# DETERMINANTS OF ANTIMICROBIAL USE, AND THEIR RESISTANCE PATTERNS OF SELECTED *ENTEROBACTERIACEAE* ISOLATED FROM COMMERCIAL POULTRY PRODUCTION SYSTEMS IN KIAMBU COUNTY, KENYA

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# DECLARATION

This thesis is my original work and has never been presented for any degree award in any other Institution/ University.

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# DEDICATION

I dedicate this work to the soul of my late Father Mr. Francis Ndukui Ngugi

My mother Josephine Munyinyi Ndukui

My Lovely Wife Priscilla Wanjiku Gakunga

My brother Gabriel Gatonye Ndukui

Thank you for your prayers, support and Love

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## LIST OF ABBREVIATIONS AND ACRONYMS

**AMR**; Antimicrobial Resistance

**SDGs** – Sustainable Development Goals

**UHC** – Universal Health Care

**DDST**; Double Disc Synergy Test
**EU**; European Union

**FAO**; Food and Agriculture Organization

**MBC**; Minimum Bactericidal Concentrations

**MIC**; Minimum Inhibitory Concentration

**OECD**; Organization for Economic Cooperation Development

**OIE**; Office International des Epizooties

**PABA**; P-amino benzoic acid

**PBPs**; penicillin-binding proteins

**US FDA**; United States Food and Drug Administration

**APUA**; Alliance for Prudent Use of Antibiotic

**CFU**; Colony-forming unit

**WGS**- Whole Genome sequencing

**PCR**- Polymerase Chain Reaction

**CLSI** – Clinical laboratory Standards Institute

**NACOSTI**-National Council of Science, Technology and Innovation

**OXA**- Oxacillin hydrolyzing Capabilities

**CTX-M** – Cefotaxime hydrolyzing Capabilities

**QNRs**- Quinolones

**SHV-** Sulfhydryl variable

**bla** – Beta lactamases

**TEM**- Temoneira

**ESBLs**- Extended spectrum β-Lactamases

**MDR**- Multi drug resistance

**TMP**- Trimethoprim

**UoN**- University of Nairobi

# ABSTRACT

Commercial poultry production is an important farming enterprise in most parts of Kenya. The treatment of enteric infections in animals has been complicated through development of antimicrobial-resistant microbes. Occurrence of Multiple forms of drug resistance patterns has rendered chemotherapy drugs less effective in management of microbial infection. Multidrug-resistant Extended-Spectrum Beta-Lactamase (ESBL) producing *Enterobacteriaceae* places great threat towards public health by raising morbidity and mortality particularly in developing countries. Accurate statistics on the frequency and mechanism of antibiotic resistance development in enteric micro-organisms isolated from poultry and persons in Kenya is scarce. This current study determined the extent of antimicrobial use, and resistance patterns in selected *Enterobacteriaceae* isolated from commercial poultry production systems in Kiambu County, Kenya.

A descriptive cross-sectional study was conducted on commercial poultry production systems of Kiambu County to ascertain the knowledge level, attitudes, and practices on antibiotics use in addition to their resistant patterns on selected *Enterobacteraceae* species. One hundred and fifty six commercial poultry farmers were purposively selected from the following sub-counties; Ruiru (n=33), Juja (n=20), Gatundu North (n=20), Gatundu South (n=19), Thika (n=27) and Kikuyu (n=37) respectively. One hundred and fifty-six (n=156) semi-structured questionnaires imprinted in a mobile Open data kit were administered to capture the knowledge level, attitudes, and practices on antimicrobial usage in commercial poultry production systems of Kiambu County. A total of 437 fecal samples (cloacal swabs (n=365), and Human stool (n=72)) were collected, followed by bacterial isolation, confirmation, antibiotics susceptibility tests, and molecular detection of ESBLs genes and Quinolones resistance (QNRs) were detected using Polymerase chain reaction (PCR) methods. Out of 156 commercial poultry farmers who participated in this study, females accounted for (64%) with 51% of them keeping layers between 50-500 birds as medium-scale farmers. Sixty-one percent (61%) of the farmers were 45 years and above, and they had obtained a secondary level of education (34%). Sixty seven percent (67%) of the farmers obtain antibiotics through self-prescription/personal experience from the drug shops. Farmers identified lack of enough veterinary officers (77%) and consultation services (72%) as key challenges in poultry production system. Sixty three percent (63%) of the respondents, indicated tetracycline as the most commonly used antibiotic in their farms, with oxytetracyclines subtype (85%) being the most preferred. Also amoxicillin (63%) was equally preferred by farmers with amoxi (88%) sub-types being the most preferred,

respectively. Nearness to veterinary services significantly (P<0.05) contributed to the use of antibiotics agents. Additionally, the area of residence, type of production, number of years in farming, and average number of birds kept also significantly (P<0.05) influenced the level of knowledge and practice on antimicrobial usage. Out of 437 fecal and stool samples collected, 591 (Cloacal isolates=544; human isolates= 47) isolates were recovered, with *E.coli* (48.9%) being the most frequently identified, followed by *Shigella spp*. (18.8%), *Salmonella spp.* (18.3%), and *Klebsiella spp.* (14.0%).There was a great occurrence of multiple resistance among isolates especially towards sulfamethoxazole (79%), trimethoprim (71%), and tetracyclines (59%), respectively. Additionally, the isolates showed the highest rate of susceptibility against cefuroxime (94%), gentamicin (93%), ceftriaxone (91%), cefepime (89%), cefotaxime (85%), ceftazidime (84%), and chloramphenicol (77%), respectively.

Out of the isolates (n=78) collected with potential ESBLs production, only 66 (84%) tested positive for the target genes with the utmost detected ESBL genetic factor from the isolates being blaOXA (n=20; 26%), followed by blaTEM (n=16, 21%), with the majority of them detected in *Escherichia coli*. In all the four enteric's bacteria-type isolates tested, the blaCTX-M gene was detected. Three (n=3) *Salmonella, and E.coli spp* respectively harbored all the five tested antimicrobial resistance (AMR) gene types. The blaTEM, blaOXA, blaSHV, and QnrS genes were not detected from Klebsiella and Shigella spps. Additionally, most of the AMR gene co-carriage was detected in both E.coli and Salmonella spps as follows blaTEM+blaOXA (n=4); blaTEM+QnrS (n=3); blaTEM+blaOXA+Qnrs (n=3), concurrently.

It was concluded that both fecal and stool materials from commercial poultry and humans can be a reservoir of multi-drug resistance enterics. This is a potential route of spreading out resistance genes amongst livestock and Humans, which pose a great danger to public health of Kiambu County residence. There is also need to strengthen antimicrobial surveillance and monitoring strategies at County level. It was recommended that the results of the current study be used in formulating strategies and informed decisions in combating antibiotic resistance and irrational usage of antibiotics in commercial poultry production in Kiambu County and other areas with similar poultry production systems in Kenya.

# CHAPTER 1: GENERAL INTRODUCTION

## 1.1 Introduction

Since year 2000, treatment of enteric disease in animals and humans has been complicated by the emergence of antimicrobial resistant pathogens. Emerging of antimicrobial resistant ESBLs producing *Enterobacteriaceae* is an undecorated threat to public health (Jaciane Marques *et al*., 2015). The quick occurrence of ESBL producing Gram-negative micro-organisms that are resistant to flouroquinolones, β-lactams and colistin has been rarely reported (Bialvaei and Samadi Kafil, 2015; Sampaio, and Gales, 2016; Kateregga *et al*., 2015). Other resistance mechanisms against beta-lactams are the surface membrane permeability change, and drug exit pumps. Over and abuse of antibiotic agents in livestock production has led to development of resistance and successive transmission of resistance genetic factor among microbes to livestock, livestock products and the environment (Rose *et al*., 2009; Thriemer *et al*., 2013; ;Davies and Davies, 2020). Conversely, prudent use of antibiotics is essential, however, tolerable approaches to accomplish this objective, and to address the challenges must be formulated and conversed (WHO, 2014). Antibiotics resistance is a plain reality across the globe, including Kenya. The problems associated with controlling antimicrobial resistance, mainly in Kenya, remain multidimensional.

Commercial poultry industry is well established and it is the leading supplier of animal protein (eggs and meat) worldwide. Poultry are moderately cheap and kept in small areas mainly providing income and protein to the families (FAO, 2002; Moreki *et al*., 2010). However, poultry diseases are some of the limiting factors to this industry. The increased poultry disease burden has accelerated the high demand and the use of veterinary antimicrobial drugs (Morley *et al*., 2005).

Majority of Kenyans depend on poultry products for livelihood and survival, therefore low production levels affect their income levels (ROK, 2010; MoLD, 2006). This affects their impact to the nation GDP and worldwide economic enhancement, this resulting to impediment of Medium term plan 111 goals as set by the government on sustainable development through agriculture. In Kenya, poultry production, is considered as a source of protein enrichment towards deficiencies caused by lack of animal proteins in the diet. The high turn-over rate and the quest for white meat have given more credence to poultry farming. Due to increased demand for white meat, by the populations, this has necessitated the large scale commercial poultry farming and leading to subsequent usage of antimicrobial agents as growth promoters. Occurrence of antimicrobial residues in food for human consumption is documented globally by numerous public health experts as being unlawful (Landers *et al*., 2012), and their ingesting could lead public health threats including: progress of resistant strains of microbes, respiratory infections, cancer, carcinogenicity, hypersensitive reaction in sensitized individuals (Tran *et al*., 2017), and distortion of intestinal microflora (Selvaraj *et al.,* 2018; Overgaard *et al.*, 2012; and European Centre for Disease Prevention and Control (ECDC), 2012).

The looming antimicrobial-resistance predicament has been perceived by policymakers, researchers, clinicians, politicians and the community at a great extent as a major challenge in control of community and hospital based infections. Extensive dissemination and progression of ESBL producing microbes has made infections that were quickly treatable as deadly again. Regrettably, supplementary to the upsurge in global resistance is failure in new drug discovery and development. According to Naylor *et al.* (2018), acquainting oneself with the history of antibiotic finding and fresh understanding of their action and the cell biology of bacterial have the ability to deliver twenty-first century medicines that are able to control infection in the resistance era (Naylor *et al*., 2018).

According to Mitema *et al.* (2001), on evaluation of antibiotics consumption in Kenya he revealed that approximately 14,600kg of active antimicrobial were annually consumed in animal food production of which tetracycline's (56%) and sulfonamides + trimethoprim (22%) accounted for nearly 78% of the antibiotics used. Similarly, this was also witnessed by Kolar *et al*. (2002) who reported high resistance due to increased incidence of tetracycline and erythromycin usage especially in *E.coli* and Enterococcus infections. This has resulted to social and scientific concern that over prescription and misuse of antibiotic has resulted to antimicrobial resistance in Africa and the entire world (Morris and Masterton, 2002). Mukasa *et al*. (2012) found that that there was a rampant misuse of antibiotic by farmers in Ngoma sub county, Uganda and called for more studies and mitigation measure to be taken to break this vicious cycle.

The usage of  antimicrobial drugs in poultry production is very important in disease treatment, prevention and growth promoting but its use must be accepted as a responsibility rather than a right when trying to improve poultry health (Rose *et al*., 2009). This will help to minimize the potential risk and hazards due to poor drug usage of antibiotics in livestock production. Therefore, usage of  veterinary antimicrobial drugs in poultry systems needs proper control through government regulatory bodies with the aim to preserve animal health, improve animal production and to protect the public (Cardona & Kuney, 2002). Therefore, the current study evaluated the antimicrobial usage, and drug resistance patterns from selected *enterobacteriaceae* isolated from commercial poultry production systems happening Kiambu County, Kenya

## 1.2 Problem statement

Globally, it is projected that by 2050, the health consequences and economic costs of AMR are estimated at 10 million annual human fatalities and a 2 to 3.5 per cent decrease (equivalent to USD 100 trillion) in global Gross Domestic Product (GDP). Kenya is considered as the hub to East and Central Africa in trade relations (Import and export of good and services). Therefore, use of antimicrobial agents has created concerns for antibiotic resistant pathogens. This has resulted due to the increasing world population and the need to cater for their nutritional need which has consequently, resulted to increased demand towards poultry proteins, which has resulted into increased poultry production systems, the traditional systems may require a dramatic expansion of chicken populations to satisfy current local and global demand for white meat and eggs. Thus, increased use of antimicrobial in commercial poultry is inevitable as growth promoters not noting that farms for commercial purposes are key facilitators of human to animal transmission of genes and zoonotic diseases. However, the country is under high disease burden due to gastrointestinal infections. This has resulted to increased dependence on veterinary antimicrobial agents to control these diseases and boost production. This has raised a growing concern that the use of antimicrobial drugs in veterinary medicine and animal husbandry may compromise human health if resistant bacteria develop in animals and are then transferred to humans via the food chain or the environment. The occurrence of antimicrobial resistance among *E.coli spp*; *Salmonella Spps; Shigella Spps and Klebsiella spp* pose a great danger to humans and poultry in management of gastrointestinal disorders and loss of economic production due to the high cost in management of this disease burden. This resistant bacteria could then cause disease that is difficult to treat in humans, lengthened hospital stay, and may also transfer the resistant gene to some other human pathogens which undermines the achievement of sustainable development goals (SDG's) and Universal health care in Kenya. In addition, molecular-based detection of the drug resistance of indicator microorganisms is a challenge, as is monitoring their circulation in hospitals and in the community. Hitherto, withdrawal period is not observed and there is laxity of programmes put in place in monitoring/surveillance of rational use of veterinary antimicrobial agents.

## 1.3 Justification of the Study

Commercial poultry production is a very important enterprise in terms of its contribution to the peoples' livelihood and food security. However, due to increased demand for animal proteins, this has resulted to increased poultry production initiatives. The traditional systems requires a dramatic expansion of chicken populations to satisfy current local and global demand for white meat and eggs. Thus, increased use of antibiotics in commercial poultry systems is inevitable as growth promoters which has resulted to antibiotics resistance. This has resulted due to inconsistency in vulnerability of *Enterobacteraceae* to different antibiotics which has turn out to be a key character preventing the effective treatment of bacterial infections. Even though resistance in these alleged normal flora microbes might not be of importance in single host species, these microbes can result to sickness in other microbes and all unaffected microbes could serve as pools of resistance genetic materials. Further to these, developing resistance among bacteria is becoming worrying, if left unrestrained, efficacy of many of the most significant antimicrobial drugs at a given point will no longer be foreseeable, and some microbial illness could once more become deadly. Even though extensive discussion is witnessed along this issue, desirable new investigation to further document the threats posed to humans and animals by antibiotic resistance is necessary because evidence based comprehensive information is fundamentally unavailable. Nevertheless, there is growing worldwide pressure to develop policies to guard the efficacy of prevailing and novel antibiotics through decreasing selection stress causing evolving resistance in microbes. Therefore, the current study has generated new information on challenges and factors that prompt commercial farmers to use antibiotics irrational and the mechanisms and resistant patterns that selected *Enterobacteraceae* have developed to the currents antibiotics in market in the management of poultry diseases.

## 1.4 Objectives

### 1.4.1 General Objective

To Determine antimicrobial use, and their resistance patterns of selected *Enterobacteriaceae* isolated from commercial poultry production systems in Kiambu County, Kenya.

### 1.4.2 Specific Objectives

i. To assess the usage of antibiotics agents in commercial poultry production systems in Kiambu County, Kenya.
ii. To establish the phenotypic basis of resistance patterns of selected *Enterobacteriaceae* isolated from commercial poultry production systems and humans in Kiambu County, Kenya
iii. To establish genotypic expression of antimicrobial resistance patterns of *Enterobactaeraceae* isolates from poultry fecal material.

## 1.5 Research hypothesis

i. The poultry farmers do not have appropriate knowledge, attitudes and practices on antimicrobial drugs use in treatment of diseases in poultry production in Kiambu County, Kenya.
ii. Antimicrobial resistance has not been developed against antimicrobial agents used in commercial poultry production systems in Kiambu County, Kenya.

iii. Extended spectrum Beta-lactamases producers and QnrS gene are are not present in fecal and stool samples collected from commercial poultry production systems of Kiambu County.

# CHAPTER 2: LITERATURE REVIEW

## 2.0 Antimicrobial/Antibiotics agents and their use

Antimicrobial agents are drugs or medicines that manage all form of diseases causing micro-organisms namely: Bacteria, parasites, Viruses and Fungi. Whereas the term antibiotics was initially defined as elements produced by one micro-organisms to impede the progress of other microbes. Due to emergence of non-natural antibiotics this terms nowadays is defined as molecules produced by a microbes or to a related substance (fashioned exclusively or partially by chemical synthesis), which in small quantities prevents the growing of supplementary microbes (Marjorie, 1999).The universal usage of antimicrobial/antibacterial drugs has enriched the well-being of man and livestock ever since the antimicrobial ideal age and the development of many nations worldwide was at need (Wieczorek and Osek, 2013). These treatments are used in both human and livestock microbial illnesses, and as feed additives to improve growth of domesticated animals. Antibiotics are also used in feed productions as preservers and in profit making ethanol to inhibit bacterial pollutants of the fermentations industry. Consequently, they are used in agriculture to prevent vegetable ailments other than in tissue cultures. Conversely, enormous usage of the antimicrobial/antibiotic agents in unreasonable mode has changed the environment of the micro-organisms, obliteration of useful microbes in the surroundings together with the normal flora in addition to aggregating the selection of the disease causing antimicrobial resilient bacteria organisms that have caused their spread worldwide (Hakem *et al.*, 2013). This has caused great financial losses in livestock industry and in humankind; furthermore it has resulted to prolonged hospitalization, adverse drug reactions, and treatment failure, raised price of treatment, and overstretched livelihood status of many persons worldwide (Morris and Masterson, 2002). These challenges are expected to intensify in forthcoming periods if there is no immediate establishment of governing measures comprising of all shareholders on the prudent use of antimicrobial/antibiotic drugs worldwide.

## 2.1 Modes of action of Antibiotic/antimicrobial agents

To understand the mode of resistance to antibiotics, it is imperative to appreciate by what method antibiotics agents act within the living systems. Antimicrobial drugs act selectively on live bacterial functions with marginal special effects experienced on the host tissues. Diverse antibiotic drugs act in diverse ways. To understand how resistance develops against antibiotics it is paramount to understand the chemical nature of the antibiotic agents. Largely, antibiotic drugs may be labeled as either those that inhibit growth (bacteriostatic) or those that kills the bacteria (bactericidal) (Jian *et al*., 2012). Bacteriostatic antibiotic drugs simply impede the growth or duplication of the germs providing the body defense system of the host time to get rid of them from the body. Total removal of the microbes in the host is dependent on the capability of the immune system. For bactericidal drugs they are able to kill the germs with or without a proficient immune system of the host, the germ will become lifeless. According to Goodman and Gilman (2006), antibiotic agents mechanism of action can be categorized further based on the structure of the bacteria or the function that is affected by the agents. These includes the following mode of action: destruction of the cell wall synthesis; suppression of ribosome function; Inhibition of nucleic acid synthesis; withholding of folate metabolism, and halting of cell membrane function.

## 2.2 Classification of antibiotics/ antimicrobials commonly present in the world market

Despite hundreds of antibiotic drugs in human-beings and livestock use, majority belong to a limited main classes; nevertheless, few of these agents are appropriate for use in food producing animals (Jian *et al*., 2012). According to WHO (2002) it recommended against the use of antibiotic drugs already approved as therapeutic agents in humans or animals as growth promoters. Strategies on developing new polices on the reducing losses witnessed from antimicrobial resistance on those drugs considered for human medicine are being developed by World Health Organization (WHO, 2002), Codex Alimentarius Commission (CAC, 2015), and the World Organization for Animal Health (2009).

Compounds with a related molecular structure and with similar modes of action against bacteria's have been entirely classified as be antimicrobial agents. However, dissimilarities within a given antimicrobial class could result due to presence of a different side chain of a fragment, which gives a different patterns of Pharmacokinetic and Pharmacodynamic actions on a drugs (WHO, 2000). Recognizable classes of most commonly available antimicrobials are as follows; Tetracyclines, sulfonamides, Macrolides, Aminoglycosides, Chloramphenicol, Penicillins, antihemintic and Fluoroquinolones.

## 2.3 Antimicrobial/antibiotic use as a global problem

The WHO, (2001) described antimicrobials as chemical substances that reduce /entirely block the growth and multiplication of bacteria. This has made them exceptional for the control of deadly infectious sicknesses caused by a variety of pathogenic micro-organisms. With this, they have modified our ability to treat infectious disease such as pneumonia, meningitis, malaria, AIDs and nosocomial contaminations. Since their great success in human-being treatment, antibiotics their usage have increased tremendously in animal disease (fish, pig and poultry largely involved) (WHO, 2001). Furthermore, subsequent discovery as growth promoters/enhancers when added to animal feeds in small doses has made them a common element of animal industry production systems. Drugs of veterinary applications are key components of food –livestock industry in which they provide great advantage to livestock health and well-being, and financial gain in animal production (Coffman and Beran, 1999). According to Mitema *et al*. (2001) the most commonly used antibiotics in food producing animals from 1995 to 1999 included; quinolones, sulfonamides, tetracyclines, Trimethoprim, nitrofurans, aminoglycosides, and β-lactams.

Despite gains associated with sub-therapeutic usage of antibiotic in improving growth and in feed proficiency which has been witnessed almost a half-century in the past, some of these products have maximized their scale of production. For a country to have a sustainable food security, agricultural production and economic empowerment, livestock industry plays a crucial role (Speedy, 2001). According to Porter *et al*. (2014), worldwide animal industry productivity developed at a rate of 2% in 1999 to exceed 233 million tonnes in 2000. In addition, he further suggested that a worldwide demand and intake of livestock products is anticipated to almost double in the subsequent 20 years. According to Speedy (2001) from 1980-2000 poultry meat production has improved from twenty three million tones to fifty seven million tonnes, while eggs production increased from 27 million tonnes to 54 million tonnes of which has virtually been witnessed in developing nations.

Kenya poultry population has grown to 31 million birds. According to MoLD (2006), Twenty two million (76 %) of these birds are free-ranging native chickens. Poultry rearing is quiet acceptable to underprivileged homes for it require little capital to operate and have low upkeep costs. In reference to WHO (2012), the issue of antibiotic resistance (AMR) has increasingly been documented as an emerging universal health threat, and the need to address AMR situation is currently accepted by numerous policy-makers, scientists, as well as by civil society organizations, including patients' advocacy groups. Even though there is developing reasoning and concern, apathy seems to continue in refining stewardship of prevailing antibiotics to avoid occurrence of new resistant microbes (Dowling *et al*., 2013). The issue of antimicrobial resistance is given less efforts in comparison to climate change, which appears to have an upsurge in scientific unanimity about its urgency in countering the impact of global warming.

Economic liability and the cost-effectiveness of changes to custody, pharmaceutical and other developments is a significant gap in the evidence-based information for policy makers in the management of AMR. According to Smith and Coast (2013a) they pointed out, that there is an incentive problem for policy makers with respect to AMR in that the problem needs to be high now and needs to be sufficient to rationalize the costs linked with solutions, such as restrictions in use of current drugs. In reference to Wilton *et al.* (2001), the economic burden attributable to AMR via reduced economic production is through increased mortality (diminishing quality of life, greater likely hood of death); increased morbidity (extended hospital stay, additional investigation, lengthened time off work, diminished quality of life).

Additionally, it is important to bear in mind that additional indirect cost can be incurred if AMR situation substantially increases this is because patients may well decide not to undertake particular medical procedures due to intensified risks involved. Individuals may also abstain from engaging in certain profitmaking activities, such as travel and trade, or experience unwanted mental effects, such as panic. It is imperative to identify the variations in the role of antibiotics drugs in health care systems across the globe (Smith *et al*., 2002). Severe infectious illness such as malaria or tuberculosis in low-income countries depends solely on antibiotic drugs at community level for their treatment, which contribute less to the burden of disease in high income countries due to their comparatively low prevalence. In comparison, hospitals in high income countries are extremely reliant on the use of antibiotic agents not only for the management of primary infections, but also for diverse aspects of secondary health care, such as cancer care or prevention of iatrogenic infection in surgical care. As a result, in well developed countries, hospital-acquired infections are a major concern (Jirka *et al*., 2014).

### 2.4 Antimicrobial residues in Veterinary practice

In 2000, Kennedy *et al*., stated that introduction of any pharmacologically active chemicals to food- generating animals unavoidably results to the manifestation of residues in food. World regulatory bodies pursues to ensure that the end-product users of the food are not prone to residues at hypothetically harmful quantities. A variety of professional bodies including the Codex Alimentatius Commission, the joint expert committee on food additives (JECFA) and the European Union (EU), have set a series of maximum residue limits (MRLs) to protect end-user on exposure to residues of known drugs to levels that cause no danger to human health (Kennedy *et al*., 2000). As long as an authorized medicine is used in agreement with its products license and as long as the withdraw timelines are checked prior to slaughter of animals (or consumption of products like eggs, milk and meat), harmful deposits should not ensue in humans. During feed production, the drugs may be carried over from treated feeds to subsequent supposedly un-medicated feeds. Unintended nourishing of such diets immediately prior to slaughter of food animal's species can translate to volatile residue concentrations (Kennedy *et al*., 2000).

