ksh……

6. How do you administer drugs to your Poultry?

1. Treat Sick only ☐ 2.Flock in water ☐ 3.Flock in feed ☐

4. Nasal drops ☐ 5. Injection ☐

7. What is the main source of feeds for your Poultry?

1. Domestic Leftovers ☐ 2.From Agro vets ☐

3.Scavenging on the compound ☐ 4 Grains ☐

PART E: ANTIBIOTIC USE FOR HUMANS

1.Do you know what antibiotic is?

1Yes ☐ 2 No ☐

2.Have you ever used antibiotics in the last 3 months?

1. Yes2.No ☐ ☐

3.What was the main reason for using antibiotics?

1 Fever ☐

2 Common cold, coughing ☐

3 When you feel sick ☐

4.What is the source of your antibiotics?

1Pharmacy by physician prescription ☐

2 Direct from chemist outlet without physician prescription ☐

3 From physician after consultation ☐

4 Household members ☐

5 Antibiotics leftovers ☐

6 Neighbour/Friend ☐

5.Who diagnosed the disease you where suffering from?

1. Byself ☐ 2.Government care provider ☐

3.Private doctor ☐ 4 Private lab ☐

6.How many days if you know or explained by your doctor should a dose take of antibiotic take?

1day ☐ 2days ☐ 3days ☐

4 days ☐ 5days ☐ More than 5 days ☐

7. How many days should a dose take?

1day ☐ 2days ☐ 3days ☐

4days ☐ 5days ☐ More than 5 days ☐

8. On average, how much do you spend on antibiotics? Ksh……

9. Are you able to afford antibiotics?

1Yes ☐ 2 No ☐

10.If No in the question above, why?.....

11.Do you take full dose of antibiotics as prescribed?

1 Yes ☐ 2No ☐

12.For how many days?

1day 2days 3days 4days 5days 6days

7days 10days 14days more than 14days

13.Why do you not adhere?

1. Distance to pharmacy ☐ 2. Cost of antibiotics ☐

3Lack of finances ☐ 4.Lack of antibiotics at the health centre ☐

5 Forgot ☐ 6. Reacted badly to medication ☐

7. Distance to the nearest chemist ☐

14.Is there a reason for not adhering to the treatment?

1 Yes ☐ 2. No ☐

Explain?.....

15.Can you recall any reason why you could not complete a dose of antibiotics in the past?

1 Yes ☐ 2 No ☐

Explain……..

16. How do you take medicine?

1 Swallow with water ☐ 2. Swallow with porridge ☐

3Swallow with juice ☐ 4ingesting with food ☐

5 Injection by doctor ☐

17. Do you use antibiotics for emergencies at home?

1.Yes ☐ 2. No ☐

18. Do you request your physician to prescribe you antibiotics for stock purpose at home?

1.Yes ☐ 2. No ☐

19. In your opinion, do physicians routinely prescribe antibiotics to treat common cold symptoms?

1Yes ☐ 2. No ☐

20. In your opinion, do physicians prescribe antibiotics over the phone to patients without examining them patient

1Yes ☐ 2 No ☐

Explain…….

21. Do have any idea that antibiotics are harmful to human beings if taken without following the correct dose?

1. Yes ☐ 2. No ☐

22. Can you mention any side affects you have experienced?

……..

……