Concentrations of drug or environmental chemicals that are detectable by analytical methods are defined as drug or chemicals residue in tissues of food producing animals (Mount, 2001). Mount, (2001) also defined residues as parent drugs or chemicals, their metabolites and their decomposition products. The quality of residues if detected is expressed by weight such as mg/kg of a tissue (Parts per billion, ppm; Parts per billion, ppb).

Medicines and chemicals that do not have a finite "tolerance level" are allowable in foods as long as the quantity is within a recognized "margin of safety". When these acceptance levels are established, they allows food with these residues to be acceptable for sale, when the residues are equal to or below the acceptable limit (Mount, 2001). Residues that exceed the "tolerance limit" are illegal and not saleable for human food. Codex Alimenarious (1994), defined withdraw time and withholding as the time frame amongst the previous administration of a drug and the collection of palatable tissue or products from a treated animal that ensures that the contents of residue in food comply with maximum residues limit for a veterinary drug (MRLD).

### 2.5 Global use of Antibiotic in poultry production systems

Drugs or medicine is defined as any element or mixture of substance manufactured, traded or characterized for use in testing, management or in prophylaxis of diseases, syndrome, abnormal physical state or symptoms thereof in man or animal (FDA, 2017).While antibiotics are drugs that are selectively toxic for bacteria as bactericidal (Killing) or bacteriostatic (growth inhibition) with minimal harm to patient (Goodman and Gilman, 2006). The usage of veterinary antibiotic agents in chicken production is very important with regards to disease control and prevention but its use must be accepted as a responsibility rather than a right when trying to improve poultry health (Andrews, 2004). This helps to minimize the potential risk and hazards due to irrational antibiotic use in poultry farming. Therefore, usage of veterinary antibiotic drugs in poultry and other animal's species need proper control through legislative bodies with broad goal to preserve animal health, improve animal production and to protect the public (FAO, 2004). In the poultry industry various types of veterinary antibiotics drugs are used, they includes but not limited to drug used in treatment and supportive therapy of infectious diseases such as coccidiosis and fowl typhoid), Infectious Bursal Disease (I.B.D or Gumboro), fowl pox and Marek's disease, among others. These includes antibiotics such as; tetracyclines (oxyveto® or OTC-plus®, Amoxicillin (Hipramox p®), Sulfadimidine (S-Dime®) and Enrofloxacine (Hipralona Enro®). All these drugs listed above calls for the absolute professional veterinary services on prudent use, proper handling and record keeping for all pharmaceutical products that may be used in farm management (Wongsamut, 2009).

### 2.6 Mechanisms that influence the use and misuse of veterinary antibiotics drugs by poultry farmers

Privatization of veterinary services has limited the access of poultry farmers to the veterinarians due to increased cost of veterinary services. Therefore, most farmers decide to treat their own birds (Ashley, 1996). Poultry farmers have little knowledge on correct antibiotic drug use, especially the recommended withdrawal periods of most drugs they use for meat or egg production (Levy, 2002). With most antibiotic drugs such as tetracyclines, amoxillin and enrofloxacin in use, poultry farmers and animal health workers cannot read and clearly understand the instructions on the labels on the drugs containers and inserts. This is because most of the instructions on drug labels are in English or other foreign language that cannot be well understood by most poultry farmers (Mukasa *et al*., 2012; Swai, 2004).

Veterinary drug dealers and sellers in drug shops often lack adequate knowledge on rational use of veterinary antibiotics. Hence, poultry farmers are not advised correctly on how to use the drug, dosage, indication, and contraindication and withdraw period (Cluff *et al*., 1975; Otupiri *et al*., 2000).

## 2.7 Antibiotics as feed additives in animal production

Feed manufacturing plants and farmers have been adding antimicrobial agents to animal's feeds since 1946, after it was recognized that use of these antimicrobial drugs triggered livestock growth quickly and the animals added mass in a short time (EFSA, 2011; Chee-Sanford, 2013). It is stated that more than 80% of all antimicrobial agents manufactured worldwide are intended for livestock use and while other are spent in aquaculture, to regulate bacterial diseases in fish and other aquatic animals (WHO, 2011; Thriemer *et al*., 2013). The antimicrobial/antibacterial agents produce an adjustment in body physiological, nutritional and metabolic processes of the faunas. According to Graham *et al*. (2005), and Giguere (2006) antibiotics are used for the following:(i) Inducement of vitamin synthesis by micro-organisms in the digestive system; (ii) lessening the total amounts of microbes (normal flora) in the digestive system hence decreasing the competition amongst microbes and host animals for nutrients; (iii) control of detrimental microbes which may be slightly pathogenic or toxin-producing; (iv) stop urease production in microbes; (v) enhanced energy usefulness of the digestive tract; (vi) downplay of microbes cholytaurin hydrolase action; (vii) nutrient saving; (viii) enrichment of nutrient pharmacokinetics mainly absorption from the digestive tract epithelium; (ix) modification of intestinal enzyme action; (x) diminished immune enhancement due to pressure triggered by overpopulation of the animals , and (xi) amendment of rumen microbial metabolism.

## 2.8 Antibiotic use in food industries and as food preservatives

Numerous micro-organisms from individuals, livestock and the environment regularly adulterate the food productions process and additional fragile foods particularly throughout processing and shipping towards the market places in contaminated conditions (FSSA, 2013). These microbes destroy and use nutrients required by human beings in the diets and also lead to putrefaction of foods resulting to business and monetary loss. In reference to Lushia and Heist, 2005; Olmstead, 2009; Bischoff *et al.*, 2009, germs pollution also upsurges the price of sanitization of the industrial facilities, this leading to irrational use of antibiotics/antibacterial to regulate microbes contamination in food manufacturing plants.

Several antimicrobial/ antibiotic agents have been used ever since 1940's in the conservation of fragile products and nutritional products. Among the habitually consumed antimicrobial agents includes chloramphenicol, oxytetracycline, penicillin acid, and streptomycin which are used to safeguard fish (Davidson and Harrison, 2002). According to FASS (2010) and Mattia *et al*. (2013), tetracyclines have been used in food industries to prevent growth of harmful microbes in poultry product, fish, canned foods, cheese, meat, sausages and other non-sterile animal products. In accordance to Davidson and Harrison, (2002); FASS, (2010), and Mattia *et al*. (2013), they support the natamycin a polyene macrolide which is manufactured through immersed aerobic fermentation of *Streptomyces natalensis* and associated species (Mattia *et al*., 2013). In emerging and under-developed nations resembling Kenya, certain confined milk storage facilities, normally use antimicrobial agents to deter milk decomposition by microbes particularly *Lactobacillus* bacteria thus exposing customers to minimal levels of these medicines thus causing selection process of antimicrobial resistant bacterial pathogens in humans, animals and environment (Alexander *et al*., 2008).

### 2.9 Antibiotic/ Antibacterial use in farming/ Agriculture

A number of antimicrobial/antibiotic drugs are frequently utilized in agriculture and agribusiness to regulate microbial illness. According to Bryden *et al*. (1994), oxytetracycline and streptomycin since 1950s have been use in horticulture to control bacterial infections in highly-valued fruits, edible greens, and trees generating wood and ornamental vegetation. Additionally, Mc manus and Stockwell (2000), informed the public that more than 30,800 pounds of streptomycin and 26,700 pounds of oxytetracycline have been used in microbial infection control in fruit trees in the United States of America. Other antimicrobial agents that have been used in agronomy includes: Chloramphenicol; Polyoxins; polyene macrolides; Cellocidin: Griseofulvin; Cycloheximide (Bryden *et al*., 1994; Falkiner, 1998; McManus and Stockwell, 2000). Nevertheless, some of these agents have been used in agribusiness and market gardening universally which has become of public health significance, ever since these agents were also used in human being and livestock to manage microbial ailments (Peeples, 2012). Due to their enormous use, this has stimulated the assortment and development of resistant microbial strains in both human being and livestock that possibly will be challenging to govern.

### 2.10 Mechanism of resistance development and spread in the biosphere

Quite a number of antimicrobial agents are combinations that are linked to natural microbiota for millions of years. Since they are recyclable can be used as food preservative to quite a few of microbes (Kümmerer, 2003; Kümmerer, 2009; Topp *et al*., 2012). In nature the microbes are subjected to sub-threshold quantities of these antimicrobial agents where selected a few are destroyed and while some cultivate resistant mechanisms to evade the medicines. These antimicrobial resistant bacteria microbes are acquired from the numerous inhabitants of microbes in nature generally by horizontal gene transfer processes. Thereafter, they reach to both human being and animals worldwide triggering severe microbial infections that escalate to high disease occurrence and death.

A number of methods have developed in microbes that gives them antimicrobial resistance. The acquired mechanism can chemically modify the antimicrobial agents and make them ineffective. The ability to make the antimicrobial agents ineffective by micro-organisms is accomplished by a swift physical elimination of the medicine from the body, or altering the specific site so that it is not identified by the antimicrobial agents, and by enzymatic inactivation of the drug by microbes which a commonly identified mechanisms of antimicrobial resistance (Anderson, 2004; Promega, 2013).

According to Anderson (2004), and Tenover (2006), antimicrobial resistance in microbes occur in two ways: Through intrinsic features of the micro-organism in the cell wall configuration that gives the natural resistant, or it may be developed by ways of transmutation in its individual DNA or through attainment of resistance from DNA of alternative origin. Inherent/ natural resistance is where microbes naturally develop resistant towards a given antimicrobial agents owing to absence of target plugs by the study drug, deficiency of carriage system for the drug thus making it not to affect microbes or the micro-organisms will naturally have little penetrability to these drugs due to the differences in the chemical nature of the medicine and the bacteria membrane features particularly for those that necessitate entry into the bacteria cell in order to induce their effect (Anderson, 2004; Promega, 2013). Secondarily, assimilated resistance is where numerous techniques are established by microbes so as to obtain resistance to the antibiotic agents. This occurs by either the infectious microbe adjusting the prevailing inherited items or the attainment of new genomic factors from a different grounds (Tenover (2006)). Perpendicular genetic factor transmission is one of major way through which microbes confer resistance. This is wherever there is unplanned transmutation incidences for antimicrobial resistance taking place in the order of $10^8$- $10^9$. This is where one in every $10^8$-$10^9$ microbe in a contamination will cultivate opposition through the course of transformation. Once resilient genetic factor have happened, they are transported unswervingly to all the microbial offspring during DNA replication in the process of perpendicular genetic factor transfer or upright evolution (Anderson, 2004). Through this remote type (non-resilient) germs are destroyed and the sturdy modified microbes withstands and grows. Secondarily, parallel gene transfer is an extra process beyond unstructured mutation that is accountable for the acquirement of antimicrobial resistance. Sideways or horizontal gene transfer (HGT) is a process in which the inherent material enclosed in small packets of DNA can be transported amongst distinct microbes of the identical species or even between diverse species (Anderson, 2004; Tenover, 2006; Promega, 2013).

According to Tenover (2006) and Promega (2013), antimicrobial invulnerability among microbes is an evolutionary impression facilitated through plasmids, transposons, and integrons that transmit DNA that encodes attack enzyme, efflux pumps, and other protective devices. Microbes can attain resistance via several processes such as stated by Torrence *et al*., 2008; Baquero *et al*., 2008; Meade-Callahan, 2011; Alexander *et al*, 2011): (i) conjugation where bacteria can join and interchange plasmids and occasionally chromosome fragments. The plasmids have a wide-range of host and are capable to cross genre lines during the gene transmission. (ii) Through a process of transfection or transduction viruses can transfer genes to bacteria and fungi, from one infected organism to the next (phage), (iii) Through microbial revolution is where a bacterium lyses in its surroundings such that some of the actively-growing microbes in that environment can acquire its DNA resulting to antimicrobial resistance that can be spread in the microbes population due to plasmids such as R- plasmids that are positively used by the beneficiary bacterium than chromosomal materials. The acquired resilient genes causes the bacterium to express the innumerable resistance processes as a way to evade the antimicrobial agents subjected to them.

According to Torrence *et al*. (2008); Todar (2011) and Promega (2013), microbes that have acquired resistance to several antibiotics expresses the following; (i) By having an enzyme that incapacitates the antibiotic agent or enzymatic modification of the antibiotic; (ii) metabolic bypass of the directed passageway or the presence of a substitute pathway for the enzyme that is inhibited by the antibiotic agent; (iii) a mutation in the antibiotic drug target, which diminishes the attachment of the antibiotic or drug repossessing by protein binding; (iv) post-transcriptional or post-translational alteration of the antibiotic target, which decreases the binding of the antibiotic or adjustment of target site ; (v) diminished reception of the antibiotic agent; (vi) active removal of the drug or active pumping of antibiotic outside the cell, and (vii) Excess production/-up-regulation of the target of the antibiotic agent.

## 2.11 Antimicrobial resistance of microbes in poultry production systems

The enteric bacteria of livestock can be responsible for a pool of antimicrobial-resilient microbes that can contaminate or colonize individuals through the food channels. In reference to Kazwala *et al*. (1990), poultry can be a source for numerous food-borne pathogens such as *Campylobacter spp* and *Salmonella spp*. Antibiotic resistance in microbes sourced from commercial slaughtered chickens, alongside free-ranging chickens, has been established in several journals, including publications emanating from the African continent namely; Bebora *et al*., 1994; Aarestrup *et al*., 2000, and Keyes *et al*., 2000. There is substantial usage of the quinolone and enrofloxacin in commercial chicken farming in some countries and there is a conviction (and accumulating evidence) that the occurrence of quinolone-resistant *Campylobacter spp*. reveals the use of these drugs in veterinary medicine (Keyes *et al*., 2000).

## 2.12 Public health concerns on veterinary antibiotic drug use in poultry systems

In this aspect two areas are of very great importance, antibiotic resistance and their residues in poultry products. Drug residues in food stuffs come from different categories of veterinary pharmaceutical products that include therapeutic agents such as antihelmintics, antiprotozoals, antibacterials , pesticides, insecticides and drug feed formulations (Hakem *et al*., 2013). However, antiprotozoals and antibacterials are the categories of antimicrobial drugs of interest to this study. When these antimicrobial drug categories are misused by poultry farmers they may accumulate in body tissues and other products like meat and eggs, hence leading to residues which definetly end up in humans (Mubito, 2014).

Antimicrobial resistance can explained as the ability of a micro/macro organism to withstand a drug which was once sensitive to slows growth or killed it (Anderson, 2004). Drug resistance in veterinary practice and in medical field is as a result of irrational drug use and in this perspective by animal health workers and poultry farmers (WHO, 2014). It's necessary to ensure that poultry farm Biosecurity or preventive measures and general poultry farm hygiene are well observed in order to minimize their overuse in poultry disease management that can lead to drug resistance especially in drugs that are used both in humans and animals like sulfur and tetracyclines drugs (Mckellar, 1998).

## 2.13 Public health implications of antibacterial residues in poultry products

The Following are principal public hazards associated with antimicrobial in meat and eggs. Tetracycline is the chief offender in stimulating sensitivity reactions whereby small amount intake of residues in these products affects the population. It's estimated that about 9-11% of the population can develop sensitivity to tetracyclines, sulfonamides, macrolides, and penicillin's ranging from mild to serious reactions as seen through consuming products with antibiotic in eggs (Pennisetum, 2009). Residues of antimicrobial agents in poultry products (meat and eggs) in surplus of the approved acceptable Minimum Residue Levels (MRLs) may add-up to the cohort of resistance in microbes found in humans. Nevertheless, the present evidence advocates that the risk is low (Sasanya *et al*., 2008). Of more concern may be that such residues could indicate inappropriate use of antimicrobials by the poultry producers (WHO, 2012). There is possibility that poultry products made for birds containing antibiotics may occasionally contain resistant strains of infectious bacteria (Mubito, 2014).

## 2.14. Enterobacteraceae

*Enterobacteriaceae* families are gram negative short rods non-spore developing bacteria and entails Salmonella, Protius, *Escherichia coli, Klebsiella*, *Shigella*, *Enterobacter*, *Citrobacter* and *Serratia* (Khan & Malik A, 2011). The process of colonization of enteric bacteria cover human and animals e.g. livestock, poultry, rodents, reptiles, and birds (Khan & Malik A, 2011).

Enteric bacteria are known for causing various diseases like enteritis and typhoid among others. These bacteria develop resistance easily to antimicrobial treatments especially when patients infected have a history of buying over the counter drugs and abusing related drugs of choice (WHO, 2014). Currently, some achievement have been made in management of GIT (gastrointestinal tract) diseases and/or infectious diarrhea through antibiotic agents. Nevertheless; quick emergence of multidrug resistance has developed progressively as an emerging problem with severe repercussions in community health according to Organization for Economic Co-operation and development (2004) and WHO (2014). Bloodstream and urinary tract infection even in patients without health associated risk factors can be associated with the intestinal carriage of MDR microbes which can persist for years without causing disease in a living being (WHO, 2014).

## 2.15 Extended Spectrum beta-Lactamases (ESBLs)

ESBLs are type of enzymes formed in microbes to breakdown third and fourth generation cephalosporin's and Monobactams. This ESBLs have been found in most members of the genera *Enterobacteraceae* and *Pseudomonas aeruginosa*, although mostly are found in *E.coli* and *Klebsiella pneumonia spps.* Four types of ESBLs have been identified namely: bla $_{OXA}$, bla $_{SHV}$, bla $_{CTX}$, and bla $_{TEM}$ (Chaudhary and Aggarwal, 2004). The ESBLs genes bla $_{TEM}$, and bla $_{SHV}$ are mostly established in *Klebsiella pneumonia* and *E.coli.* However, TEM-1 is mostly expressed in gram-negative *Enterobacteraceae*, this enzyme is responsible for hydrolysis of penicillins and first generation cephalosporins (Chaudhary and Aggarwal, 2004). Enzyme bla $_{SHV}$ has been associated with *Klebsiella pneumonia spps* and it accounts for 20% plasmid mediated ampicillin resistance according to Bradford (2001).CTX-M and bla-OXA are another rising groups of ESBLs arising from *Enterobacteraceae* and of great importance to mechanism of antibiotic resistance.

# CHAPTER 3: DETERMINATION OF ANTIMICROBIAL USAGE IN COMMERCIAL POULTRY PRODUCTION SYSTEMS IN KIAMBU COUNTY, KENYA.

## 3.1 INTRODUCTION

The importance of commercial poultry production in various households in developing nations cannot be overstated, as it has turned out to be a popular initiative that contributes immensely to income, food security, and national economy (FAO, 2010; FAO, 2004). The demand for poultry products stands on the upward scale due to the growth in earnings, suburbanization, nutritional benefits such as protein, micronutrients and higher poly-unsaturated fatty acids and less cholesterol (FAO, 2002). Lately, the Kenya government, highlighted the poultry production systems as a key sector for necessitating one of the big four agenda on food safety, security and nutrition, and on the millennium goal mid-term plan 111 on lessening poverty and enhancement in nourishment due to its short production interval, great rate of productivity, minimal land needed, low economic value, minimal cultural/ religious taboos, and manure generation which supplements crop-animal subsystems. In addition, increased awareness and prudent use of antimicrobial agent in management of poultry disease, and promotion of commercialization of poultry, will in the long run address the increasing demand for poultry and their products, which, in turn, will lessen poverty and improve food security.

Due to increased poultry product demand and the urge for white meat, commercial poultry production systems have been embraced across the country which has resulted to increase demand for antimicrobials which are  used for therapeutic purposed, for diseases prevention, as growth promoters, and with their use been intensified in many devloping  countries (Geidam *et al*., 2012; Morley *et al.*, 2005).  According to Mellon *et al*. (2001), about 70% of entire antibiotics are used for non-therapeutics purposes in reference to the Union of Concerned Scientists in the USA. Furthermore, according to Cohen (1998), 80% of antibiotics used in poultry production worldwide are unnecessary. This was further cemented by Apata (2009) who claimed that this complex approach of antibiotic use in livestock production amongst the volumes of antibiotics used with the rate of bacterial resistance development indicates that the more we use them indiscriminitively the more we tend to lose them. Therefore, in order to establish the level of knowledge, attitudes and Practices on antibiotic use amongst commercial poultry production systems in Kenya, the present study was undertaken.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Study area

This study was undertaken in six- purposively selected sub-counties of Kiambu which accommodate an area of 13,191 km², and is situated to northern part of Nairobi and west of Mt. Kenya (Fig 3.1) and it's estimated to have 4,383,743 inhabitants in reference to 2019 population census and it has got 12 sub-counties. The high population density in this area, favors poultry production more than other types of livestock production. This is because Kiambu is a predominantly an intensive small holder production region, however the pattern and extent of antimicrobial in this county significantly differs from other regions of Kenya. According to the report by Nyaga (2007), Kiambu County also has got 5351 (broiler farmers) and 1185 (layer farmers) who are for commercial purposes and 12633 for dual purpose activities.

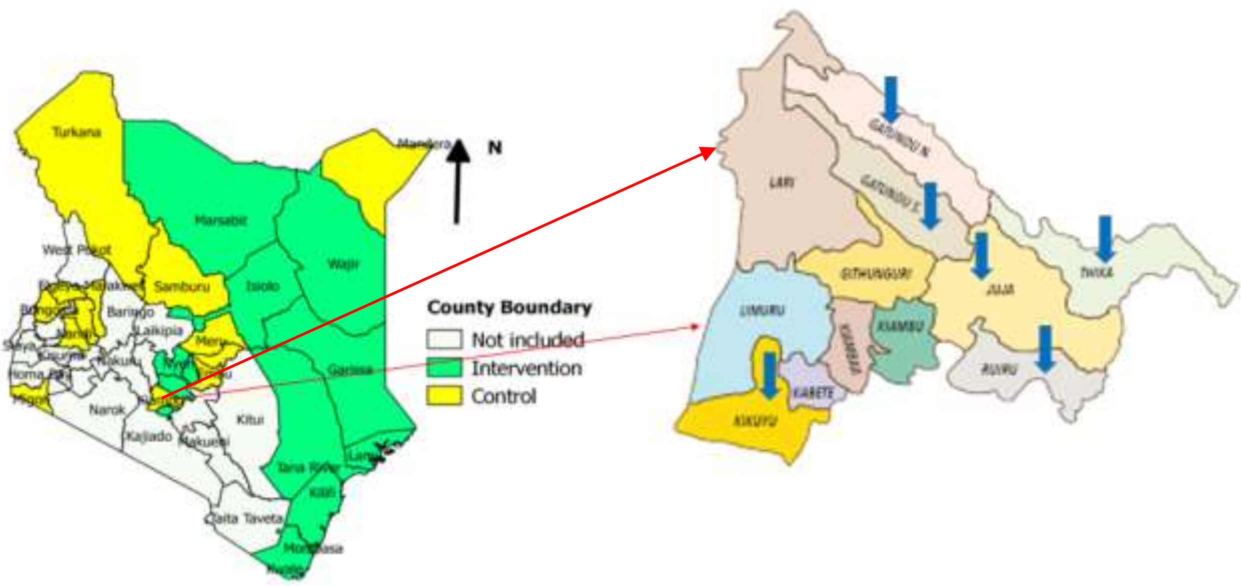



Fig 3.3: Map of Kenya showing Kiambu County with study areas being shown with blue arrow

Source: Geomaps Kenya

### 3.2.2 Study population

Commercial poultry farmers who reared both broilers and layer birds in the 6 purposively selected sub-counties of Kiambu County, Kenya.

### 3.2.3 Study design

A community-based cross-sectional study was employed. A two-stage cluster sampling procedure was used. The sub-counties (the primary sampling unit) were first identified as: Kikuyu; Ruiru; Gatundu North; Gatundu South; Thika and Kiambu and homesteads (the secondary sampling unit) in the selected sub-county were purposively selected. The veterinary extension officers, Community based animal health workers (CBAHW), and village guides were used to give direction and in questionnaire administration to the farmers.

## 3.3 Questionnaire administration

Prior to the day of data collection the Semi-structured questionnaire imprinted on Open-data kit (https://enketo.ona.io/x/#5bk4b2WE) was pretested on 10 adult commercial farmers to validate is operation. On the predefined field day (November, 2020 to February 2021), the field team explained the project objectives and methods to adults participants of the homesteads who consented to take part in data collection, and informed consent was sought from all respondents individually. Inclusion criteria was an adult inhabitant of a selected homestead, rearing poultry for commercial production. It was made very clear that inclusion was optional. Exclusion criteria was any one below 18 years and did not participate in commercial poultry production.The formal constructed interviews were conducted using a survey designed with a questionnaire imprinted in a mobile Open data kit (https://enketo.ona.io/x/#5bk4b2WE), which was used to capture the level of knowledge, attitudes, and practices on antimicrobial usage in commercial poultry production systems of Kiambu County. The questionnaire had both closed and open-ended questions. Each interview varied between approximately 20 minutes up to 1 hour.

## 3.4 RESULTS

### 3.4.1 Socio-demographic characteristics of commercial poultry farmers in Kiambu County, Kenya.

A total of 156 commercial poultry farmers from the six selected Sub-counties of Kiambu participated as follows in the study Gatundu North (13%), Gatundu south (12%), Juja (13%, Kikuyu (24%), Ruiru (21%) and Thika (17%). The female accounted for two-third (64%) of the study participants. Majority of the farmers were aged above 45 years (61%). In the study Sub-counties most of the poultry farmers were married (91%). Overall, (34%) of the poultry farmers had attained secondary school education; 22 % diploma education; 18%- primary; 13% certificate; Degree - 10%; Masters -1 % and 3%- no formal education respectively. Majority of the farmers are from the Kikuyu Sub-county (24 %), followed by Ruiru (21%) with Gatundu South (12%) with the least number of poultry farmers. More than half of the farmers were layer farmers (51%) with the majority having been in poultry farming for more than 5yrs (44%). It was noted that most farmers kept on average 50-500 (67%) number of birds with layers farming being the most preferred type of production (47%). The study also showed that most farmers prefer keeping both feed (46%) and income (42%) records as indicated in Table 3.1 below.

**Table 3.1: Socio-demographic characteristics of poultry farmers in Kiambu County, Kenya (n=156).**

| Study Variable | Total percentage (%), n=156 |
|---|---|
| **Gender** | |
| Male | 36% (56) |
| Female | 64% (100) |
| **Age (Yrs)** | |
| 15-25 | 1% (2) |
| 26-35 | 11% (17) |
| 36-45 | 27% (42) |
| >45 | 61% (95) |
| **Marital status** | |
| Single | 9% (14) |

| | |
|---|---|
| Married | 91% (142) |
| Divorced | 0% (0) |
| **Level of education** | |
| Primary | 18% (28) |
| Secondary | 34% (53) |
| Certificate | 13% (20) |
| Diploma | 22% (35) |
| Degree | 10% (15) |
| Master's degree | 1% (1) |
| No formal education | 3% (4) |
| **Region** | |
| Gatundu North | 13% (20) |
| Gatundu south | 12% (19) |
| Juja | 13% (21) |
| Kikuyu | 24% (37) |
| Ruiru | 21% (33) |
| Thika | 17% (26) |
| **Designation** | |
| Layer farmer | 51% (80) |
| Broiler farmer | 17% (26) |
| Multipurpose farmer | 32% (50) |
| **No of years in poultry farming** | |
| Less than 1yr | 4% (6) |
| One year | 3% (5) |
| Two years | 10% (16) |
| Three years | 16% (24) |
| Four years | 8% (12) |
| Five years | 17% (26) |
| >5yrs | 44% (67) |
| **Average No. of birds kept** | |
| < 50 | 1% (2) |
| 50- 500 | 67% (105) |
| 501-1,000 | 21% (33) |

| | |
|---|---|
| 1001-5000 | 10% (16) |
| **Type of production** | |
| Native Multipurpose | 36% (56) |
| Layers | 47% (73) |
| Broiler for meat production | 17% (27) |
| **Types of record** | |
| Health care (Drug & treatment record) | 8 % (12) |
| Feed records | 46% (71) |
| Income record | 42% (65) |
| No records | 5% (8) |

Source: Field data collected from November 2020 – February-2021

**Key: n=156; % percentage; < less than; >greater than**

### 3.4.2 Role played by farmers on the usage of veterinary antimicrobial in commercial poultry farming systems in Kiambu County

**Table 3.2** presents results on factors influencing the choice of antimicrobial drugs in commercial poultry production systems in Kiambu County. Eighty seven percent (87%) of the farmers indicated that they obtain their antimicrobial drugs through prescription by veterinary officers. However, 67% of the farmers buy or obtain these drugs although personal experience while 8% of the farmers obtain antimicrobial agents through their fellow farmers. 86% of the farmers pointed out that they are able to get advice and follow them (85%) on how to use this antimicrobial agent from Veterinarians (84%), Drug shop sellers (57%), From fellow Farmers(32%), From CBAHW of the area (21%),and from others (1%) successively. It was also noted that 85% of the farmers are satisfied with the advice they get from the listed personnel and 46%, 33%, and 21% of farmers have to walk medium distance (3-4 km), short distance (1-2km) and long distance (above 5km) respectively to obtain this veterinary services as shown in table 3.2 below.

**Table 3.2: Factors influencing choice of antimicrobial drugs by poultry farmers in Kiambu County**

| (N=156) Variables | Frequency (%) |
|---|---|
| **How were the antimicrobial obtained?** | |
| Through prescription by the veterinary officer | 87% (136) |
| Personal experience | 67% (104) |
| Fellow farmer | 8% (13) |
| **Do you get advice on how to use antimicrobial agents?** | |
| Yes | 86% |
| No | 14% |
| **If yes from whom?** | |
| Drug shop seller | 57% |
| Veterinarian | 84% |
| From a CBAHW in the area | 21% |
| From a fellow Farmer | 32% |
| All of the above | 1% |
| Others (workers, Human clinics) | 1% |
| **Do you follow the advice given?** | |
| Yes | 85% |
| No | 15% |
| **Were you satisfied with the quality of advice provided?** | |
| Yes | 85% |
| No | 15% |
| **What is the nearest distance from where you get your veterinary services?** | |
| Short distance (1-2km) | 33 % |
| Medium distance (3-4km) | 46% |
| Long distance (Above 5km) | 21% |

**Key: %-percentage; Km-Kilometer; CBAHW-Community based animal health worker; n-sample size.**

**Fig 3.2** presents the result who does the diagnoses of the sick birds in the farms. From the results both the veterinarian (65%) and the farmer (65%) have equal preponderance of carrying the diagnosis of the sick birds from the farms, followed by the community based animal health worker (26%). The farm worker (5%) and fellow farmer (3%) with the least percentage of undertaking diagnosis of the sick birds as indicated in the fig 3.2 below.

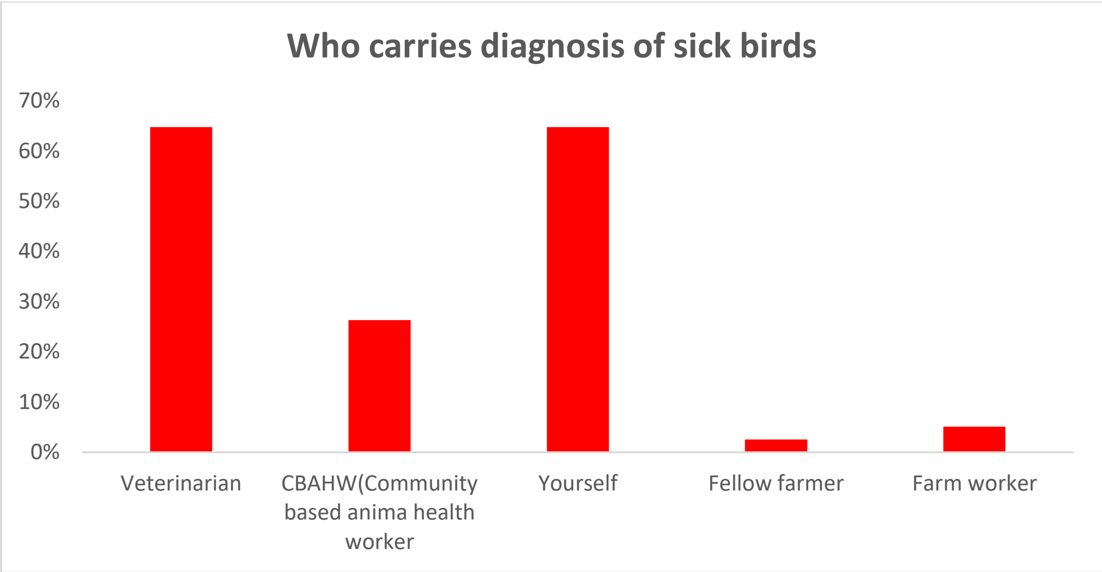



**Fig 3.2: Distribution of persons who carries the diagnosis of the sick birds in the poultry farms**

**Table 3.3** represents results on the level of knowledge on scrutinizing and assessing major challenges facing Kiambu commercial poultry farmers on antibiotic use. Seventy two percent (72%) and 49%, of farmers indicated that antibiotics were expensive for them to afford as well as lack of money to buy drugs was a challenge, respectively. We also noted that 51% and 34% of the farmers lacked understanding on proper usage of antibiotics, and unfamiliar languages used in the inserts respectively. Lack of specific measuring containers (58%), lack of veterinary officers (77%) and consultative services (72%) were the key challenges that the farmers faced as shown in table 3.3 below.

**Table 3.3: Challenges fronting Kiambu County Commercial poultry farmers in selection and use of antibiotics**

| (N=156) Variables | Frequency (%) |
|---|---|
| **What are the major challenges in accessing antimicrobials?** | |
| Drugs not available in the market | 12% |
| Long distances from the source | 22% |
| Lack of money to buy the drugs | 49% |
| They are expensive | 72% |
| All of the above | 30% |
| No challenges | 1% |
| **What challenges are there regarding the use of antimicrobials?** | |
| Lack of knowledge on antibiotic drug use | 51% |
| Unfamiliar language used in the inserts on their use | 34% |
| Lack of efficacy and effectiveness to treat infections | 14% |
| No challenges | 21% |
| **What are the challenges regarding dosage formulation?** | |
| Dosage calculation and estimation difficult | 15% |
| Hard to follow dosage guideline on the leaflet | 22% |
| Lack of specific measuring containers | 58% |
| No challenges | 8% |
| **What are challenges regarding quality of the drug?** | |
| Some drugs are fake | 1% |
| Some drugs are very expensive | 94% |
| No challenges | 5% |
| **What are some of the challenges regarding service providers?** | |
| Lack of veterinary officers | 77% |
| Lack of CBAHW | 28% |
| Lack of consultation services | 72% |
| No challenges | 13% |

**Key: CBAHW- Community based animal; health worker; %-percentage**

**Source:** Field data November 2020 to February 2021.

**Table 3.4** presents results of the most stocked and preferred type's antimicrobial agents by farmers in the management of diarrhea, sudden death, emaciation, and swollen eyes with watery discharge in commercial poultry production systems of Kiambu County. We found that Tetracycline's (60%), Amprolium ®powder (52%), Enrofloxacine (51%), and Sulfadimidine (40%) are the most commonly preferred agents in the management of this condition with Oxytetracyclines(86%) and Amoxi (91%) being the most used sub-types as shown in table 3.4 below.

**Table 3.4: Most Preferred types of antimicrobial agents stocked and used in the management of diseases in commercial poultry production in Kiambu County**

| Variables | (N=156) Frequency (%) | | | | |
|---|---|---|---|---|---|
| | **Type stocked** | **Diarrhea** | **Sudden Death** | **Emaciation** | **Swollen eyes** |
| None of the above | 1% | 1% | 2% | 54% | 3% |
| Tetracycline's (1a. Tetracyclines, 1b. Oxytetracyclines 1c. doxycycline | 63% | 60% | 56% | 29% | 60% |
| Amoxicillin (2a. Hipramox p® powder, 2b. Amoxi, 2c. Sacox, 2d, Panax,) | 32% | 29% | 29% | 42% | 30% |
| Sulfadimidine (3). sulfadimethoxine) | 48% | 40% | 40% | 52% | 44% |
| Enrofloxacine (4a) Hipralonaenro.S®) | 50% | 51% | 51% | 53% | 51% |
| Amprolium ®powder (5a) Amprolium hydrochloride) | 45% | 52% | 51% | 23% | 51% |
| Diaziprim ®powder | 21% | 24% | 23% | 17% | 21% |
| Poltricin ®powder | 13% | 17% | 17% | 5% | 13% |
| Penicillin G/Benzylpenicillin | 6% | 6% | 6% | 32% | 6% |
| Erythromycin/Tylosin | 28% | 32% | 30% | 24% | 31% |
| Neomycin sulfate/ Streptomycin/ Spectinomycin | 26% | 21% | 23% | 1% | 24% |
| OTC dawa | 4% | 1% | 1% | 6% | 1% |

| | | | | | |
|---|---|---|---|---|---|
| Aliseryl | 21% | 5% | 5% | 3% | 6% |
| Colesultrix | 3% | 3% | 3% | 22% | 3% |
| Miramed | 7% | 22% | 22% | 3% | 21% |
| If not indicated on the list | 1% | - | - | 8% | 2% |
| **Please confirm which type of Tetracyclines? (n=98)** | | | | | |
| 1a. Tetracycline's | 9% | 9% | 9% | 9% | 9% |
| 1b. Oxytetracyclines | 85% | 86% | 86% | 86% | 86% |
| 1c. Doxycycline | 35% | 31% | 31% | 31% | 31% |
| **Please confirm which type of Amoxicillin (n=49)** | | | | | |
| Hipramox p® powder | 18% | 13% | 13% | 13% | 13% |
| Amoxi | 88% | 91% | 91% | 91% | 91% |
| Sacox | 2% | 2% | 2% | 2% | 2% |

**Key:** ®- Trade name; %-Percentage; n- sample size **Source:** Field data November 2020 to February 2021.

**Fig 3.3** present photos of the most commonly mentioned and used antibiotics across the six purposively selected sub-counties of Kiambu County in management of poultry diseases as shown below.


**Fig 3.3: Photos taken of antibiotics usage by commercial poultry farmers in Kiambu County Kenya**

### 3.4.3 Alternative natural remedies used by commercial poultry farmers in disease management in Kiambu County

**Table 3.5** presents knowledge, approaches and undertakings about alternative natural remedies used to manage poultry disease in Kiambu County. Ninety two percent (92%) of the farmers who responded said that they use natural herbal remedies in the management of poultry diseases. Most of the farmers indicated that they use *Aloe vera* (100%), *Capsicum annum* (67%), with both Livergenplus and *Moringa Oelifera* having 8% response to use. The farmers further highlighted that they use this natural remedy for both disease treatment and as growth promoter (58%), with treatment scoring 33%. The diseases that are mostly treated with natural remedies are Newcastle (78%), Coccidiosis (64%), diarrhea (50%), and fowl typhoid (21%) respectively as shown in table 3.5 below.

**Table 3.5: Alternative remedies to conventional medicine used in poultry disease management in Kiambu County**

| (N=156) | |
|---|---|
| **Variables** | **Frequency (%)** |
| **Do you use alternative remedies/treatment apart from these antimicrobials?** | |
| Yes | 8% |
| No | 92% |
| **Name the alternative natural remedies you use to treat your birds (n=12)** | |
| *Aloe vera* | 100% |
| *Capsicum annum* | 67% |
| Livergenplus | 8% |
| *Moringa oleifera* | 8% |
| **What purpose do you use these natural remedies for in poultry production? (n=12)** | |
| For treatment | 33% |
| To promote growth | 8% |
| All of the above | 58% |
| **Which diseases are they applied to if they are used for treatment/ prophylaxis? (n=14)** | |
| Coccidiosis | 64% |
| Newcastle | 71% |
| Diarrhea | 50% |
| Gumboro | 7% |
| Immune Booster | 7% |
| Fowl Typhoid | 21% |
| Growth Promoter | 7% |
| Liver Toxicity | 7% |

**Key: %-percentage,** ®-Trademark, n-sample size; Source: Research data November 2020 to February 2021.

### 3.4.4 Knowledge and practice of farmers withdraw period after treating poultry antibiotics

**Table 3.6** presents results about knowledge and practices on the observation of withdraw period post-antibiotic use in commercial poultry production. Eighty two (82%), indicated that they understood the importance of observing withdrawn period post-antibiotic use. It was further noted that 91% of farmers observe withdraw period to ensure the safety of animal production towards the consumer. This claim was further supported by 7% of the farmers indicating that as the withdraw period is not observed, the quality of the poultry products will be affected. In addition, more than half of the farmers (51%) seemed not to understand the health implications of antimicrobial residues due to consuming products with residues. Majority of the poultry farmers attributed the health implication of consuming products with residues due to allergies (58%), toxicity to human beings (34%) with the least of farmers indicating lack of product marketability and un-palatability (1%) respectively due to residues presence as indicated in Table 3.6 below.

**Table 3.6: Knowledge and practice of farmers withdraw period after treating poultry antibiotics**

| (N=156) | |
|---|---|
| **Variables** | **Frequency (%)** |
| **Do you think it's important to observe withdraw period? (n=156)** | |
| Yes | 82% |
| No | 18% |
| **If yes, why do you think it is important to observe withdraw period (n=128)** | |
| Food safety | 91% |
| To prevent drug resistance | 4% |
| To prevent deaths and allergic rxns | 1% |
| Quality of poultry product | 7% |
| Palatability | 5% |
| Marketability | 2% |
| To give time for the birds to utilize drug in their bodies | 1% |
| **Do you know any health implication associated with consuming animal products contaminated with antimicrobial residues? (n=156)** | |
| Yes | 49% |
| No | 51% |
| **If yes, which ones (Health implications)? (n=76)** | |
| Toxicity to Human being | 34% |
| Allergies | 58% |

| | |
|---|---|
| Resistance to antimicrobial drugs | 6% |
| Lack of market | 1% |
| Diarrhea | 49% |
| Unpalatability | 1% |

**Key: %-percentage,** ®-Trademark, n-sample size; **Source**: Research data November 2020 to February 2021.

### 3.4.5 Possible solutions mentioned by the poultry farmers that could help to curtail the challenges they are facing poultry production systems in Kenya.

**Table 3.7** presents the results of the possible solutions that were given by farmers towards curtailing some of the challenges they face in commercial poultry production in Kiambu County. More than half of the farmers suggested the need for continued training of the farmers on prudent use of antibiotics (57%) in the management of commercial poultry diseases. Furthermore, 28% of the farmers suggested the need to have well-trained extension veterinary officers who will be guiding and providing consultative services to the farmers on prudent usage of antibiotic agents together with the management of commercial poultry diseases. The study identifies that, accessibility and availability of markets for poultry products (19%), provision of cheap antimicrobial drugs (17%), more effective drugs (6%), affordable feeds (3%) and government regulation and control of poultry feed prices (2%) were some of the major solutions that would help to tame down the major challenges there poultry framer face in their daily production systems respectively as shown in table 3.7 below.

**Table 3.7: Some of the possible solutions suggested by commercial poultry farmers in Kiambu County that might scale down the problems they face in poultry farming**

| (N=156) | |
|---|---|
| **Variables** | **Frequency (%)** |
| **What are the possible solutions to the above-mentioned challenges facing poultry farmers with the use of antibiotic agents and in management of poultry production systems in Kenya?** | |
| Training of farmers on prudence use of antibiotics | 57% |
| Well trained Extention veterinary officers | 39% |
| Market accesibility/availability | 19% |
| Cheap drugs | 17% |
| Affordable feeds | 3% |
| Government to control and regulate food price | 2% |
| Introduction of another source of food | 1% |
| More effective drugs | 6% |
| To observe withdraw to poultry farmer | 1% |
| None | 1% |

**Key: %-percentage,** , n-sample size; **Source of data**: Research data November 2020 to February 2021.

### 3.4.6 Relationship between socio-demographic factors and the challenges in accessing and use of antimicrobial agents stocked as analyzed by Chi-square.

**Table 3.8** shows the results of the relationship among socio-demographics factors and the challenges commercial poultry farmers face on their access. The results revealed that there was a statistical significance association between nearness to veterinary services, number of years in poultry farming, area of residence, type of production, and numbers of birds kept ($p<0.05$) and the challenges that farmers face in accessing antimicrobial agents. The rest of the sociodemographs (age, marital status, and level of education) had no significant ($p>0.05$) statistical association with challenges affecting farmers towards accessing antimicrobial agents as indicated in Table 3.8 below.

**Table 3.8: Relationship between socio-demographic factors and the challenges in accessing and use of antimicrobial agents stocked.**

| Study Variable | Chi square ($X^2$); (P-Value<0.05) | |
|---|---|---|
| | **Accessing Antibiotics** | **Use of antibiotics** |
| **Gender** | $X^2$=7.907 (0.161) | $X^2$=1.900 (0.591) |
| Male | | |
| Female | | |
| **Age (Yrs)** | $X^2$=5.271 (0.984) | $X^2$=14.668 (0.100) |
| 15-25 | | |
| 26-35 | | |
| 36-45 | | |
| >45 | | |
| **Marital status** | $X^2$=2.990 (0.701) | $X^2$=1.173 (0.760) |
| Single | | |
| Married | | |
| Divorced | | |
| **Level of education** | $X^2$=29.132 (0.511) | $X^2$=20.037 (0.331) |
| Primary | | |
| Secondary | | |
| Certificate | | |
| Diploma | | |
| Degree | | |
| Master's degree | | |
| No formal education | | |
| **Region/area of residence** | $X^2$=11. 934 (0.290) | $X^2$=82.037 ($0.000^*$) |
| Gatundu North | | |
| Gatundu south | | |
| Juja | | |
| Kikuyu | | |
| Ruiru | | |
| Thika | | |
| **Designation** | | $X^2$=13.715 ($0.033^*$) |

| | | |
|---|---|---|
| Layer farmer | | |
| Broiler farmer | | |
| Multipurpose farmer | | |
| **No of years in poultry farming** | $X^2$=20.484 (0.903) | $X^2$=36.723 (0.006$^*$) |
| Less than 1yr | | |
| One year | | |
| Two years | | |
| Three years | | |
| Four years | | |
| Five years | | |
| >5yrs | | |
| **Average No. of birds kept** | $X^2$=8.394 (0.907) | $X^2$=17.047 (0.048$^*$) |
| < 50 | | |
| 50- 500 | | |
| 501-1,000 | | |
| 1001-5000 | | |
| **Nearness to veterinary services** | $X^2$=32.105 (0.000*) | $X^2$=15.471 (0.017$^*$) |

**Key: $X^2$-Chi-square; $^*$-Statistically significance; df-degrees of freedom**

### 3.4.7 Relationship between area/region of residence designation and knowledge, attitudes, and practices on the type of antimicrobial agents stocked by farmers as analyzed by Chi-square

**Table 3.9** shows results of the relationship among regions of residence and the knowledge, attitudes, and practices on the type of antimicrobial agents stocked by farmers. The area of residence statistically significantly ($p<0.05$) influences majority of the parameters that were used to access the level of understanding and awareness on the kind of antimicrobial agents stocked by farmers in Kiambu County as displayed in Table 3.9 below.

**Table 3.9: Association amongst area/region of residence designation and knowledge, attitudes, and practices on the type of antimicrobial agents stocked by farmers as analyzed by Chi-square**

| Study variables | Chi square ($X^2$), (df) (P-value)) | |
|---|---|---|
| | **Areas of residence** | **Designation** |
| Type of antimicrobial agents stoked by the farmers | 157.572(75) (0.000*) | 55.052(30) (0.04*) |
| Type/class of antimicrobial currently used by farmers | 181.069 (80) (0.000*) | 59.393 (32) (0.02*) |
| Use of antimicrobial to treat Diarrhea (Profuse, acute, Chronic) | 190.117 (80) (0.000*) | 60.229(32) (0.02*) |
| Use of antimicrobial to treat sudden death | 188.834 (85) (0.000*) | 46.163(34) (0.80*) |
| Use of antimicrobial to treat emaciation | 192.520 (80) (0.000*) | 42.273(32) (0.105*) |
| Use of antimicrobial to treat swelling eyes with watery discharge | 260.757 (85) (0.000*) | 75.306(34) (0.000*) |
| What production type of birds do you have? | 53.102(15) (0.000*) | 163.557(6) (0.000*) |
| What type of records do you keep? | 30.002(15) (0.012*) | 20.134(6) (0.003*) |
| Who carries diagnosis of sick birds/ tells bird are sick | 59.354(25) (0.000*) | |
| How do you tell that the birds are sick? | 48.668(25) (0.003*) | |
| Who does the treatment of your sick birds? | 87.087(20) (0.000*) | 18.303(8) (0.019*) |
| What is your source of antimicrobial? | 32.612(20) (0.037*) | |
| How were the antimicrobial obtained? | 22.425(10) (0.013*) | |
| If yes, From who? | 48.857 (25) (0.003*) | |
| What are the major challenges in accessing antimicrobial agents? | 38.877(25) (0.038*) | |
| What challenges are there regarding the use of antimicrobials? | 82.037(15) (0.000*) | 13.715(6) (0.033*) |
| What are the challenges regarding dosage formulation? | 45.720(15) (0.000*) | |
| What are some of the challenges regarding the service provider? | 53.903(15) (0.000*) | 22.242(6)( 0.001*) |
| Why do you think it is important to observe withdraw periods | 58.650(30) (0.001*) | |
| Knowledge on effect of drug residues presence in poultry products | 82.579(35) (0.000*) | |

| | | |
|---|---|---|
| What are the possible solutions to the above-mentioned challenges facing poultry farmers on the use of antimicrobial in Kenya? | 139.771(50) (0.000*) | 32.937(20) (0.034*) |

**Key: X2-Chi-square; *-Statistically significance; df-degrees of freedom**

### 3.4.8 Relationship between age of farmers, level of education, experience, and knowledge, attitudes, and practices in commercial poultry production systems of Kiambu County as analyzed by Chi-square

**Table 3.10** represents the results on how age statistically significance ($p<.05$) influences on who does the treatment of sick birds, and knowledge and practice on the importance of observation of withdrawn periods as displayed in Table 3.10 beneath.

**Table 3.10: Association amongst age of farmers, level of education, experience, and knowledge, attitudes, and practices in commercial poultry production systems of Kiambu County as analyzed by Chi-square**

| Study variables | Chi square ($X^2$), (df), (P-value) | | |
|---|---|---|---|
| | **Age** | **Education level** | **Experience** |
| Who does the treatment of your sick birds? | 30.833(12) (0.002*) | 39.967(24), (0.022*) | 36.739(24) (0.046*) |
| Why do you think it is important to observe withdraw periods | 29.465(18) (0.043*) | - | - |
| Knowledge on effect/impact of drug residues presence in poultry products | - | 136.446(42) (0.000*) | 68.418(42) (0.006*) |
| What challenges are there regarding the use of antimicrobials? | - | - | 36.723(18) (0.006*) |

**Key: $X^2$-Chi-square; *-Statistically significance; df-degrees of freedom**

### 3.4.9 Impact of the number of birds stocked in commercial poultry production on knowledge, attitudes, and practices on antimicrobial use as analyzed by Chi-square

**Table 3.11** presents the results on how the size of stocked birds influences knowledge, attitudes, and practices on antimicrobial use by poultry commercial farmers of Kiambu County. The results reveals that there was a significance ($p<.05$) association about the number of birds stocked with the factors presented that were used to access the level of understanding and awareness on antimicrobial usage as shown in Table 3.11 below.

**Table 3.11: Impact of the number of birds stocked in commercial poultry production on knowledge, attitudes, and practices on antimicrobial use as analyzed by Chi-square**

| What average number of birds do you keep? | Chi square ($X^2$), (df) | P-Value |
|---|---|---|
| What production type of birds do you have? | 20.271(9) | 0.016* |
| What type of records do you keep? | 20.339(9) | 0.016* |
| Who carries diagnosis of sick birds/ tells the bird is sick? | 25.599(15) | 0.042* |
| Who does the treatment of your sick birds? | 33.132(12) | 0.001* |
| What challenges are there regarding the use of antimicrobials? | 17.047(9) | 0.048* |
| What are the possible solutions to the above-mentioned challenges facing poultry farmers on the use of antimicrobial agents in Kenya? | 53.514(30) | 0.005* |

**Key: $X^2$-Chi-square; *-Statistically significance; df-degrees of freedom**

### 3.4.10 Impact of distance to access veterinary services in commercial poultry production on knowledge, attitudes, and practices on antimicrobial use as analyzed by Chi-square

**Table 3.12** presents the results on how the distance travelled to access veterinary services influence the level of awareness, understanding and practices on antimicrobial use in commercial poultry production systems. The results revealed a significance association between the distances travelled to access veterinary services with knowledge on challenges affecting farmers on accessing antimicrobial agents, their use, and on regarding the service providers' accessibility as presented in Table 3.12 below.

**Table 3.12: Impact of nearness to veterinary services on knowledge, attitudes, and practices on antimicrobial use as analyzed by Chi-square**

| What is the nearest from where you get your veterinary services? | Chi square ($X^2$), (df) | P-Value |
|---|---|---|
| What are the major challenges in accessing antimicrobials? | 32.105 (10) | 0.000* |
| What challenges are there regarding the use of antimicrobials? | 15.471(6) | 0.017* |
| What are some of the challenges regarding the service provider? | 21.198(6) | 0.002* |

**Key: $X^2$-Chi-square; *-Statistically significance; df-degrees of freedom**

## 3.5 DISCUSSION OF RESEARCH FINDINGS

The usage of antimicrobial drugs in poultry production is essential in disease treatment, prevention, and growth promoting, but its use must be accepted as a responsibility rather than a right when trying to improve poultry health (Rose *et al.*, 2009). This will help to minimize the potential risk and hazards due to improper use of antimicrobial agents in livestock production. Therefore, usage of veterinary antibiotic drugs in poultry systems needs proper control through legislation bodies with the broad goals being to preserve animal health, improve animal production, and to protect the public (Cardona & Kuney, 2002). Resistance to antimicrobial agents is significantly growing in almost all populations. This increase can be associated due to lack of adequate knowledge and poor practice on the prudence, usage of antibiotic agents in the management of poultry diseases, and inappropriate attitudes towards their usage for prophylaxis and as growth booster. This has promptly added to the occurrence of Multi-drug resistant microbes and accumulation of residues in poultry products, hence affecting the international market trade. This being the first cross-sectional study to be undertaken in Kiambu County on the level of awareness, attitudes, and practices on antimicrobial usage in commercial poultry production will help to unravel potential mitigation and measures in policy making to help curb antimicrobial resistance across the country.

The study demonstrated that 2/3$^{rd}$ of the farmers were Females (68%), married (91%), aged above 45 years, and had attained secondary education level and they preferred keeping layer birds. This agrees according to FAO (2009), that most farmers prefer keeping layers in medium and large scale under intensive management. This is attributed to the great demand for eggs in urban areas and the high profit margin associated to layers than broilers. This could further be attributed to the reason that most women are left in homestead while taking care of the livestock and the majority are above 45yrs age, which could be as a result of the retirement plan scheme because poultry care does not require a lot of capital and space to invest in. This agrees with a similar study carried out by Calvin *et al*. (2020) in Tanzania. We further noted that Kikuyu sub-county was leading in commercial layer production systems seconded by Ruiru to Gatundu south having the least of poultry production activity. This agrees with the following studies undertaken by Okello *et al*. (2015); Mercy *et al.* (2014); (ROK, 2010). It was further noted that the majority of the farmers are small-scale farmers (101-500 birds) which agrees with the study carried out by Nyaga (2007). This could be as a result of little capital to keep large scale production. We also noted that the majority of the farmers prefer to keep feed and income records compared to antibiotic records. This could be explained by the need of farmers to maximize on profit and lessen expenditure as antibiotics are sometimes little concern due to ignorance and not knowing the great danger they can expose to both animals and humans.

In the study, we found that the most of the Socio-demographic characteristics were not statistically significant ($p>.05$) to influence the ability to access antimicrobial agents, however, nearness to veterinary services significantly ($P<.05$) contributed to the usage of antibiotics in the farms. The study found that the area/ region of residence was significantly ($P<0.05$) connected with the challenges that farmers were facing from antimicrobial use, with Kikuyu farmers less affected due to their extensive history of commercial poultry production. This agrees with a research that was carried out by Mercy *et al.* (2014) who found that the majority of Kiambu farmers came from Kikuyu Sub-County.

In the study we noted that there was a substantial ($P<0.05$) association between the level of experience and who treats the sick birds, challenges on the usage of antibiotic agents and on knowledge on the effects of eating poultry products with the presence of residues. The years of experience in commercial poultry production had positive impacts on the level of awareness, understanding, and practices on antimicrobial use in poultry production systems. Higher experience level it would help most of the farmers to observe, withdraw periods, and ensure prudence use of antimicrobial agents in poultry farming. We further noted that commercial farmers who kept medium and large scale stock significantly ($p<.05$) had adequate knowledge and experience on antimicrobial agents use, record keeping, proper way of disease diagnosis and treatment and knowledge on possible solutions to avert problems that affect them in commercial poultry farming. This could be attributed to the heavy investment they have engaged in and they could take any measures to avert any situation that could halt their production.

From the study, we found that most of the farmers acquire this antibiotic through prescription by veterinary officers, which disagrees with a research undertaken by Lindonne Glasgow *et al*. (2019), who found that the majority of antibiotics users are self-prescribers. Furthermore, we noted that most of the farmers claim that they follow the advice they get from the veterinary officers on the use of antimicrobial agents which disagrees with research undertaken by Calvin Sindato *et al.* (2020) who claimed most of the antibiotic users do not follow the guidelines of the prescriber. We also found that the majority of the farmers walk medium distances (3-4km) in order to access this antimicrobial agent. The need for close access to veterinary services is very crucial to commercial poultry farmers to ensure they get the right advices and services on time and this will help to stem-down the irresponsible use of antimicrobial agents (Aniroot Nuangmek *et al*. 2016). It was further noted that the majority of cases of sick birds are noted and reported by the farmers themselves who lacked credible skills, instead of engaging well-trained veterinary extension field officers. This could be attributed due to the lack of enough veterinarians and consultative services to poultry farmers who mostly own small and medium scale farms. This is more so supported by the results in Fig 3.3, which indicate that both the veterinarian and the farmer have an equal propensity of diagnosing sick birds, hence the farmer uses his experience instead of well trained personnel to treat and diagnose the sick. This could be one of the ways of promoting irrational use of antimicrobial agents by untrained persons. Additional, we identified that the majority of the farmers get their antimicrobial agents from drug shops through personal experience, this agrees with a research undertaken in Thailand by Aniroot Nuangmek *et al*. (2016) who found that the majority of farmers understood the need for professional antimicrobial prescription but due to ignorance they disregarded this practice. This further supports that most of commercial poultry farmers have poor attitudes and practices that exhibit inappropriate behavior towards antimicrobial use.

Furthermore, the study found that the majority of the farmers still find most of the antibiotics to be expensive for them to afford despite them having inadequate knowledge on the proposed use of antimicrobial agents. The commercial poultry farmers further claimed that antibiotics have inserts that have unfamiliar language and they do not have proper measuring containers that would culminate with either overdose or under a dose of drugs. This might lead to a lack of effectiveness and efficiency of the antibiotics as was claimed by the famers. Proper and adequate knowledge is very critical to the farmers to help ensure prudence and usage of antibiotics hence preventing forthcoming occurrence of antibiotic resistance. This is corresponding to a research undertaken by Casal *et al.* (2007), suggesting the need for efforts to increase farmer's awareness on biosecurity as a major input in stemming antimicrobial resistance in livestock production. Lack of enough veterinary extension officers and consultative services towards the use and preparation of antimicrobial agents among poultry farmers was also immensely experienced by the farmers. This could contribute greatly to the improper use of these drugs among the farmers in Kiambu County and across Kenya. In reference to research undertaken by Aniroot Nuangmek *et al*. (2016), he noted that adequately trained veterinarian officers are essential for upholding proper antibiotic use on farms, due to their prevailing worthy character among farmers, and are perfectly suitable to perform as a waterway for enlightening livestock keepers about antibiotics resistance and the right preparations of these agents in livestock disease management.

Similarly, we found that antimicrobial use is a common practice among commercial poultry farmers in Kiambu County. The types/class of antimicrobial agents that we found to be commonly used and preferred by commercial poultry farmers of Kiambu County were Tetracycline, Enrofloxacine, Sulfadimidine, and Amprolium compounds respectively, with oxytetracyline and Amoxi sub-types of these drugs being highly mentioned. This could be associated with high cases of bacterial and protozoal infestation in poultry production. This same class of drugs were mentioned to be having used previously in the last one year and currently in the farms. We also found that in the same order the drugs are used to manage diarrhea (acute, profuse, and chronic), emaciation, sudden death, and swelling of eyes with watery discharge. Oxytetracycline and sulfadimidine are a broad spectrum antibacterial drug and Amprolium is used to treat coccidiosis making them to be on high demand in commercial poultry production. This creates an increased dependence on antibiotics as a substitute for good management practices such as good animal husbandry and housing hygiene (Rice and Straw, 1992). According to a study carried out in Ethiopia by Gebeyehu, Bantie, and Azage (2015) and in Uganda by Ocan *et al.* (2014), they found that continuous use of antimicrobial agents is a vital determinant of antibiotic resistance, mostly when they are used incorrectly. Therefore, it is important to stipulate that antimicrobial agents are at great danger of developing bacteria resistance, and high multidrug resistance in the near future (Muhie, 2019).Regarding Austin, Kristinsson, and Anderson (1999), the capacity of drug use is a vital to selection pressure motivating changes on the extent of antibiotic resistance in the society.

According to this study, majority of the farmers observed withdrawing period's post-antibiotic use in their commercial poultry production systems to ensure the safety of the products to human health. However, the majority of the poultry farmers seemed not to understand the implication of having antibiotics residues in poultry products in human health. According to Mitema *et al.* (2001), in a study undertaken in Kenya they found that there was high level of antimicrobial residue contaminants in livestock meat, this could be elucidated by the reason that most of the farmers in this research could not understand the health implication of this residue in animal products. In accordance to Bagley (1997), this could be associated due to lack of adequate awareness creation by all stakeholders on the public importance of drug residues. Allergies and diarrhea from eating poultry products contaminated with drugs residues were highly implicated in this study and according to Mubito (2014); (WHO, 2012), this can constitute a potential hazard to the public health. This signifies that there is a need to create awareness and sensitize the commercial poultry farmers on the implication of not observing withdraw periods and the detrimental effects of selling poultry products with antibiotic residues.

Due to the high prices of conventional antimicrobial agents, most of the commercial poultry farmers seemed to understand the usage of natural herbal remedies in the controlling of diseases in their production systems**.** *Aloe vera, Capsicum annum*, Livergen plus, and *Moringa oleifera* preparations were noted to be used in treatment of the following disease conditions in commercial poultry production; Newcastle, coccidiosis, fowl typhoid and diarrhea. The use of alternative natural products in animal disease management has been promoted since they are cheap, readily available, efficacious, and regarded as safe (WHO, 2008); Ndukui *et al.* (2014).

According to the study results, we found that the majority of the farmers proposed the need for adequate training on prudence, and use of antimicrobial agents, the impacts of not observing the withdraw periods, and the impacts of antibiotics residues on human well-being and the marketability of their products to the world market. These trainings to farmers would help to halt the escalating problem of antimicrobial resistance. We also found that there is a need for more adequately trained veterinary extension officers to provide consultative services to these farmers on prudent use of antibiotics in management of poultry diseases. This agrees with a research carried out by Aniroot Nuangmek *et al*. (2016), who proposed need for a thorough and continuous efforts to train farmers on proper antimicrobial agent used in poultry production. He further states that the availability of well-trained veterinarians to the poultry farmers is a key intervention towards knowledge dissemination on prudence and application of antimicrobial agents. Furthermore, we realized that since the majority of farmers are small scale holder, there is a need for County government and the state government to provide funds to upscale their production systems which will result in a high source of income generation. There is also need for the government to control the prices of both antibiotics and feed, which have become a big challenge to the commercial poultry production system which agrees with an investigation carried out by Ling *et al.* (2011) who stated that economic cost and benefits to the farmers are the first reasons making judgment on antimicrobial use, since they are much worried about financial implication than prudence use of this drugs. In 2004, Tollefson in his study suggested that there is need of high quality regulatory government agencies in developing countries like Kenya to help in the management of an emerging threats such as AMR. According to Lindonne Glasgow *et al.* (2019), under-developed and developing nations mostly are confronted in distributing acceptable funds and establishing strategies to resolve gaps in awareness and practices on the use of antibiotics in animal food production activities. Antimicrobial usage in animals' production remains unfettered, resulting to incorrect usage and a world-wide surge in antimicrobial resistance, hence the need for the government of the day to take caution and influence policies to stem down AMR. This current study underscores the understanding of the factors that lead to antibiotic misappropriation in commercial poultry production systems in Kiambu County, Kenya, and the outcomes show there is a need for cooperation between the Ministry of Agriculture and Livestock Development, with the County government and the Ministry of Health to ascertain factors that promote antimicrobial use in the poultry and livestock industry by developing and distributing procedures to assess the usage of antimicrobial agents, and upgrade AMR surveillance in humans and livestock's in Kiambu County.

# CHAPTER 4: DETERMINATION OF ANTIMICROBIAL RESISTANCE PATTERNS OF SELECTED *ENTEROBACTERIACEAE* ISOLATED FROM COMMERCIAL POULTRY PRODUCTION SYSTEMS IN KIAMBU COUNTY, KENYA

## 4.1 INTRODUCTION

In the last 20 years, the management of enteric illness in humans and poultry sector has been problematical due to emergence of antimicrobial resistant pathogens. Emerging of antimicrobial resistant ESBLs producing Enterobacteriaceae is a severe threat to community health (Jaciane Marques *et al.*, 2015). The rapid surge in the prevalence of ESBL producing *Enterobacteraceae* pathogens that are resilient to fluoroquinolones, aminoglycosides, β-lactams and colistin has been rarely reported (Bialvaei & Samadi Kafil, 2015);(Sampaio, Gales, Sampaio, & Gales, 2016);(Kateregga, Kantume, Atuhaire, Lubowa, & Ndukui, 2015). Other resistance mechanisms against beta-lactams are the outer membrane permeability change and efflux pumps. The irrational use of antibiotics in livestock has led to development of resistance and successive transmission of resistance inheritable factor among microbes to livestock, livestock products and their surroundings (Rose *et al*., 2009); (Thriemer *et al*., 2013),(Davies & Davies, 2010). In contrast, prudent use of antimicrobial agents is a prerequisite, though adequate mechanisms to realize and mitigate this dream, necessity to be developed and communicated (WHO, 2014). Antimicrobial resistance is a blunt reality through the world, as well as in Kenya. The strategies connected with regulating antimicrobial resistance, mostly in Kenya, are multidimensional.

Commercial poultry industry is well established and it is the leading supplier of animal protein worldwide in form of meat and eggs. Poultry are moderately cheaper and kept in small areas basically providing income and protein to the families (FAO, 2002; Moreki *et al*., 2010). However, poultry diseases are some of the limiting factors to this industry. The increased poultry disease burden has accentuated the high demand and the use of veterinary antimicrobial drugs (Morley *et al*., 2005).

Majority of Kenyans depend on poultry products for livelihood and survival, therefore low production levels affect their income levels. This affects their involvement to the country GDP and global economic development, halting the achievement of the Medium term plan 111 as set by the government on sustainable development through agriculture. In Kenya, poultry production is amongst the steadiest way of improving the animal protein insufficiency in the diet. The increased need, and hunt for white meat has surmounted additional credibility to poultry farming. In order to meet the raised demand for white meat large scale poultry production and successive use of antimicrobial agents as growth promoters has been observed. Incidence of antibiotic residues in foodstuff is documented world-over by numerous public health experts as being unlawful (Landers *et al*.,2012) and their ingestion could lead to public health threats including: emergence of resilient strains of microbes to antibiotics, respiratory infections, cancer, carcinogenicity, hypersensitive reaction in sensitized individuals (Tran *et al*., 2017) and distortion of intestinal microflora (Selvaraj et al., 2018; Nair *et al*., 2010; Overgaard *et al*., 2012; European Centre for Disease Prevention and Control (ECDC), 2012).

The imminent antimicrobial-resistance predicament has been embraced by clinical researchers, legislators, and the civic at large. The progression and extensive dissemination of ESBL producing micro-organisms has created sicknesses that used to be easily curable to become deadly again. Regrettably, supplementing the escalation in worldwide resistance is lack of antimicrobial drug discovery. In order to deliver twenty-first century medicines that are able to suppress diseases in the drug resistance era, we need to embrace experiences from the past on antimicrobial discovery, development, and fresh understanding of antibiotic action and the cell biology of the microorganisms (Naylor *et al*., 2018). According to Rose *et al*. (2009), the use of antimicrobial drugs in poultry production is very important in disease treatment, prevention and growth promoting but its use must be accepted as a responsibility rather than a right when trying to improve poultry health. This will help to minimize the potential risk and hazards due to poor usage of antimicrobial agents in livestock production. Therefore, use of veterinary antibiotic drugs in poultry systems needs proper control through legislation bodies with broad goal to preserve animal health, improve animal production and to protect the public (Cardona & Kuney, 2002). Therefore, the study aims to evaluate the antibiotic resistance patterns of selected *Enterobacteriaceae* isolated from commercial poultry production systems and humans in Kiambu County, Kenya.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Study area

Kiambu County covers an area of 13,191 km² and is located to northern part of Nairobi and west of Mt. Kenya and it’s estimated to have 4,383,743 inhabitants according to 2009 population census. The high population growth in this area, favors commercial poultry production more than other types of livestock production. This is because Kiambu is a predominantly an intensive small holder production region, it pattern and extend of antimicrobial use significantly differs from other regions of Kenya. According to the report by Nyaga (2007), Kiambu County also has got 5351 (broiler farmers) and 1185 (layer farmers) who are for commercial purposes and 12633 for dual purpose activity.

#### 4.2.2 Study Population

The current study targeted farmers practicing Commercial poultry production systems in Kiambu County and therefore keeping Broiler, layers and Improved Kienyeji birds for business.

#### 4.2.3 Study Design

A laboratory based cross-sectional study was undertaken from November 2020, to February 2021 in six purposively selected Sub-counties namely; Juja, Ruiru, Thika, Gatundu South, Gatundu north and Kikuyu in Kiambu County, Kenya. Four hundred and Thirty seven (437) cloacal swabs (Broiler, layers and Improved Kienyeji) and stool samples from the farmers were collected and submitted to the Centre for Microbiology Research –KEMRI for analysis. From this samples 591 isolates of target enterobacteriaceae (*E.coli; Salmonella spp; Shigella Spp and Klebsiella spp*) were identified.

#### 4.2.4 Sample size determination

This was calculated according to (Kehinde & Daniel, 2015). Using this formula, the sample size calculated was estimated to be 400 fecal samples at CI of 95%. The fecal samples were distributed among farmers and chicken (Layers; broilers; Improved Kienyeji),

$$n = \frac{\mathrm{t}^2 * \mathrm{p}(1-\mathrm{p})}{\mathrm{m}^2}$$

Where; Estimated prevalence=P, Margin of errors (Standard deviation of 0.05) =M, Confidence level at 95% (Standard deviations of 1.96) =T. n = required sample size. n≈ 400 approximated fecal samples. This fecal and stool samples were be distributed among Farmers and poultry (broiler; layers; Kienyeji) respectively.

### 4.2.5 Sample collection

Four hundred and thirty seven (n=437) fecal and stool (human) samples were collected from the six purposively selected representative sub-counties in Kiambu County. Around five grams of fresh fecal samples was aseptically gotten from Humans (n=72), and chicken cloacal swabs (n =365) were collected for this study from the various farms. Each fecal and cloacal swabs was placed in Amies and SF transport media, stored in cool box and transported to the Centre of Microbiology Research (CMR)-KEMRI, in about 6 hours. Upon arrival to the lab the samples were incubated at $37^0$C overnight for 18 to 24 hours. The incubated Amies and Selenite F (SF) samples were mixed uniformly. A sterile wire loop picked a loopful mixture separately from both Amies and SF with streaking on MAC and SS agar differential culture plates to form distinct colonies as shown in fig 4.1. The petridishes were Incubated at $37^0$C overnight between 18 to 24 hours, followed by reading to identify the enteric's of interest (*E.coli*, *Klebsiella Pneumonia, Salmonella spps and Shigella spps*) using morphological characteristics. The morphologically identified isolates were stocked in tryptic soy broth and kept in -$20^0$C refrigerator awaiting for biochemical identification, Antibiotic susceptibility testing (AST) and molecular genotyping.

### 4.2.6 Step wise outline of cloacal swab and fecal sample collection and preparation from the field to the Laboratory

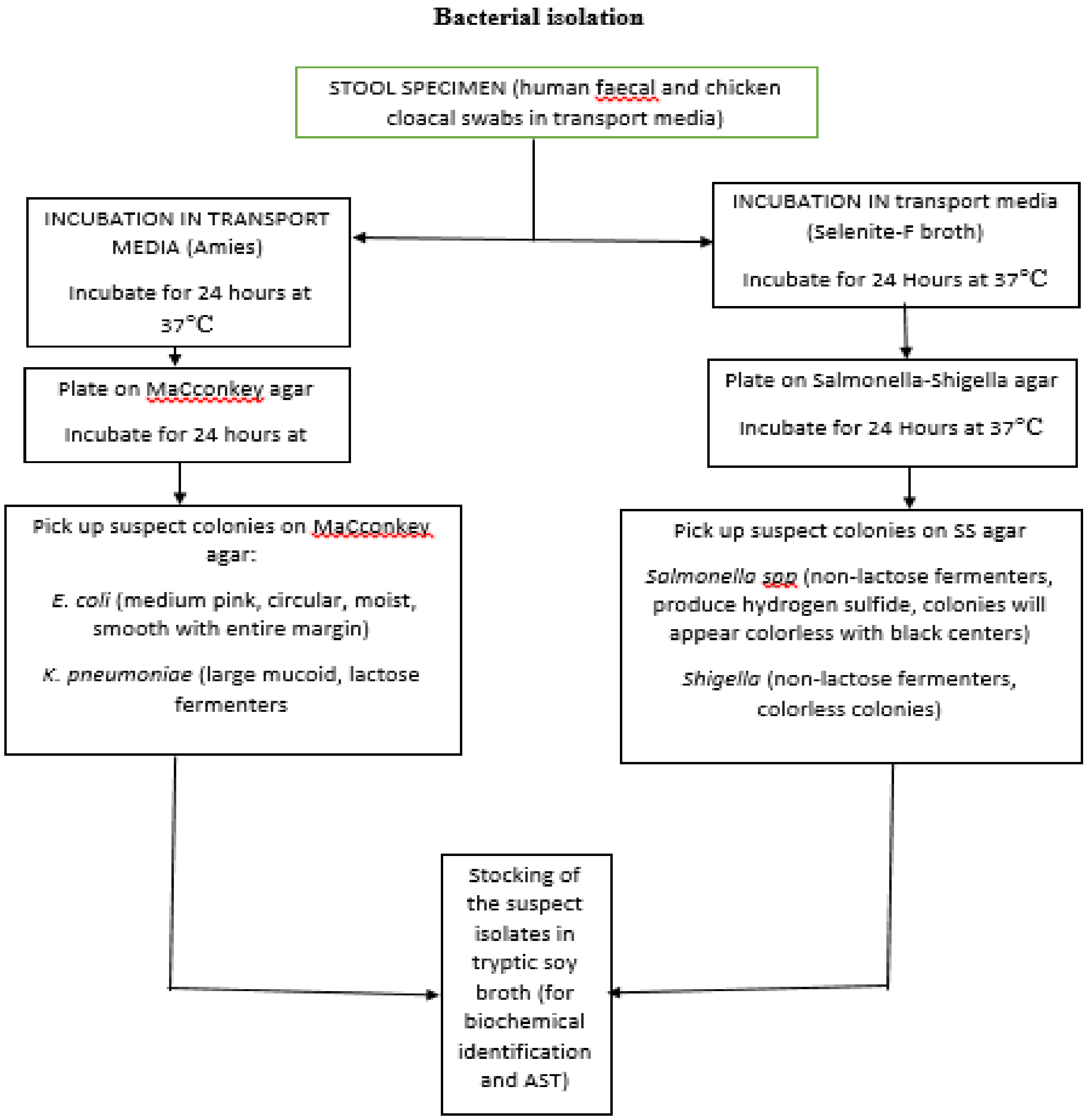



**Fig 4.1: A flow Diagram for the Culture of *Salmonella spp*, *Shigella spp*, *Escherichia spp* and *Klebsiella spp* from cloacal swabs and stool Sample.**

### 4.2.7 Biochemical identification of the isolates

Uncontaminated clusters of bacteria were designated and sub-cultured on nutrient agar slant and were promptly prepared for biochemical tests. Renowned traditional biochemical tests and selective media for bacteria documentation were engaged (Bassetti *et al*., 2013).The isolates were identified using MacConkey broth (Sigma-Aldrich), and Simmons citrate agar (Sigma_Aldrich), catalase, sugar fermentation (Triple sugar iron (TSI) agar), Indole, citrate utilization, urease production, and motility tests. The recognized bacterial species were stored in a 20% glycerol/medium mixture at −80 °C at the Molecular and microbiology laboratory of Centre for Microbiology Research- KEMRI.

#### 4.2.8 Bacterial isolation

A sum of 591 isolates including *Escherichia coli* (n=289); *Klebseilla pneumonia* (n=83); *Salmonella spps* (n=108) *and Shigella spp* (n=111) were isolated from stool and fecal obtained from farmers practicing commercial poultry production in Kiambu County- Kenya from November, 2020 to February, 2021. In totality, the isolates were gotten under approved ethical standards as per Clinical and Laboratory standard institute guidelines (CLSI, 2013). The isolates were identified and verified using standard biochemical reactions including: growth on MacConkey agar, citrate utilization, Voges Proskauer, methyl Red, and motility tests as per Amira Mohamed EL-Ganiny *et al*. (2016); Koneman *et al*. (1997).

#### 4.2.9 Antibiotic susceptibility tests

Antimicrobial resistance patterns of *E.coli*; *Shigella spps*; *Salmonella spps* and *Klebsiella spps* was ascertained through the disc diffusion method on Muller –Hinton agar according to CLSI, (2013); CLSI, (2021). The antibiotic discs were obtained from Oxiod (USA). Thirteen antibiotics were tested and distributed among plate A and B.  Plate A had the following: Amoxacillin-Clavullanic acid (AMC,10ug); Ampicillin (AMP-25µg); Ceftriaxone (CRO); Cefiximine (CFM); Cefotaxime (CTX);  Cefepime (FEP,); Ceftazidime-Cefpodoxime (CAZ/CPD) and plate B had the following:  Ciprofloxacin (CIP, 5 µg); Trimethoprim (TMP); Gentamicin (GEN, 10 µg); Tetracycline (TET-100 µg); Sulfamethoxazole (SMX-200 µg); Chloramphenicol(CHL, 30µg); Ceftazidime (CAZ). The isolated microbes were plated on the dried Mueller Hinton agar plate. Using a sterilized forceps impregnated antibiotic were placed on the surface of the agar, and incubated at 35$^{o}$C overnight. Using a ruler, the antibiotic inhibition zones were measured to the nearest millimeter (mm) from the center of the disk to a point on the circumference of the zone where a distinct edge was seen. For all isolates the Zones of inhibition were determined and interpreted as per breakpoints. Phenotypic uncovering of ESBLs production was identified by a double-disk synergy test (DDST). Presence of ESBL production was identified through enhancement of the inhibition zone between the disks containing Amoxacillin-Clavullanic acid (AMC) and Cefotaxime or Ceftazidime.

#### 4.2.10 Quality Controls

The quality control were run using *E.coli* ATCC25922. The cultures were classified as sensitive, intermediate and resistance on the foundation of diameter of zones of inhibition. According to Saidi, Khelef and Kaidi (2014), isolates resistant to three or more antimicrobial were considered to be Multi-resistance in this current study.

#### 4.2.11 Data analysis and Presentation

The inhibition zone diameter were measured by use of divider and a ruler, and the mean, and standard errors of three replicates calculated, analyzed and keyed into EPICOLLECT5 (https://five.epicollect.net/project/ekss-ast/data) and thereafter WHONET for data analysis with results presented in form of tables and graphs.

### 4.3 RESULTS

Out of 437 fecal and stool samples collected, 591 isolates were recovered with *E.coli* (48.9%) being the most frequently identified, followed by *Shigella Spp.* (18.8%), *Salmonella spp.* (18.3%), and *Klebsiella Spp*. (14.0%) as indicated in table 4.1 below.

**Table 4.1: Selected species of enterobacteriaceae isolated from commercial poultry production systems and Humans in Kiambu County**

| Bacteria species | Bacteria isolated | |
|---|---|---|
| | **Number (n=591)** | **%** |
| *Escherichia coli* | 289 | 48.9 |
| *Klebsiella species* | 83 | 14.0 |
| *Salmonella species* | 108 | 18.3 |
| *Shigella species* | 111 | 18.8 |
| Total | 591 | 100 |

**Key:** %-percentage; n-sample size; spp-species

**Figure 4.2** present the characteristic growth of *Escherichia Coli* and *Klebsiella spp* on Macconkey agar and the growth of *Salmonella spp*. and *Shigella spp*. on *Salmonella* and *Shigella* Agar.

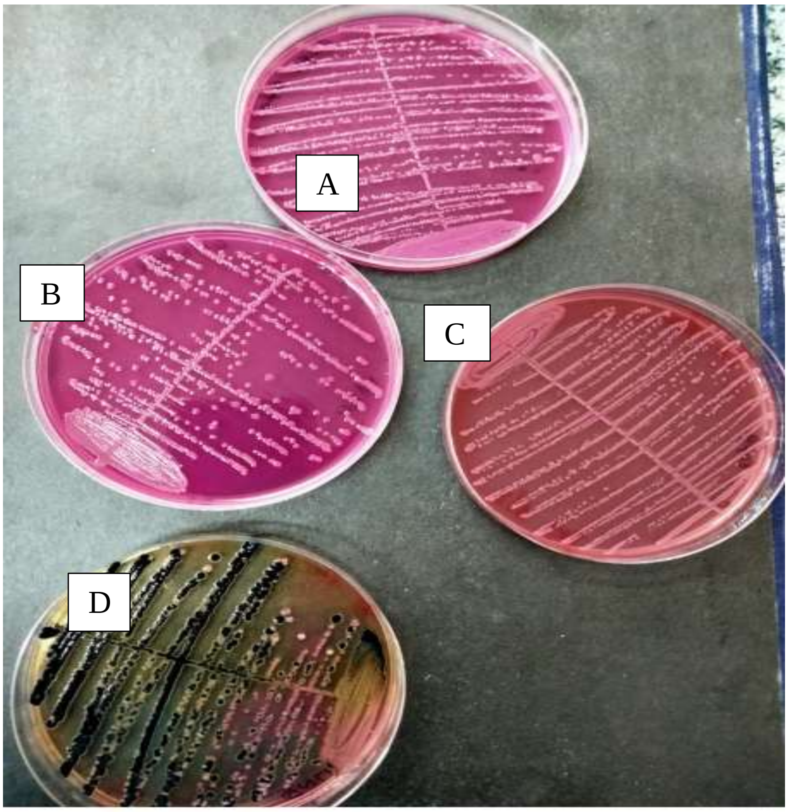



**Fig 4.2: Growth characteristics of the four Enterobacteraceae species (A-*Klebsiella spp*; B-*E.coli*; C-*Shigella spp*; D- *Salmonella spp*.) selected for this study**

**Figure 4.3** shows the principal investigator undertaking some of the laboratory procedure at the Centre for Microbiology research -KEMRI

**Fig 4.3: Principal researcher working in the Centre for microbiology Research (CMR-KEMRI**

**Fig 4.4** presents results of the biochemical tests that were carried out to phenotypically identify the four *Enterobacteraceae* of research interest.

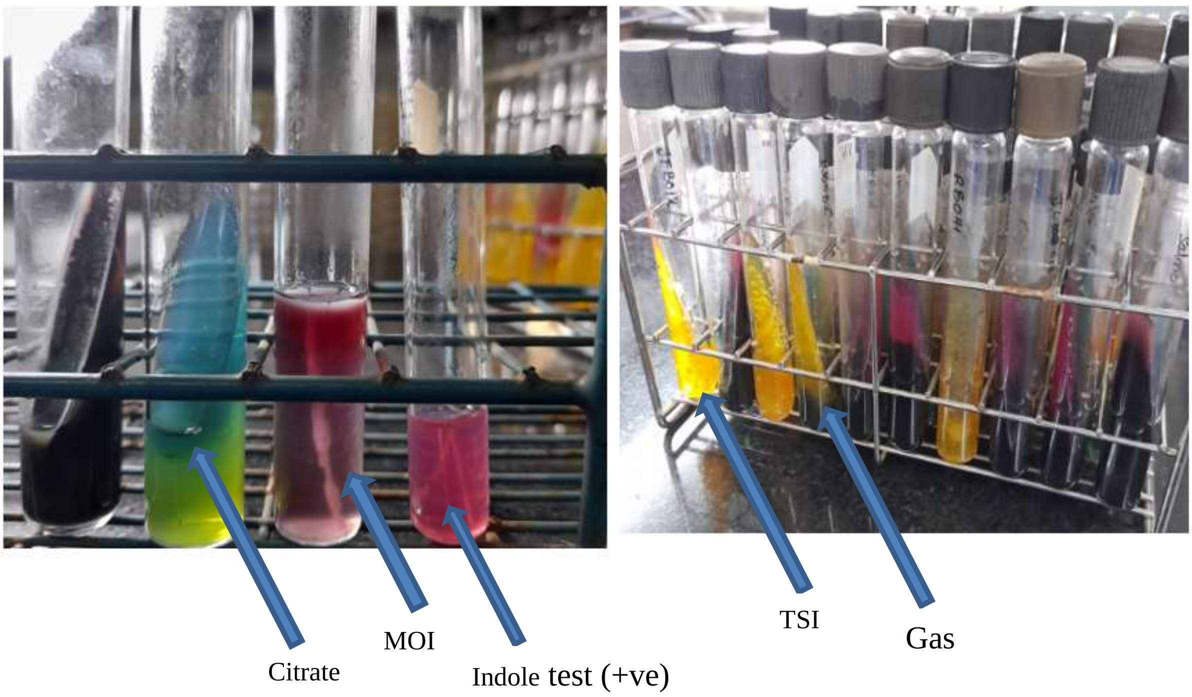


**Fig 4.4: Biochemical test performed to identify the isolates**

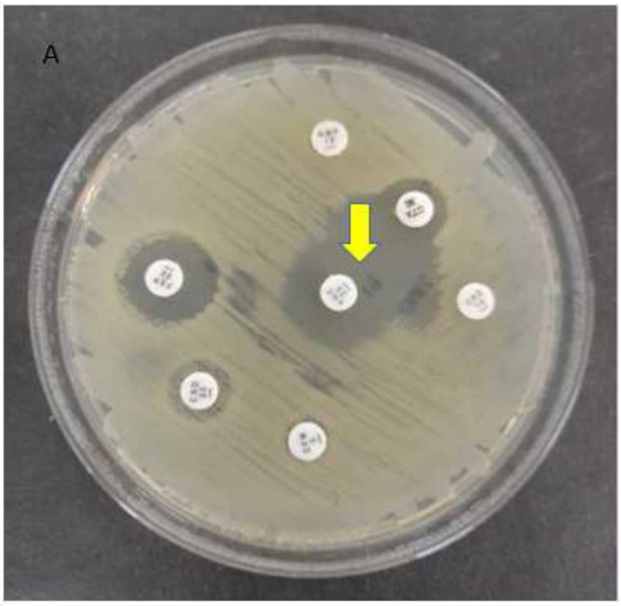
**Table 4.2** present results of antibiotics resistance profiles of 591 *Enterobacteriaceae* isolates against to 13 antibiotics. The results indicates that there is multi-drug resistance across the various classes of antibiotics used among the four selected enterobacteriaceae. In general, the antibiotics susceptibility profiles of the selected *Enterobacteriaceae* species showed the highest rate of susceptibility against Cefuroxime (94%), Gentamicin (93%), Ceftriaxone (91%), Cefepime (89%), Cefotaxime (85%), Ceftazidime (84%), Chloramphenicol (77%), followed by Amoxicillin/Clavulanic acid and Ciprofloxacin (56%) with least susceptibility identified in Ampicillin (46%); Trimethoprim and Tetracycline (28%) and sulfamethoxazole (17 %). The isolates were most resistance to Sulfamethoxazole (79%), Trimethoprim (71%), Tetracyclines (59%), Ampicillin (49%) and Amoxicillin/Clavunallic acid (39%) correspondingly as indicated in table 4.2 below.

**Table 4.2: Antimicrobial resistance profiles of 13 antibiotics agents tested against to the four selected Enterobacteriaceae of interest**

| Organisms | Name of antibiotic | Number | %R | %I | %S | %R at 95%C.I. |
|---|---|---|---|---|---|---|
| Selected spps | Ampicillin | 591 | 49 | 5 | 46 | 45.1-53.3 |
| Selected spps | Amoxicillin/Clavulanic acid | 591 | 39 | 4 | 56 | 35.5-43.5 |
| Selected spps | Cefuroxime | 591 | 3 | 2 | 94 | 2.0-5.1 |
| Selected spps | Ceftazidime | 591 | 5 | 10 | 84 | 3.8-7.6 |
| Selected spps | Ceftriaxone | 591 | 4 | 5 | 91 | 2.8-6.3 |
| Selected spps | Cefotaxime | 591 | 7 | 8 | 85 | 4.9-9.2 |
| Selected spps | Cefepime | 591 | 3 | 7 | 89 | 2.1-5.3 |
| Selected spps | Gentamicin | 590 | 4 | 2 | 93 | 3.0-6.5 |
| Selected spps | Ciprofloxacin | 590 | 17 | 27 | 56 | 13.7-19.9 |
| Selected spps | Sulfamethoxazole | 589 | 79 | 4 | 17 | 75.0-81.8 |
| Selected spps | Trimethoprim | 590 | 71 | 1 | 28 | 67.5-74.9 |
| Selected spps | Chloramphenicol | 591 | 15 | 8 | 77 | 12.2-18.1 |
| Selected spps | Tetracycline | 591 | 59 | 13 | 28 | 55.1-63.2 |

**Key:** %-p; R- resistance; I-intermediate; S-susceptibility; CI-confidence interval; selected spps (*E.coli*, *Shigella spps*, *Salmonella Species*, and *Klebsiella spps.*)

**Fig 4.5** present results of augmentation of the inhibition zone in *E.coli* amongst the disks encompassing Amoxacillin-Clavullanic acid (AMC) and Cefotaxime or Ceftazidime showing presence of ESBL production, with a plate (B) where there was no growth indication.



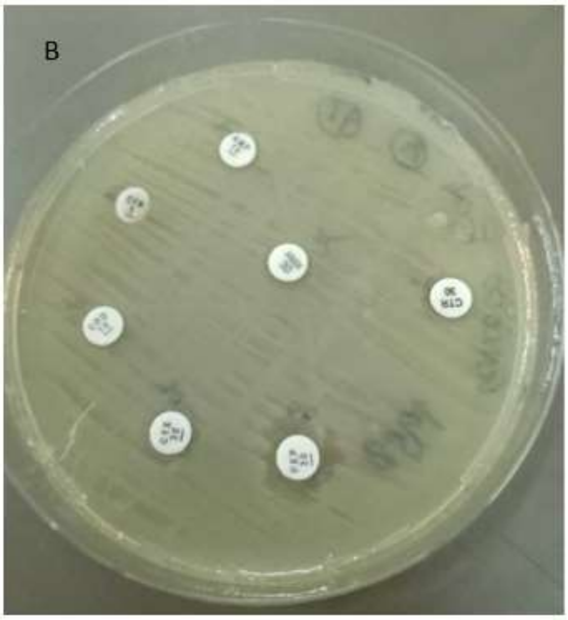
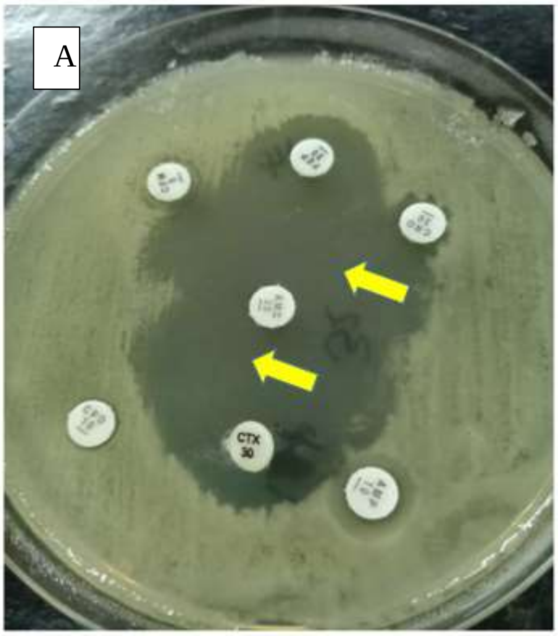




**Fig 4.5: Arrow in plate A indicate zone of synergy between Amoxi-Clavunalic acid and Cefotaxamine (CTX) as an indication of ESBL Genes production in E.coli while plate B show no  zone of inhibition in E.coli by the various antibiotic discs.**

**Fig 4.6** presents' results of enhancement of the inhibition zone in *Klbessiella spp* (A)and *Salmonella spp* (B) amongst the disks having Amoxacillin-Clavullanic acid (AMC) and Cefotaxime or Ceftazidime showing the presence of ESBL production.



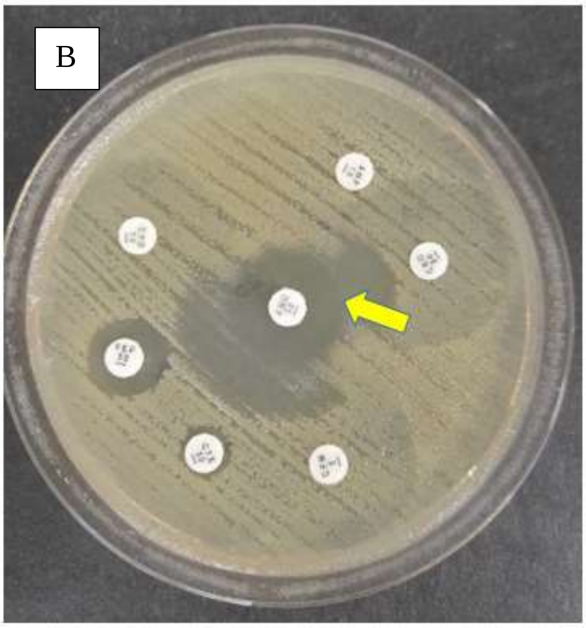



**Fig 4.6: Arrow A and B indicate zone of synergy between Ampicillin, Cefotaxamine (CTX) and Ceftriaxone as an indication of ESBL Genes production in Klebsiella spp and Salmonella spp respectively.**

**Table 4.3** present the findings of antimicrobial resistance outline of the 591 bacterial isolates tested against 13 antibiotics. Most the isolates were resistance to sulfamethoxazole (SMX), Trimethoprim (TMP), Tetracycline's (TCY) and Ampicillin (AMP) as show in table 4.3 below.

**Table 4.3: Antibiotic resistance levels in various bacterial isolates (n=591)**

| **Antimicrobial agent** | ***Escherichia coli*** **(n = 289)** | ***Klebsiella sp.*** **(n = 83** | ***Salmonella sp.*** **(n = 108** | ***Shigella sp.*** **(n = 111)** |
|---|---|---|---|---|
| **AMC %R** | 45 | 2 | 7 | 84 |
| **AMP %R** | 48 | 60 | 39 | 55 |
| **CAZ %R** | 4 | 6 | 6 | 8 |
| **CHL %R** | 15 | 11 | 24 | 10 |
| **CIP %R** | 20 | 11 | 16 | 13 |
| **CRO %R** | 5 | 6 | 2 | 5 |
| **CTX %R** | 7 | 4 | 7 | 7 |

| | | | | |
|---|---|---|---|---|
| **CXM %R** | 3 | 4 | 1 | 5 |
| **FEP %R** | 2 | 5 | 3 | 5 |
| **GEN %R** | 4 | 5 | 6 | 4 |
| **SMX %R** | 82 | 72 | 77 | 77 |
| **TCY %R** | 56 | 58 | 82 | 48 |
| **TMP %R** | 78 | 60 | 67 | 68 |

**Key:** R- Resistance; %-Percentage; n- sample size; AMC- Amoxi-Clavunallic; Ampicillin (AMP),Ceftazidime (CAZ); (CHL); CIP-Ciprofloxacin; Ceftriaxone (CRO); Cefotaxime(CTX); Cefuroxime(CXM); Cefepime (FEP); Gentamicin(GEN); SMX-sulfamethoxazole; TCY-Tetracycline; TMP- Trimethoprim

**Figure 4.7** shows percent distribution of resistance to the 13 established antibiotics against the isolates. The figure indicates most isolates were vulnerable to Ceftazidime and Cefotaxime. However, most of isolates were resistance to Sulfamethoxazole, Trimethoprim, Tetracycline's and Ampicillin respectively.

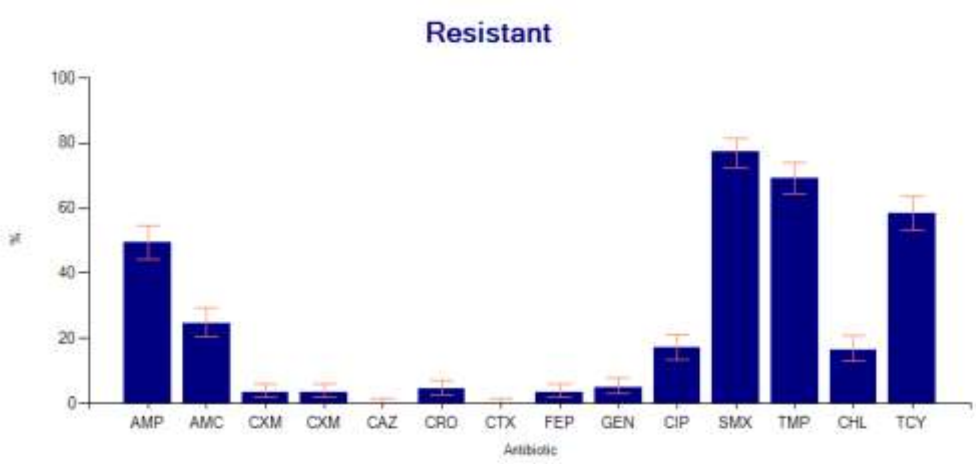



**Fig 4.7: Graph showing %resistance towards the tested antimicrobial agents**

**Figure 4.8** shows the results of % resistance by *Escherichia coli* to the 13 established antibiotics. Majority of the *Escherichia Coli* were resistance to Sulfamethoxazole, Trimethoprim, Tetracycline, Ampicillin and Amoxi-Clavunalic acid, consecutively as displayed in fig 4.8 below.

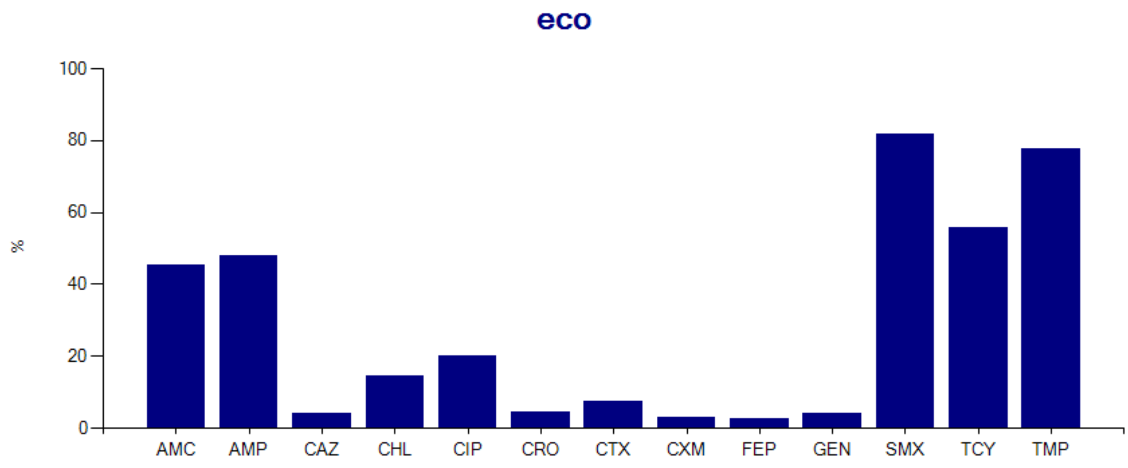



**Fig 4.8: Graph showing %resistance towards the tested antimicrobial agents by E.coli spp.**

**Figure 4.9** presents the results of % resistance *Klebsiella Spp*. to the 13 tested antibiotics. Majority of the *Klebsiella Spp*. isolates were resistance to Sulfamethoxazole, Trimethoprim, Tetracycline, and Ampicillin consecutively as displayed in fig 4.9 below.

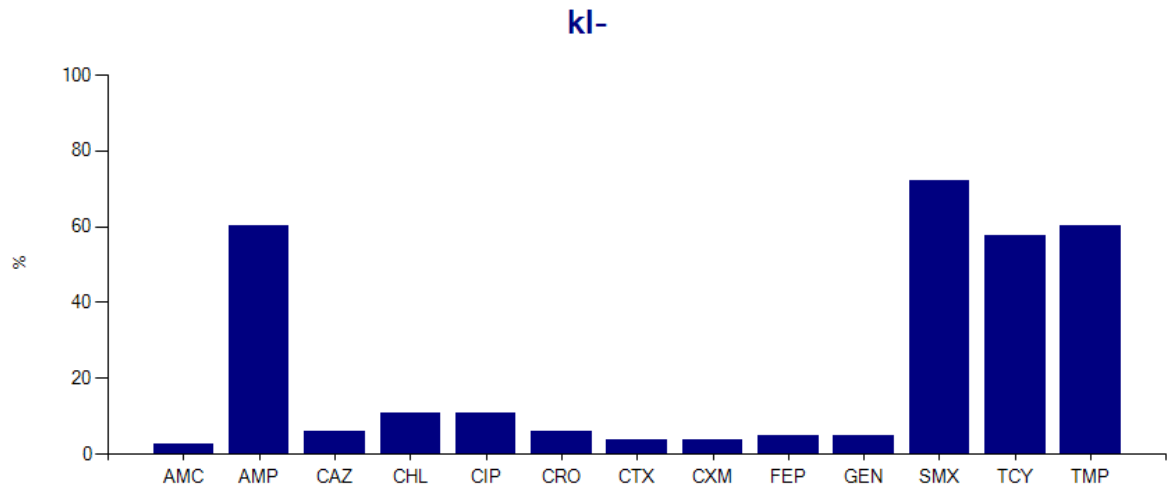


**Fig 4.9: Graph showing %resistance towards the tested antimicrobial agents by Klebsiella spps**

**Figure 4.10** presents the results of % resistance *Salmonella Spp*. to the 13 tested antibiotics. Majority of the *Salmonella Spp*. were resistance to Tetracycline, Sulfamethoxazole, Trimethoprim, and Ampicillin Respectively as displayed in fig 4.10 below.

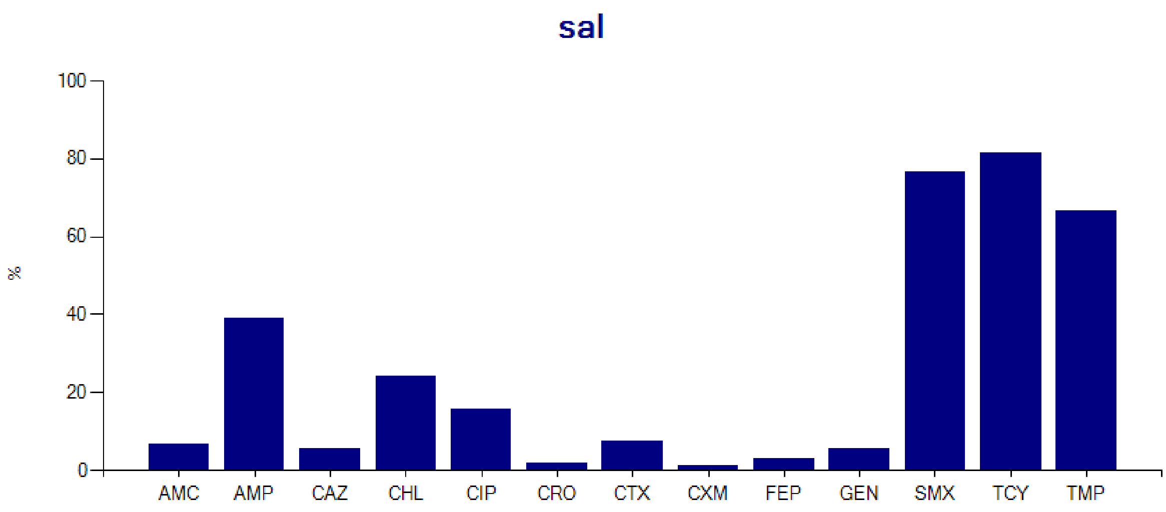



**Fig 4.10: Graph showing %resistance towards the tested antimicrobial agents by Salmonella spps**

**Figure 4.11** presents the results of % resistance *Shigella Spp*. to the 13 tested antibiotics. Majority of the *Shigella Spp*. were resistance to Amoxi-Clavunallic acid (AMC), Sulfamethoxazole (SMX), Trimethoprim (TMP), Ampicillin (AMP) and Tetracycline (TCY) respectively as displayed in fig 4.11 below.

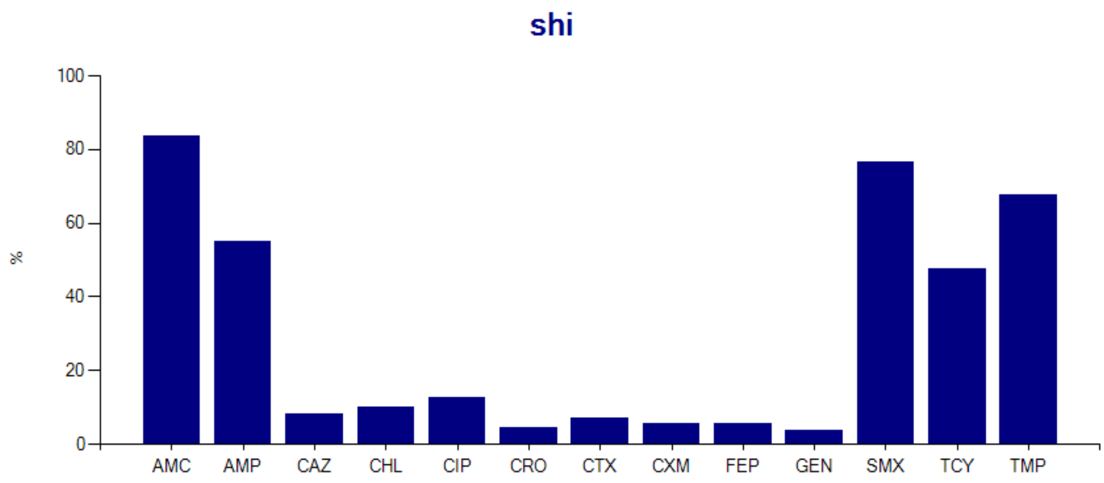



**Fig 4.11: Graph showing %resistance towards the tested antimicrobial agents by Shigella spps**

**Table 4.4** indicates the distribution of the isolates according to the species, source of the sample and their antibiotic resistance profiles. From the study we found that most isolates across the four species of interest were from Layers and Improved Kienyeji birds. Furthermore, majority of the *E.coli* (n=125) isolates were from layer birds followed by Improved Kienyeji (n=92), Broiler birds (n=55) and Broiler farmer (n=17), respectively. We further, noted that most of *Klebsiella spp.* isolates emanated from Improved Kienyeji (n=27), Broiler birds (n=23), layer birds (n=22) and Broiler farmer (n=11), consecutively. For the *Salmonella Spp* majority of the isolates were from Layer birds (n=45), followed by Improved Kienyeji (n=31), Broiler birds (n=26 and Broiler farmer (n=6). For the *Shigella spp.* isolates most were from layers (n=39) and Improved Kienyeji (n=31) birds, with broiler bird having (n=24) and Broiler farmer (n=13), respectively. Resistance to Sulfamethoxazole, Trimethoprim, Tetracycline's, and Ampicillin was witnessed in all the four isolates used in this study. Both *E.Coli*, and *Shigella spp.* also displayed great resistance to Amoxi-Clavunallic acid as indicated in table 4.4 below.

**Table 4.4: Antibiotic resistance of bacterial isolates from the various sample types (n=491)**

| Organism | Local specimen code | Number of isolates | AMC %R | AMP %R | CAZ %R | CHL %R | CIP %R | CRO %R | CTX %R | CXM %R | FEP %R | GEN %R | SMX %R | TCY %R | TMP %R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Escherichia coli | Broiler | 55 | 29 | 56 | 6 | 15 | 29 | 7 | 7 | 6 | 4 | 2 | 87 | 56 | 80 |
| | Broiler farmer | 17 | 59 | 53 | 12 | 18 | 18 | 12 | 12 | 12 | 6 | 6 | 94 | 41 | 88 |
| | Improved Kienyeji | 92 | 39 | 49 | 5 | 15 | 19 | 5 | 8 | 2 | 3 | 5 | 84 | 59 | 82 |
| | Layers | 125 | 55 | 42 | 2 | 14 | 18 | 2 | 6 | 2 | 1 | 4 | 77 | 55 | 72 |
| Klebsiella spp. | Broiler | 23 | 9 | 70 | 13 | 22 | 26 | 9 | 9 | 13 | 17 | 13 | 87 | 61 | 78 |
| | Broiler farmer | 11 | 0 | 64 | 9 | 0 | 0 | 18 | 0 | 0 | 0 | 9 | 36 | 46 | 36 |
| | Improved Kienyeji | 27 | 0 | 52 | 4 | 11 | 4 | 4 | 4 | 0 | 0 | 0 | 67 | 63 | 56 |
| | Layers | 22 | 0 | 59 | 0 | 5 | 9 | 0 | 0 | 0 | 0 | 0 | 82 | 55 | 59 |
| Salmonella spp. | Broiler | 26 | 0 | 46 | 4 | 23 | 8 | 0 | 8 | 4 | 4 | 4 | 72 | 81 | 73 |
| | Broiler farmer | 6 | 0 | 50 | 17 | 17 | 17 | 0 | 0 | 0 | 0 | 0 | 50 | 50 | 50 |
| | Improved Kienyeji | 31 | 10 | 39 | 7 | 36 | 19 | 3 | 10 | 0 | 3 | 3 | 87 | 87 | 68 |
| | Layers | 45 | 9 | 33 | 4 | 18 | 18 | 2 | 7 | 0 | 2 | 9 | 76 | 82 | 64 |
| Shigella spp. | Broiler | 24 | 92 | 58 | 13 | 17 | 29 | 13 | 8 | 4 | 4 | 0 | 83 | 54 | 79 |
| | Broiler farmer | 13 | 85 | 46 | 15 | 15 | 8 | 8 | 15 | 15 | 15 | 8 | 69 | 31 | 62 |
| | Improved Kienyeji | 35 | 77 | 57 | 9 | 3 | 11 | 3 | 9 | 9 | 6 | 3 | 86 | 54 | 69 |
| | Layers | 39 | 85 | 54 | 3 | 10 | 5 | 0 | 3 | 0 | 3 | 5 | 67 | 44 | 62 |

**Key:** R- Resistance; %-Percentage; n- sample size; AMC- Amoxi-Clavunallic; Ampicillin(AMP), Ceftazidime (CAZ); CHL-Chloramphenicol; CIP-Ciprofloxacin; CRO-Ceftriaxone; Cefotaxime (CTX); Cefuroxime (CXM); Cefepime (FEP); Gentamicin (GEN); SMX-sulfamethoxazole; TCY-Tetracycline; TMP- Trimethoprim

**Table 4.5** indicates the finding on the distribution of the isolates according to the species, region of the sample collection and their antibiotic resistance profiles. The results indicates that most of the *E.coli spps* came from Gatundu South (n=60), Juja (n=51), followed by Thika and Gatundu north (n=48), respectively. We also noted that most of the *Shigella spp* came from Juja (n=41), with *Salmonella Spp* coming from Ruiru (n=19). Majority of the *Klebsiella spp* were isolated from Ruiru (n=19), and Thika (n=16), accordingly. We found that *Klebsiella spp* from Gatundu South were 100% resistance to Ampicillin as per *Shigella Spp* which had also 100% resistance to Amoxi-Clavunallic acid. Most of the isolates irrespective to area of origin had high resistance to Sulfamethoxazole, Trimethoprim and Tetracycline's, and Ampicillin respectively. *Klebsiella spp, Shigella Spp, and Salmonella Spp* from Gatundu North were all resistant to Ceftriaxone (CRO), Cefotaxime (CTX), Gentamicin (GEN), Cefuroxime (CXM), and Cefepime (FEP), consecutively. We also noted that Klebsiella *spp, Shigella Spp, and Salmonella Spp* from Ruiru we all susceptible to Ceftriaxone (CRO), Cefotaxime (CTX), and Cefuroxime (CXM) respectively as indicated in table 4.5 below.

**Table 4.5: Antimicrobial resistance profiles of bacteria isolates tested against antimicrobial agents as per Sub-counties in which isolates were collected.**

| Organism | Location | Number of isolates | AMC %R | AMP %R | CAZ %R | CHL %R | CIP %R | CRO %R | CTX %R | CXM %R | FEP %R | GEN %R | SMX %R | TCY %R | TMP %R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E. coli | Gatundu North | 48 | 71 | 42 | 2 | 17 | 25 | 4 | 13 | 2 | 2 | 6 | 75 | 67 | 75 |
| | Gatundu south | 60 | 63 | 47 | 3 | 12 | 15 | 2 | 5 | 2 | 3 | 7 | 80 | 53 | 75 |
| | Juja | 51 | 12 | 47 | 10 | 16 | 16 | 12 | 12 | 8 | 6 | 4 | 75 | 35 | 67 |
| | Kikuyu | 39 | 49 | 59 | 3 | 23 | 18 | 3 | 5 | 3 | 0 | 0 | 90 | 51 | 84 |
| | Ruiru | 43 | 9 | 49 | 2 | 14 | 28 | 5 | 2 | 2 | 2 | 2 | 95 | 58 | 86 |
| | Thika | 48 | 63 | 46 | 4 | 8 | 21 | 2 | 6 | 2 | 0 | 4 | 81 | 71 | 83 |
| Kleb. sp. | Gatundu North | 9 | 0 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 78 | 89 |
| | Gatundu south | 11 | 0 | 100 | 18 | 18 | 18 | 18 | 9 | 9 | 9 | 18 | 82 | 82 | 82 |
| | Juja | 14 | 14 | 57 | 14 | 0 | 14 | 14 | 7 | 14 | 14 | 0 | 57 | 36 | 43 |
| | Kikuyu | 14 | 0 | 43 | 0 | 36 | 7 | 7 | 0 | 0 | 0 | 0 | 86 | 64 | 50 |
| | Ruiru | 19 | 0 | 53 | 5 | 5 | 16 | 0 | 0 | 0 | 5 | 11 | 53 | 37 | 47 |
| | Thika | 16 | 0 | 63 | 0 | 6 | 6 | 0 | 6 | 0 | 0 | 0 | 88 | 69 | 69 |
| Salmonella sp. | Gatundu North | 10 | 10 | 30 | 10 | 20 | 30 | 0 | 0 | 0 | 0 | 0 | 70 | 90 | 70 |
| | Gatundu south | 22 | 18 | 55 | 0 | 14 | 9 | 0 | 5 | 0 | 0 | 14 | 82 | 68 | 82 |
| | Juja | 19 | 0 | 26 | 5 | 26 | 32 | 0 | 5 | 5 | 5 | 0 | 74 | 90 | 63 |
| | Kikuyu | 26 | 4 | 35 | 4 | 27 | 15 | 0 | 15 | 0 | 4 | 4 | 73 | 77 | 54 |
| | Ruiru | 7 | 0 | 71 | 0 | 29 | 14 | 0 | 0 | 0 | 0 | 14 | 100 | 100 | 86 |
| | Thika | 24 | 4 | 33 | 13 | 29 | 4 | 8 | 8 | 0 | 4 | 4 | 75 | 83 | 63 |
| Shigella sp. | Gatundu North | 5 | 100 | 20 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 60 | 80 |
| | Gatundu south | 12 | 83 | 50 | 8 | 8 | 17 | 0 | 8 | 8 | 8 | 17 | 75 | 50 | 67 |
| | Juja | 41 | 95 | 56 | 15 | 7 | 22 | 10 | 10 | 5 | 7 | 0 | 78 | 49 | 76 |
| | Kikuyu | 16 | 94 | 25 | 0 | 13 | 0 | 6 | 6 | 13 | 6 | 0 | 69 | 25 | 38 |
| | Ruiru | 23 | 61 | 91 | 0 | 4 | 9 | 0 | 0 | 0 | 0 | 4 | 96 | 70 | 91 |
| | Thika | 14 | 71 | 43 | 14 | 14 | 7 | 0 | 14 | 7 | 7 | 7 | 50 | 29 | 36 |

**Key: Key:** R- Resistance; %-Percentage; n- sample size; AMC- Amoxi-Clavunallic; Ampicillin (AMP), Ceftazidime (CAZ); Chloramphenicol (CHL); Ciprofloxacin (CIP); CRO-Ceftriaxone; Cefotaxime (CTX); Cefuroxime (CXM); Cefepime (FEP); Gentamicin (GEN); SMX-sulfamethoxazole; TCY-Tetracycline; TMP- Trimethoprim

### 4.3 DISCUSSION OF RESEARCH FINDINGS

Ever since the discovery of antimicrobial/ antibiotics agents over eighty years ago, they have protected immeasurable lives from contagious illnesses and changed the current medical techniques, including surgery, cancer management and organ transplant. This significant gains, over the years are deteriorating due to the slow but steady spread of antibiotics resistance - whereby bacteria turn antimicrobial agents ineffective, which might take the world back to a pre-antibiotic era. Although, this antimicrobial resistance problem has been aggravated by the extensive use of antibiotics in the human health sector, in recent years there has been acknowledgement of the problems emanating from greater use of these miracle agents in food-animal production (WHO, 2014). The occurrence of antibiotic-resistant has become a major public health concern. The usage of antibiotics/antimicrobial in any aspect, such as in infection management and growth promotion in livestock, can hypothetically lead to extensive propagation of antibiotic-resistant bacteria (Guerra *et al*., 2002)

The occurrence of multi-antimicrobial resistant in enteric pathogens presents a severe danger to the healthcare and Livestock farming systems in Kenya as these microbes can disseminate from the surroundings to the hospital establishment resulting to community acquired infections. In current research we determined the antimicrobial resistance patterns of selected *enterobacteriaceae* obtained from human and poultry in commercial production systems in Kiambu County, Kenya and assessed their extent of distribution among the representative Sub-Counties.

Overall in the research we found that most of the isolates were *E.coli* (48.9 %), followed by *Shigella spp* (18.8%), respectively. This agrees with the study carried out by Buxton and Frazer (1977), and Bebora (1979) that found that *Escherichia coli* was the most common microbe in animal fecal and human stool samples. This is further supported by a study that was carried by Njagi (2003) who found *E.coli* prevalence of 40.2%. In this study it is found that there is high occurrence of single and multi-drug resistance (MDR) among the 13 antibiotics tested across the four enteric's. This resistance was highest among, Sulfamethoxazole (79%), Trimethoprim (71%), Tetracyclines (59%), Ampicillin (49%) and Amoxicillin/Clavunallic acid (39%). This agrees with a study carried out by Deng (2017), and Nyabudi *et al.* (2017), who found these antibiotics to be most commonly used in poultry production and to have developed resistance. Similarly, this has been established by notable researchers such as: Allorechtova *et al*. (2012); Gakuya *et al*. 2007; Ombui *et al*. 2000 who found multi-drug resistance in the above mentioned drugs in the same order. Hence, this calls for joint effort for fight against MDR and X-DR by advocating prudent use of antimicrobial agent in animal production. However, the four enteric's were also highly susceptible to the following antibiotic; Cefuroxime (94%), Gentamicin (93%), Ceftriaxone (91%), Cefepime (89%), Cefotaxime (85%), Ceftazidime (84%), Chloramphenicol (77%), followed by Amoxicillin/Clavulanic acid and Ciprofloxacin (56%) respectively.

In addition, the study found that both Shigella spp and *Escherichia coli* isolates displayed great resistance to Amoxi-Clavunallic acid as indicated in table 4.5. The results also indicates, most of the *E.coli spps* came from Gatundu South, Juja followed by Thika and Gatundu north, respectively. The study indicated that most of the *Shigella spp* came from Juja, with *Salmonella Spp* coming from Ruiru. Majority of the *Klebsiella spp* were isolated from Ruiru and Thika accordingly. We found that *Klebsiella spp* from Gatundu South were 100% resistance to Ampicillin as per *Shigella Spp* which had also 100% resistance to Amoxi-Clavunallic acid. This could be explained by the extensive use of amoxillin in this study areas. Most of the isolates irrespective to area of origin had high resistance to Sulfamethoxazole, Trimethoprim and Tetracycline's, and Ampicillin respectively. These indicates that the microbes could have been subjected to these antimicrobial agents a bit earlier, henceforth acting as a discriminatory force for resistance. Previous studies by Ansari and Khartoon (1999; Kariuki *et al* (1999), found that high resistance realized to tetracycline as one of the broad spectrum antibiotics was due to it use as feed supplements and its irrational use can lead to development of antimicrobial resistance. This could further be attributed to either to its irrational use for therapeutic treatment, sub therapeutic preventive measure as feed additives to promote growth or for use as disinfectants (Esipisup, 2009). Several reports by Al-Bahry *et al.* (2001) indicated that multiple resistances are now more common than resistance to a single antibiotic. This occurrence can be linked to the genes that are responsible for multiple resistances that are carried on the same plasmid. For example, in gram negative organisms, resistance is commonly controlled by genetic factor that are customarily associated with large plasmids which are conjugative. According to a study carried out by Tricia *et al*. (2006), He postulated that these plasmids often carry antibiotic resistance gene, heavy metals resistance genes and/or other pathogenic factors such as toxins, hence the choice for any of these factors selects for the plasmid which contain them.

From this study we found that Cefotaxime (CTX), Ceftriaxone (CRO) Gentamicin (GEN), Cefuroxime (CXM), and Cefepime (FEP), were all resistance against *Klebsiella spp, Shigella Spp,* and *Salmonella Spp* isolated from Gatundu North, consecutively. This agrees with a study carried by Zahraei and Farashi (2006) who found an association between emergency of flouroquinolones resistance zoonotic pathogens with subsequent use of approved veterinary Antimicrobial agents in livestock production. This indicates there is irrational usage of antimicrobial agents in poultry farming systems in Kenya. According to Amy *et al.* (2007), they stated that misuse and misappropriation of antimicrobial agents in poultry production systems can lead to an emerging alarm on it cause to extended spectrum of resistance to antibiotics by enteric pathogens. This can be a potential source of resistant genes which can be conveyed to human beings pathogens via conjugation. We also noted that Klebsiella *spp, Shigella Spp, and Salmonella Spp* from Ruiru were all susceptible to Ceftriaxone (CRO), Cefotaxime (CTX), and Cefuroxime (CXM) respectively as indicated in table 4.5.

According to the results we found that most of the isolates across the four enteric species of interest were from Layers and Improved Kienyeji birds. In the study, majority of the *Escherichia coli* isolates were from layer birds followed by Improved Kienyeji, Broiler birds and Broiler farmer respectively. This is because most of the farmers in this areas preferred keeping layers birds for eggs and for improved Kienyeji to supply both meat and eggs.. We further, noted that most of *Klebsiella spp.* isolates emanated from Improved Kienyeji, and Broiler birds. For the *Salmonella Spp* majority of the isolates were from Layer birds and Improved Kienyeji. For the *Shigella spp.* isolates most were from layers) and Improved Kienyeji birds. This can be explained by the reason that most farmers prefer keeping layer and improved Kienyeji birds as compared to broilers. This enteric pathogens causes infections in the blood stream, surgical wounds, urinary tract infections (UTIs), and respiratory tract infections and also cause recurrent diseases such as cancer and diabetes. McEwen and Fedorka Cray (2002), stated that freely and straightforwardly spread of MDR *Enterobacteriaceae* from contaminated animal food sources and contact surfaces makes them a subject of public health concern.

Therefore, the current study vindicate the essence for uninterrupted surveillance of antimicrobial resistance by *Enterobacteriaceae spps* in order to approximate the occurrence of infection, the danger of infection, cost implications and probable management possibilities accessible for these multidrug resistant pathogens. Additionally, it is imperative to ascertain the bases and the means of contaminations coined to the patterns of transmission of this drug-resistant Entero-pathogens in Kenya for a suitable appreciation of the dynamic forces involved and measures to avert epidemics in the public arena.

# CHAPTER 5: MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM BETA-LACTAMASES (ESBLS) AND QUONOLONES (QNRS) PRODUCERS FROM SELECTED *ENTEROBACTAERACEAE* STRAINS ISOLATED FROM COMMERCIAL POULTRY PRODUCTION SYSTEMS IN KIAMBU COUNTY, KENYA

## 5.1 INTRODCUTION

The occurrence and spread of ESBLs-producing *Enterobacteriaceae* from livestock and humans has developed into an important community health concern worldwide (LIU *et al*., 2017). The widespread usage of antibiotic agents in disease managements in human, veterinary, and agriculture has ominously led to the selection and global dissemination of resistant genes in the *Enterobacteriaceae* family over the previous years (Sheikh *et al*., 2015). It has been of great concern owing to the irrational usage of expanded-spectrum antimicrobial agents in livestock feeds for disease prevention, prophylaxis and treatment. Potential under-dosage of these medicines in animals may lead to development and transmission of resistant strains of microbes in the nature, which constitutes a dangerous hazard to the community health. These practices in EU member states' countries have been regarded as illegal since they resulted in the transmission of ESBL-producers in modern times. According to Ojer-Usoz *et al*. (2017), this transmission has led to undesired treatment outcomes of serious nosocomial infections resulting to extended hospitalization and at times mortality. The spread and extensive use of β-lactams in poultry and livestock production has continuously rendered this class of antibiotics less effective against both livestock and Human infections. These ESBLs producing superbugs have been isolated from poultry production systems, humans and domestic farm animals, which increases their ability to share these genes through genetic elements namely; transposons, plasmids, and integrons. According to Dolejska *et al.* (2011), this elements may result to the conversion of non-pathogenic microbes into resistant pools in the normal bacterial ecosystem. According to Abrar *et al*. (2019), most enterics obtain ESBL genetic factors through transmutation or horizontal transfer of plasmids, which culminates into oxyimino-cephalosporin resistance, with majority of the ESBL-encoding genes being $bla_{\text{CTX-M}}$, $bla_{TEM}$, $bla_{SHV,}$ and $bla_{OXA}$. In reference to Bush and Jacoby (2020), Extended Spectrum Beta-Lactamases are categorized owing to their prime arrangements and substrate profiles into diverse families such as the TEM-, the SHV-, the OXA-, and the CTX-M -family. When this resistant genes occurs in enteric microbes this increases the susceptibility of these micro-organisms for extended resistance to several beta-lactam drugs. It is on this basis that the current research was undertaken to molecular characterize the existence of $^{bla}$TEM, $^{bla}$ CTX-M, $^{bla}$ OXA, $^{bla}$SHV, and *Qnrs* from fecal isolates of *E.coli*, *Shigella spp, Salmonella Spp,* and *Klebsiella spps* in commercial poultry production systems of Kiambu County, Kenya.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Area of Study

This laboratory-based cross-sectional research study was carried out in purposively selected six sub-counties of Kiambu County –Kenya namely; Ruiru, Juja, Gatundu North, Gatundu South, Thika, and Kikuyu being the major commercial poultry production centers. A total of 437samples that consisted of farmers' fecal samples (n=72) and cloacal swabs [(Broiler (n=80), Layer (n=160), and Improved Kienyeji (n=145) in commercial poultry production systems were collected in Kiambu County, Kenya, between November -2020 to February 2021. From the samples analyzed, a sum of 591 non-repetitive isolates of *Escherichia coli* (n=291), *Klebsiella spp* (n=83), *Salmonella spp* (n=108), and *Shigella spp* (n=110) were phenotypically identified.

### 5.2.2 Selection of bacterial isolates for ESBL screening

The identification of the recovered enteric bacterial isolates was performed through traditional bacteriological methods and biochemical tests as guided in the CLSI (2005) guidelines with an API 32E system (bioMerieux SA, Marcy l'Etoile, France) in reference to Wei and Charles (2005). The isolates were stored at −80°C in MicroBank cryovials containing 20% glycerol (Pro-Lab Diagnostics, Round Rock, TX, USA). *K. Pneumoniae* ATCC 700603, and *E. coli* ATCC 25922 were used as control strains in the study. The carriage of ESBL and QnrS gene was screened on seventy-eight ESBL-positive isolates which included 48 species of *Escherichia coli*, 7 species of *K.Pneumonia spp*, 28 species of *Salmonella spp*, and 5 species of *Shigella spp.* These bacteria genera were chosen on their phenotypic resistance profiles towards β-lactams and fluoroquinolone antimicrobial tested as described in previous related research according to Gundran *et al*., (2019).

### 5.2.3 DNA extraction through boiling methods

According to methods by Solberg *et al.* (2006), a distinct pure colony of every revived target bacterial isolate was suspended in 0.5ml of extraction buffer (100µl of 1 ml buffer Tris Borate and 2 µl of 0.5 EDTA). Thereafter, 400 µl buffer suspension known as reaction mixer in Eppendorf tube was boiled for 10 minutes at $100^0$C. Post-boiling process centrifugation was done at 14,000 rpm for five minutes at $4^0$C. This was followed with DNA-supernatant stored at $-20^0$C for later use as a DNA template for PCR amplification.

### 5.2.4 Molecular characterization of ESBLs resistant genes

Out of 591 bacterial isolates from the Poultry and human fecal samples obtained in Juja, Ruiru, Kikuyu, Thika, Gatundu North, and Gatundu South sub-counties of Kiambu County, only 78 isolates showed the presence of ESBL genes and their presence was detected using polymerase chain reaction technique. The Inheritable factor targeted were $bla_{TEM}$, $bla_{CTX\text{-}M}$, $bla_{OXA}$, $bla_{SHV}$, and Qnrs. The DNA extract of each sample had to use a template for uncovering of *bla* TEM, *bla* CTX-M, *bla* OXA, *bla* SHV, and Qnrs as described in the methods of Brody and Kern (2004). In every reaction, a total volume of 26µl of the reaction mixture per gene was mixed in the Eppendorf tube as follows; 12µl Qiagen master mix, 12µl DNA's free PCR water, 1µl forward primer, 1µl reverse primer, and finally 2µl DNA. The following PCR amplification settings were used; Initial denaturation at 95ºC for 5minutes, denaturation at 94ºC for 1minute, Annealing at 60ºC for 1minute, initial extension at 72ºC for 30 seconds, and final extension at 72ºC for 5minutes for 35 cycles using a GeneAmp® PCR system 9700 thermocyclers. However, the amplification condition varied slightly depending on the primer type and manufacturers' recommended annealing temperature. The PCR primer as indicated in Table 1 were used as per manufacturers' guidelines. Separation of PCR amplicons was done using 1.5 % agarose gel (Agarose Hi-Res standard) stained with Sybr green (Sigma-Aldrich) in 1X TBE buffer at 100 volts for 1 hour. Gel viewing was done use a UV Gelmax® imager and extended productive size compared against a 1kb plus DNA ladder (Invitrogen).

**Table 5.1: Primers sequences and annealing temperatures for the ESBL gene used in the study**

| AMR gene | Primer | Primer sequence | Expected product size | Reference |
|---|---|---|---|---|
| *bla* CTX-M | CTX-M - Forward | 5'-ATGTGCAGYACCAGTAARGTKATG GC -3' | 592 BP | (Gundran *et al*., 2019) |
| | CTX-M - Reverse | 5'-TGGGTRAARTARGTSACCAGAAYCA GC GG-3' | | |
| *bla* OXA | OXA-F | 5'-ATGAAAAACACAATACATATCAACT TC GC-3' | 280 bp | (Taneja *et al*., 2012). |
| | OXA-R | 5'-GTGTGTTTAGAATGGTGATCGCAT T-3' | | |
| *bla* TEM | TEM-F | 5'-ATGAGTATTCAACATTTC CG-3' | 867 bp | (Gootz *et al*., 2009). |
| | TEM-R | 5'-CCAATGCTTAATCAGTGA CG-3' | | |
| Qnrs | Qnrs-F | 5'-GCAAGTTCATTGAACAGG GT-3' | 428 bp | (Pons *et al*., 2014). |
| | Qnrs-R | 5'-TCTAAACCGTCGAGTTCGGCG-3' | | |
| *bla* SHV | SHV-A | 5'-ATGCGTTATWTTCGCCTGTGT-3' | 861 bp | (El-Shazly *et al*., 2015). |
| | SHV-B | 5'-TTAGCGTTGGCAGTGCTC G-3' | | |

**KEY: *bla*- B-lactam; CTX-M- Cefotaxime hydrolyzing capabilities; TEM-Temoneira; OXA-; SHV-; Qnrs- F- forward; R-reverse;**

### 5.2.5 Data analysis and presentation

The results from this section were present in form of percentages while the qualitative data was presented in form of gel electrophoreses images.

### 5.2.6 Research ethical approval

The research proposal was presented at the department of Public Health, Pharmacology and Toxicology (UoN) to seek permission to undertake the research and for accreditation, thereafter to the faculty of veterinary medicine board of postgraduate studies (UoN), and to NACOSTI (NACOSTI/P/21/8761) and from County Government of Kiambu Livestock, Fisheries and Veterinary services (KCG/ALF/ RESEARCH/VOL.1/49) for approval

## 5.3 RESULT

From the results it was found that majority of the ESBLs producing isolates were from improved Kienyeji (32%; n=25) poultry samples while humans/farmers fecal samples isolates had the least ESBL genes carriage at 14% (n=11). The ESBL genes detections was common in isolates from Juja sub-county (28%; n=22), followed by Kikuyu (22%;n=17), Gatundu south (20.5%; n=16) with the least from Ruiru (6.4%; n=5) and Gatundu North (3.8%; n=3), respectively. Our study showed that majority of the ESBLs genes were identified from both broiler (n=24) and improved Kienyeji (n=23) poultry systems with $^{bla}$OXA (n=20), and $^{bla}$TEM (n=16) genes being most present. Out of the 78 isolates with potential ESBLs production only 66 were positive for the tested genes as presented in table 5.2 below.

**Table 5.2: Distribution of the isolates and Extended Spectrum Beta-Lactamases genes in the commercial poultry production systems of Kiambu County**

| **Area of sample collections** | **Layers (27%; n=21)** | **Broilers (27%; n=21)** | **Improved Kienyeji (32%; n=25)** | **Humans/farmers (14%; n=11)** | **Total number** |
|---|---|---|---|---|---|
| Juja | 4 | 10 | 4 | 4 | **22** |
| Kikuyu | 3 | 1 | 13 | 1 | **17** |
| Ruiru | 1 | 2 | 1 | 1 | **5** |
| Thika | 7 | 4 | 2 | 1 | **14** |
| Gatundu North | 1 | 1 | - | 1 | **3** |
| Gatundu South | 5 | 3 | 5 | 3 | **16** |

| | | | | | |
|---|---|---|---|---|---|
| **Total no of isolates** | **21** | **21** | **25** | **11** | **78** |
| **ESBLs Genes detected** | | | | | |
| $^{bla}$CTX | 3 | 4 | 4 | 1 | **12** |
| $^{bla}$TEM | 3 | 6 | 5 | 2 | **16** |
| $^{bla}$Qnrs | 3 | 6 | 3 | 1 | **13** |
| $^{bla}$OXA | 4 | 6 | 9 | 1 | **20** |
| $^{bla}$SHV | 1 | 2 | 2 | 0 | **5** |
| **Total no. of genes** | **14** | **24** | **23** | **5** | **66** |

**Key:** % -percentage; n= sample size; *bla*-Beta lactamase

**Table 5.3** present results of Genomic distribution of the ESBL genes among four target *Enterobacteriaceae Spps* isolated from commercial poultry production systems. A total of 78 bacterial isolates that indicated ESBLs production were exposed to PCR test for the manifestation of $^{bla}$TEM ; $^{bla}$CTX-M ; $^{bla}$OXA; $^{bla}$SHV and QnrS genetic factor. The $^{bla}$CTX-M was identified in all the four enteric's bacteria isolates tested. Twenty one percent (21% )of isolates showed presence of $^{bla}$TEM gene with majority detected in *E.coli*. In both *E.coli* and *Salmonella SPPs* all the five genes were detected from these isolates. The $^{bla}$TEM, and QnrS genes were not detected from *Klebsiella* and *Shigella spps*. Additionally, the $^{bla}$OXA and $^{bla}$SHV were not detected in Klebsiella spps and Shigella spps respectively as indicated in table 5.3 below.

**Table 5.3: Percentage Genomic spread of the ESBL genes among four target Enterobacteriaceae Spps isolated from commercial poultry production systems.**

| Organism | total isolates screened | AMR genes n (%) | | | | |
|---|---|---|---|---|---|---|
| | | **blaTEM** | **blaCTX-M** | **blaOXA** | **blaSHV** | **QnrS** |
| *E. coli* | 42 | 11(26) | 8(19) | 12(29) | 3(7) | 8(19) |
| *Klebsiella species* | 7 | 0 | 1(14) | 0 | 1(14) | 0 |
| *Salmonella species* | 24 | 5(21) | 2(8) | 7(29) | 1(4) | 5(21) |
| *Shigella species* | 5 | 0 | 1(20) | 1(20) | 0 | 0 |
| *All* | 78 | 16(21) | 12(15) | 20(26) | 5(6) | 13(17) |

**Key: *bla*-Beta-lactamases; %- percentage; AMR-antimicrobial resistance; Qnrs-quinolones resistance genes; CTX-M- Cefotaxime-Munich;**

**Fig 5.1** presents the percentage distribution of ESBLs and Qnrs genes among the 78 isolates. Twenty six percent of the isolates had $^{bla}$OXA, 17% (Qnrs), 16% ($^{bla}$TEM,) 12% ($^{bla}$CTX-M), and 5% ($^{bla}$SHV) respectively as shown in fig 5.1 below.

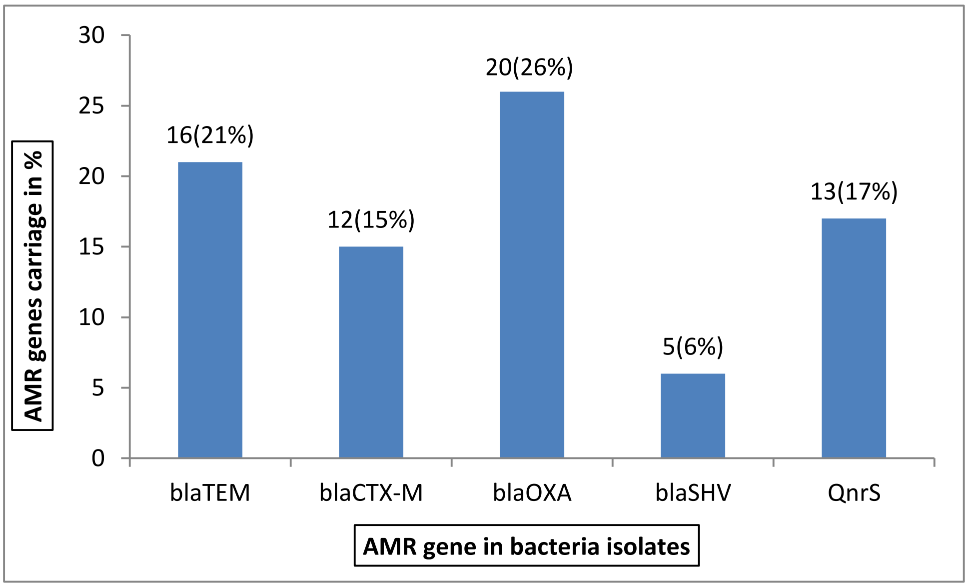


**Fig 5.1: Percentage distribution of the AMR genes carriage among the isolates**

**Table 5.4** presents the results of the co-carriage of the AMR genes among the four selected enteric's in this study. Most of the AMR gene co-carriage was witnessed in both *E.coli* and *Salmonella spps* as follows $^{bla}$TEM+$^{bla}$OXA (n=4); $^{bla}$TEM+QnrS; $^{bla}$TEM+$^{bla}$OXA+Qnrs . Four percent (4%) showed presence of $^{bla}$TEM+OXA co-carriage among *E.coli* and *Salmonella spps.* $^{bla}$TEM+CTX-M+OXA and $^{bla}$OXA+QnrS AMR gene co-carriage was on observed in *Salmonella spps.* Also $^{bla}$TEM+CTX-M, CTX-M+SHV, and TEM+SHV+QnrS AMR gene carriage were only noticed in *E.coli* isolates. Both *Shigella* and *Klebsiella spps* showed no AMR gene co-carriage as indicated in table 5.4 below.

**Table 5.4: Antimicrobial resistance gene co-carriage among the isolates**

| Organism | Total isolates screened | AMR genes co-carriage n(%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **TEM+CTX-M+OXA** | **TEM+OXA** | **TEM+QnrS** | **TEM+OXA+QnrS** | **TEM+CTX-M** | **CTX-M+SHV** | **TEM+SHV+QnrS** | **OXA+QnrS** |
| *E. coli* | 42 | 0 | 2(5) | 2(5) | 2(5) | 1(2) | 2(5) | 1(2) | 0 |
| *Klebsiella species* | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Salmonella species* | 24 | 1(4) | 2(8) | 1(4) | 1(4) | 0 | 0 | 0 | 1(4) |
| *Shigella species* | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| All | 78 | 1(1) | 4(5) | 3(4) | 3(4) | 1(1) | 2(3) | 1(1) | 1(1) |

**Key: *bla*-Beta-lactamases; %- percentage, CTX-M- cefotaxime hydrolyzing capabilities; TEM-Temoneira; OXA-; SHV-; Qnrs-**

**Fig 5.2** presents results showing the amplicons for the positive isolates with $^{bla}$TEM genes among the isolates. With 11 *E.coli* and 5 *Salmonella spps* isolates testing positive for the $^{bla}$TEM gene.

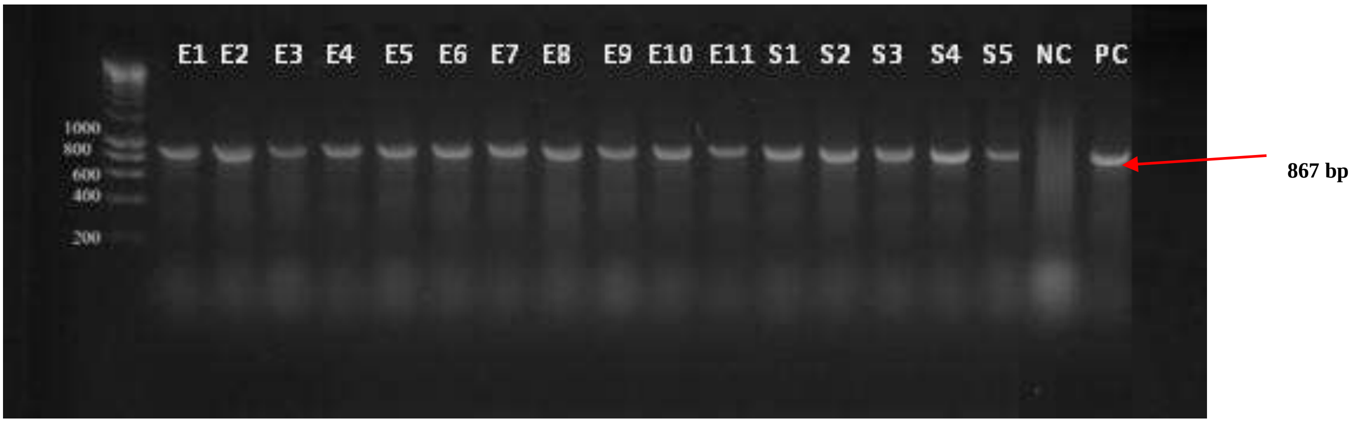



**Fig 5.2: Amplicons for the positive isolates with blaTEM genes among the isolates. Key: M-Molecular weight markers (100-5000 bp); E - *Escherichia coli* isolate; S - Salmonella *spp* isolate; Shig – *Shigella* species; Negative control (NC); Positive control (PC)**

**Fig 5.3** presents results showing the amplicons on behalf of the positive isolates with $^{bla}$CTX-M genes among the isolates. With 8 *E.coli,* 2 *Salmonella spps,* 1 Shigella and 1 Klebsiella isolates testing positive for the $^{bla}$CTX-M gene.

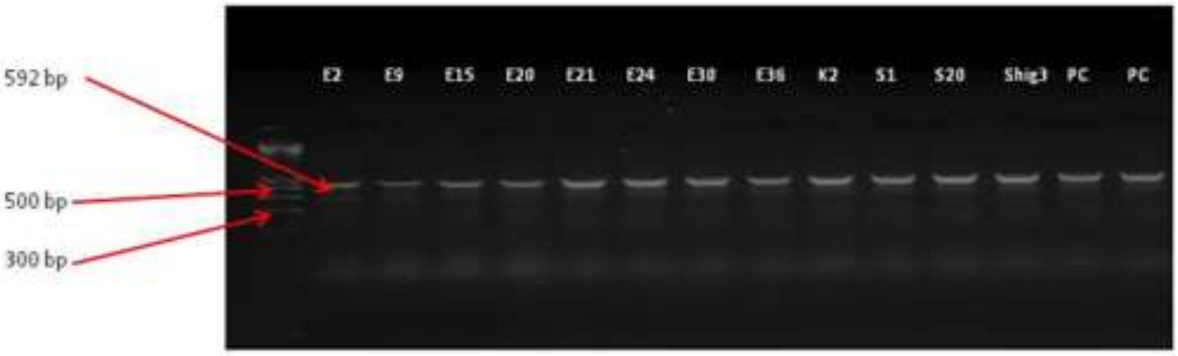
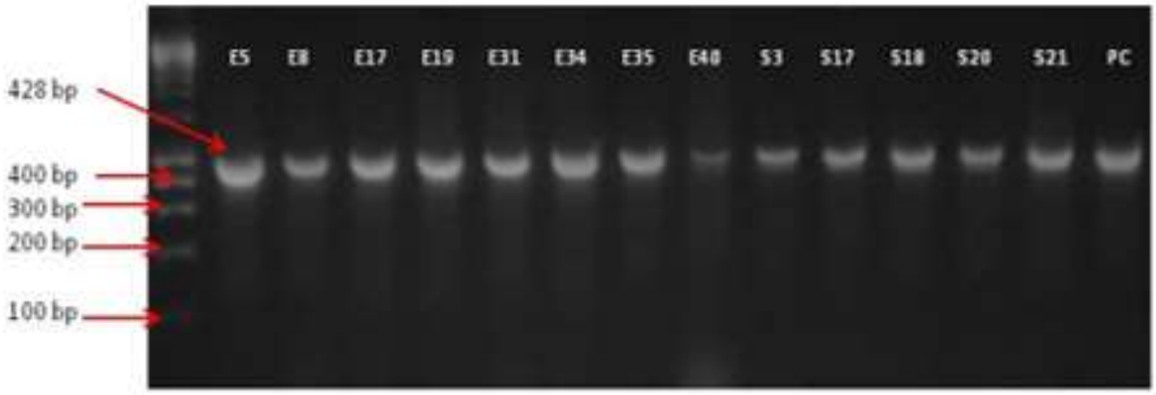



**Fig 5.3: LM-DNA Ladder (100bp for gene size determination), for electrophoretic reaction with positive isolates for blaCTX-M genes among the isolates. Key: M**-Molecular weight markers (100-5000 bp; DNA ladder); **E -** *Escherichia coli* isolate; **S -** Salmonella *spp* isolate; **Shig** – *Shigella* species; Negative control (**NC**); Positive control (**PC**)

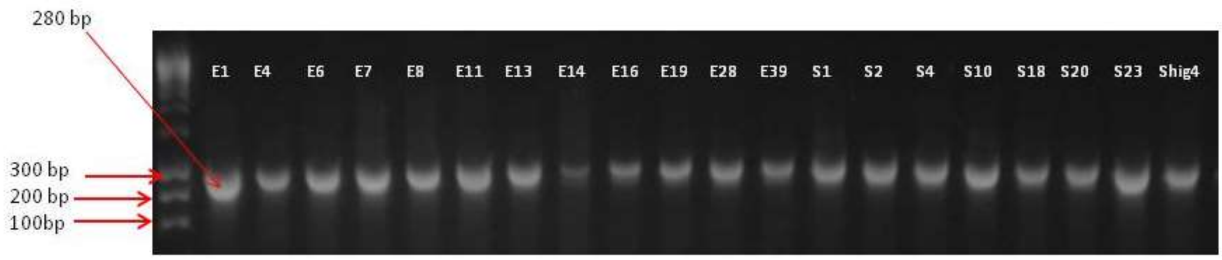
**Fig 5.4** presents results showing the electrophoretic reactions for the positive isolates with Qnrs genes. We found that 8 *E.coli,* and 5 *Salmonella spps* isolates testing positive for the Qnrs gene.




**Key: M**-Molecular weight markers (gene ruler 100-5000 bp DNA ladder)**; E -** Escherichia *coli* isolate; **S -** *Salmonella spp* isolates; **PC-** Positive control

**Fig 5.4: LM-DNA Ladder, for electrophoretic reaction with positive isolates for Qnrs genes among the isolates**

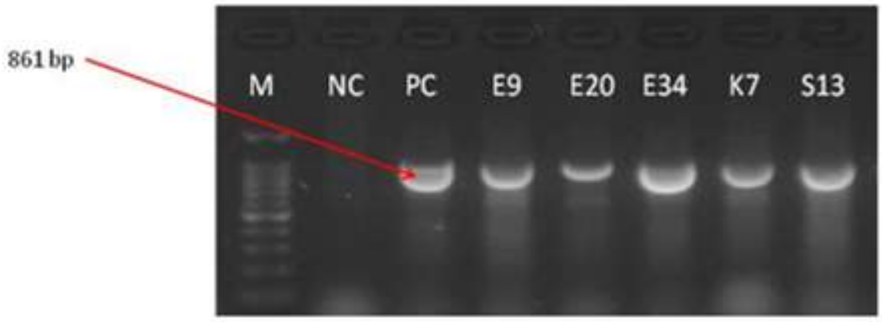
**Fig 5.5** presents results indicating the electrophoretic reactions used for the positive isolates with ***bla*****OXA** genes. We found that 12 *E.coli*, 7 *Salmonella spps* and 1Shigella spps isolates testing positive for the blaOXA gene.




**Key: M**-Molecular weight markers (100-5000 bp; DNA ladder)**; E -** *Escherichia coli* isolate; **S -**Salmonella *spp* isolates; **Shig -** *Shigella spp*

**Fig 5.5: LM-DNA Ladder, for electrophoretic reaction with positive isolates for blaOXA genes among the isolates**

**Fig 5.6** presents results of the electrophoretic reactions for the positive isolates with ***bla*SHV** genes. We found that 3 *E.coli,* 1 *Salmonella spp* and 1 *Klebsiella spp* isolates tested positive for the blaSHV gene.




**Key: M**-Molecular weight markers (100-5000 bp; DNA ladder); **E -** *Escherichia coli* isolate; **S -**Salmonella *spp* isolates; ***K****-Klebsiella spp*; Negative control (**NC**); Positive control(**PC**)

**Fig 5.6: LM-DNA Ladder, for electrophoretic reaction with positive isolates for blaSHV genes among the isolates**

### 5.4 DISCUSSION OF RESEARCH FINDINGS

Research-based knowledge and conceptualization of antibiotic resistance patterns among bacterial isolates from livestock and humans are paramount for endorsements in the experimental antibiotic management of infections. The present research describes the molecular detection of ESBLs producers among *E. coli*, *Shigella spp*, *Salmonella spp.* and *K. pneumonia spp* isolates from commercial poultry production systems of Kiambu County, Kenya. Data from our recent related work recorded high antimicrobial resistances among the present bacterial isolates especially towards Sulfamethoxazole (79%), Trimethoprim (71%), Tetracyclines (59%), Ampicillin (49%), and Amoxicillin/Clavunallic acid (39%). Our results were consistent with findings of previous studies that have also recorded high AMR profiles towards Sulfamethoxazole, and Trimethoprim among *E.coli* isolates from poultry samples as per studies carried out by Ahmed *et al*., 2013; Ahmed OB *et al*., 2013; Kariuki *et al*., 2006;

Ombui *et al*., 2000; Kikuvi *et al.*, 2007b. These findings could be a reflection of AMR emergence as result of widespread empirical use of antimicrobial agents with similar modes of actions (active molecules) to human drugs in the management of poultry diseases prophylaxis and as growth promoters (WHO, 2014).

Over the past decades, ESBL generating entero-pathogens, especially *E. coli*, *Salmonella*, *Shigella*, and K. *pneumonia* have occurred as serious challenge to both community, and hospital-acquired infections, globally. Manifestation of these ESBLs amongst livestock and human isolates significantly differs globally and locally and is promptly varying with time (Ibrahim AL-Subol and Nihad Youssef, 2015; Tofteland *et al*., 2007). According to this study, ESBL phenotypes were present in 78 isolates (13.2%) out of 591 isolates. The study demonstrates that majority of the ESBLs producing isolates were gotten from improved Kienyeji (n=25) poultry production system with the least number of isolates gotten from humans/farmers fecal samples (n=11). We further found that most of the isolates came from the Juja sub-county (n=22). We further found that majority of the ESBLs genes were identified from both broiler (n=24) and improved Kienyeji (n=23) poultry systems with $^{bla}$OXA (n=20), and $^{bla}$TEM (n=16) genes being most present. According to Frère *et al*. (1992), *Enterobacteraceae* species exert their antibiotic resistance mechanisms through plasmid-mediated production of extended-spectrum β-lactamases (ESBLs) which disable β-lactam-antibiotics including Cephalosporins and monobactams by hydrolyzing their β-lactam ring.

In this study, out of 78 isolates which showed potential ESBLs production, only 66 were positive for the tested genes. Additionally, there was high occurrence of ESBL generation by *E. coli* (54%) and *K. pneumonia* (31%) isolates, in commercial poultry production systems of Kiambu County. Similarly, this has also been found in Kenya by Ajak Deng *et al*. (2017) who found 31.4 % *E.coli*; Mutsami *et al*., 2011; Njagi 2003), Syria (Ibrahim AL-Subol and Nihad Youssef (2015), Sudan (Ahmed *et al*., 2013); Jordan (Batarseh *et al.*, 2013), India (Goyal *et al*., 2009) and Nihad Youssef (2015). Moreover, in countries like Nigeria (Alo *et al*., 2012) and China (Wang *et al*., 2012) great occurrence of ESBLs producers have been reported. This high occurrence of ESBLs producers in our study could be related with extensive use of third-generation cephalosporin's in poultry farming systems in Kenya.

The present study, found that $^{bla}$OXA (26%) and $^{bla}$TEM (21%) were the most identified, this agrees with a study carried in Brazil and disagrees with the previous studies by Naseer and Sundsfjord (2011) who found $^{bla}$CTX to be the most predominant. In year 2000, SHV and TEM genes persistently remained to be the dominant variants of ESBL; however, CTX-M enzymes have occupied their position over the last decades. Maybe, this could ascertained to the fact that ESBL genes are situated on a plasmid that can be transported from one microbe to another, slightly easily, and can integrate hereditary material coding for other resistance genes. We also noted that both *E.coli* and *Salmonella* isolates were resilient to fluoroquinolones as established by the presence of the Qnrs gene. This may well be connected with increased irrational use of fluoroquinolones in commercial poultry production, as Zahraei and Farashi (2006) witnessed in their study.

Furthermore, we found a high co-carriage of AMR resistance genetic factors among *E.coli* and *Salmonella* isolates with $^{bla}$TEM+OXA, $^{bla}$TEM+QnrS, and $^{bla}$TEM+OXA+QnrS being the most occurring. This can be elucidated by the point that these genetic markers are situated on plasmids and can be carried from one bacteria to another and hence coding resistance to other antimicrobial classes. Additional, enterobacterials found in animals and the human intestinal tract have been important reservoirs for resistance genetic factors resulting to spreading of ESBL-producers inside the community especially if ESBL genes are coded by resistance pathogenic bacteria leading to hospital and community infections. In this study, our genomic assessment on 78 established ESBL phenotype strains by PCR exposed that 85% (n=66) of the isolates were positive for at least one of the studied genes. According to XI *et al.* (2009), antimicrobial-resistant microbes and resilient inheritable factors are deliberated to be ecological pollutants and accountable for a persistent community health crisis all over the world. The well-being problems associated with antimicrobial-resistant microbes are more about constrained therapeutic remedies in most emerging countries that are deprived access to good quality treatment, thus, emphasizing infection as a significant source of morbidity and mortality in the society.

In conclusion, the study results advocate for the significance of ESBL-generating *Escherichia coli.,* Salmonella *spp., Shigella spp.,* and *K. pneumonia spp.*, as common causes of infectious diseases among the commercial poultry production systems of Kiambu County, Kenya. Furthermore, majority of multidrug-resilient micro-organisms necessitate to be taken into account especially when selecting therapeutic agents. Simultaneously, the unceasing local monitoring of resistance pathways is essential to handpick an empirical antimicrobial therapy adequately. Supplementary research designed at disentangling the molecular mechanisms of resistance will better understand the epidemiology connected with ESBL-producing species of *Enterobacteriaceae.*

# CHAPTER 6: OVERALL DISCUSSION, CONCLUSIONS, STUDY LIMITATIONS AND RECOMMENDATIONS

## 6.1 Overall discussion

Continued use of antimicrobial drugs in poultry production is essential in disease treatment, prevention, and growth-promoting, but its use must be accepted as a responsibility rather than a right when trying to improve poultry health (Rose *et al*., 2009). This will help to minimize the potential risk and hazards due to poor drug usage of antibiotics in livestock production. Therefore, the usage of veterinary antibiotic drugs in poultry systems needs proper control through legislation bodies with the broad goals to preserve animal health, improve animal production, and protect the public (Cardona & Kuney, 2002). Resistance to antimicrobial agents is significantly growing in almost all populations. This increase can be associated due to a lack of adequate knowledge and poor practice on the prudence use of antibiotics in the management of poultry diseases, and inappropriate attitudes towards their usage for prophylaxis and as growth promoters This greatly contributes to the occurrence of Multi-drug resilient microbes and offload of residues in poultry products, hence affecting the international market trade. This being the first cross-sectional research to be undertaken in Kiambu County on the level of awareness, attitudes, and practices on antibiotic use and their resistance patterns in commercial poultry production will help to unravel potential mitigation and measures in policy making to help curb antimicrobial resistance across the country.

The current study demonstrated that 2/3rd of the farmers were Females (68%), married (91%), aged above 45 years, and had attained secondary education level and they preferred keeping layer birds. This agrees according to FAO (2009), that most farmers prefer keeping layers on a medium and large scale under intensive management. This is attributed to the great demand for eggs in urban areas and the high profit margin associated with layers than broilers. This could further be attributed to reason that most women are left in the homestead taking care of the livestock and being that majority are above 45yrs age, which could be as a result of the retirement plan scheme as poultry keeping does not require a lot of capital and space to invest in. This agrees with research undertaken by Calvin *et al*. (2020) in Tanzania. The study demonstrated that Kikuyu sub-county was leading in commercial layer production systems seconded by Ruiru with Gatundu south having the least of poultry production activity. This resonate with a research undertaken by Okello *et al.*, 2015; Mercy *et al*., 2014; ROK, 2010. It was further noted that the majority of the farmers are small-scale farmers (101-500 birds) which agrees with the study carried out by Nyaga (2007) which found that the majority of the farmers in Kiambu undertake small-scale farming. This could be as a result of little capital to keep large-scale production. The study found that the majority of the farmers prefer to keep feed and income records compared to antibiotic records. This could be explained by the need of farmers to maximize profit and lessen expenditure as antibiotics are sometimes little concern due to ignorance and not knowing the great danger they can expose to both animals and humans.

In the study, Socio-demographic characteristics did not significantly ($p>.05$) influence the ability to access antimicrobial agents, however, nearness to veterinary services significantly ($P<.05$) contributed to the usage of antibiotic agents in the farms. Concurrently, the area/ region of residence was statistically considerably ($P<0.05$) linked with the challenges that the farmers were facing from antimicrobial use, with Kikuyu farmers less affected due to their extensive history of commercial poultry production. This agrees with a research that was conducted by Mercy *et al*. (2014) who found that the majority of Kiambu farmers came from the Kikuyu Sub-County.

From the present research, most of the farmers acquire this antibiotic through prescription by veterinary officers, which disagrees with a investigation carried out by Lindonne Glasgow *et al.* (2019), who found that the majority of antibiotics users are self-prescribers. Furthermore, most of the farmers claim that they follow the advice they get from the veterinary officers on the use of antimicrobial agents which disagrees with an investigation carried out by Calvin Sindato *et al*. (2020) who claimed most of the antibiotics users do not follow the guidelines of the prescriber. We also found that the majority of the farmers walk medium distances (3-4km) to access this antimicrobial agent. There is a need for close access to veterinary services which is very crucial to commercial poultry farmers to ensure they get the right advice and services on time and this may help to stem down the irresponsible use of antimicrobial agents (Aniroot Nuangmek *et al*. 2016). It was further noted that the majority of cases of sick birds are noted and reported by the farmers themselves who lacked credible skills, instead of engaging well-trained veterinary extension field officers. This could be attributed due to the lack of enough veterinarians and consultative services to poultry farmers who mostly own small and medium-scale farms.  This could be one of the ways of promoting irrational use of antimicrobial agents by untrained persons. Additional, the study identified that the majority of the farmers get their antimicrobial agents from drug shops through personal experience, this agrees with a research undertaken in Thailand by Aniroot Nuangmek *et al*. (2016) who found that most farmers understood the need for professional antimicrobial prescription but due to ignorance they disregarded this practice. This further exonerates that most commercial poultry farmers have inaccurate attitudes and practices that exhibit inappropriate behavior antimicrobial use.

Furthermore, the majority of the farmers still find most of the antibiotics to be expensive for them to afford despite them having inadequate knowledge on the proposed use of antimicrobial agents. The commercial poultry farmers further claimed that antibiotics have inserts that have unfamiliar language and they do not have proper measuring containers that would culminate with either overdose or under a dose of drugs. This might lead to a lack of effectiveness and efficiency of the antibiotics as was claimed by the farmers. Proper and adequate knowledge is very critical to the farmers to help ensure prudence and usage of drugs hence preventing imminent occurrence of drug resistance. This agrees with a study undertaken by Casal *et al*. (2007) who suggested that there is a need for efforts to increase farmer's awareness of biosecurity as a major input in stemming antimicrobial resistance in livestock production. Lack of enough veterinary extension officers and consultative services towards the use and preparation of antimicrobial agents among poultry farmers was also immensely experienced by the farmers. This could contribute greatly to the improper use of these drugs among the farmers in Kiambu County and across Kenya. In reference to a study carried out by Aniroot, Nuangmek *et al*. (2016), he noted that adequately trained veterinarian officers are crucial to stimulating correct drug use in farms, due to their prevailing good characters amongst farmers, and are perfectly well-matched to perform as a waterway for enlightening farmers about drug resistance and the right presentation of antibiotic agents in livestock disease management.

Similarly, antimicrobial use is a common practice among commercial poultry farmers in Kiambu County. The types/class of antimicrobial agents that we found to be commonly used and preferred by commercial poultry farmers of Kiambu County were Tetracycline, enrofloxacin, sulfadimidine,  and amprolium compounds respectively, with Oxytetracycline and Amoxi sub-types of these drugs being highly mentioned. This could be associated with high cases of bacterial and protozoal infestation in poultry production. This same class of drugs was mentioned to be having used previously in the last year and currently in the farms. oxytetracycline and sulfadimidine are broad-spectrum antibacterial drugs and amprolium is used to treat coccidiosis making them be on high demand in commercial poultry production. This creates an increased dependence on antibiotics as a substitute for good management practices such as good animal husbandry and housing hygiene (Rice and Straw, 1992). According to a study carried out in Ethiopia by Gebeyehu , Bantie , and Azage,  (2015), and Uganda (Ocan *et al*. 2014), they found that continuous use of antimicrobial agents is a key determinant of antibiotic resistance, particularly when used incorrectly. Therefore, it is important to stipulate that these antimicrobial agents are at elevated threat of developing bacteria resistance and high multidrug resistance shortly (Muhie, 2019). According to Austin, Kristinsson and Anderson, (1999), the bulk of drug used is a key selection pressure motivating changes in the frequency of antimicrobial resistance inside the society.

According to the results, the majority of the farmers proposed the need for adequate training on prudence, and the use of antimicrobial agents, the impacts of not observing the withdraw periods, and the effects of antibiotics residues on human health and the marketability of their products to the world market. These acts of seminars and training to farmers would help to halt the escalating challenges emanating from antimicrobial resistance. Additionally, a need for more adequately trained veterinary extension officers to provide consultative services to these poultry farmers on the appropriate use of antimicrobial drugs and management of poultry diseases. This agrees with a study undertaken by Aniroot Nuangmek *et al.* (2016), who proposed the need for a thorough and continuous effort to train farmers on the proper antimicrobial agent used in poultry production. He further states that the availability of well-trained veterinarians to the poultry farmers is a key intervention towards knowledge dissemination on prudence and application of antimicrobial agents. Furthermore, the majority of farmers are small-scale holders, there is a need for the County government and the state government to provide funds to upscale their production systems which will result in a high source of income generation. There is also a need for the government to control the prices of both antibiotics and feed, which have become a big challenge to the commercial poultry production system which agrees with an investigation that was carried out by Ling *et al.* (2011) who stated that economic cost and benefits to the farmers are the first reasons making a judgment on antimicrobial use since they are much worried about financial implication than prudence use of this drugs. In 2004, Tollefson in his study suggested that there is a need for high-quality regulatory government agencies in developing countries like Kenya to help in the management of emerging threats such as AMR. According to Lindonne Glasgow *et al*. (2019), emerging nations are mostly challenged in allotting sufficient funds and introducing guidelines to mitigate gaps in knowledge, awareness, and practices in animal food production enterprise. Antimicrobial use in animal production stands unfettered, resulting to incorrect use of drugs and a global escalation in antibiotic resistance, hence the need for the government of the day to take caution and influence policies to stem down AMR. The current study underscores the importance of understanding the reasons that necessitates antibiotic misuse in commercial poultry production systems in Kiambu County, Kenya, and the study outcomes show that there is a need for collaborative efforts amongst the Ministry of Agriculture and Livestock Development, with the County government, and the Ministry of Health to ascertain factors that promote antimicrobial misuse in the livestock industry and human medicine by developing and distributing procedures to monitor the use of antimicrobial agents and upgrade AMR surveillance in humans and livestock.

Overall in the present study most of the isolates were *E.coli* (48.9 %), followed by *Shigella spp* (18.8%), consequently. This agrees with the study by Buxton and Frazer (1977), and Bebora (1979) who found *E.coli* was the most common bacteria in animal and human fecal samples. This is further supported by a study that was carried by Njagi (2003) who found E.coli prevalence of 40.2%. In this study, we demonstrate that there is raised occurrence of single and multi-drug resistance (MDR) among the 13 antibiotics tested across the four enteric's. This resistance was highest among, Sulfamethoxazole (79%), Trimethoprim (71%), Tetracyclines (59%), Ampicillin (49%), and Amoxicillin/Clavunallic acid (39%). This agrees with a study carried by Deng (2017), and Nyabudi *et al*. (2017), who found these antibiotics to be most commonly used in poultry production and to have developed resistance. Similarly, this is expressed by other research such as Allorechtova *et al*. (2012); Gakuya *et al*. 2007; Ombui *et al.* 2000. This calls for joint effort for a fight against MDR and X-DR by advocating prudent use of the antimicrobial agent in animal production. However, the four enteric's were also highly susceptible to the following antibiotic; Cefuroxime (94%), Gentamicin (93%), Ceftriaxone (91%), Cefepime (89%), Cefotaxime (85%), Ceftazidime (84%), Chloramphenicol (77%), followed by Amoxicillin/Clavulanic acid and Ciprofloxacin (56%) respectively.

The study found that *Klebsiella spp, Shigella spp*, and *Salmonella Spp* from Gatundu North were all resistant to Cefotaxime, Ceftriaxone, Gentamicin (GEN), Cefuroxime (CXM), and Cefepime (FEP), consecutively. This agrees with a study carried by Zahraei and Farashi (2006) who found an association between emergency of fluoroquinolones resistance zoonotic pathogens with subsequent use of approved veterinary Antimicrobial agents in livestock production. This indicates there is extensive irrational use of antimicrobial drugs in commercial poultry production farming in Kenya. Overuse and misuse of antimicrobial agents in commercial poultry farming systems would lead to an emerging concern on its influence to high resistance to antimicrobial agents by pathogenic microbes as stipulated by Amy *et al.* (2007). This can be a potential source of resilient inheritable factor which can be transmitted to human bacteria via conjugation. The majority of the results of the isolates across the four enteric species of interest were from Layers and Improved Kienyeji birds. Further to this, most of *E.coli* isolates were sourced from layer birds followed by Improved Kienyeji, Broiler birds, and Broiler farmers respectively. This is because most of the farmers in these areas preferred keeping layers of birds for eggs and for improved Kienyeji to supply both meat and eggs. Also, the majority of the farmers were hesitant and did not consent to provide a stool sample for culture. We further, noted that most of Klebsiella spp. isolates emanated from Improved Kienyeji, and Broiler birds. For the Salmonella Spp majority of the isolates were from Layer birds and Improved Kienyeji. For the Shigella spp. isolates most were from layers) and Improved Kienyeji birds. Infection in the bloodstream, surgical sites, urinary tract infections (UTIs), respiratory tract infections, and also frequent infections such as cancer and diabetes can be linked to *Enterobacteraceae* pathogens. Due to their easy way of spread MDR *Enterobacteriaceae* from contaminated animal food sources and contact surfaces makes them a major public health distress (McEwen and Fedorka Cray, 2002).

Therefore, the current study reinforces the need for unrelenting surveillance of antibiotic resistance by Enterobacteriaceae spps to project the frequency of infection, the risk of infection, cost, and promising treatment options existing for these multidrug-resistant pathogens. Additionally, it is imperative to ascertain the causes and the sources of contaminations coined to the patterns of spread of this drug-resistant *Enterobacteriaceae* in Kenya for a suitable understanding of the mechanism involved and techniques to prevent outbreaks in the community. It is also imperative, to understand the dynamics underlying drugs resistant *Enterobacteriaceae* isolated from Commercial poultry production systems and Human fecal materials to have an impression of the pattern of the distribution of these drug-resistant bacteria's.

Evidence-based Awareness on antibiotic resistance patterns amongst bacterial isolates from livestock and humans is paramount for empirical-based recommendations in the experimental antibiotic management of diseases. The present study ascertain the molecular detection of ESBLs producers among *Escherichia coli, Shigella spp, Salmonella spp.* and *K. pneumonia* spp isolates, from commercial poultry production systems of Kiambu County, Kenya. From our previous study (James *et al*., 2021b) we found that there was a high incidence of multiple resistance amongst isolates especially to Sulfamethoxazole (79%), Trimethoprim (71%), Tetracyclines (59%), Ampicillin (49%), and Amoxicillin/Clavunallic acid (39%) gotten from commercial poultry production systems of Kiambu County with E.coli being the most prevalent organism identified. Our results were consistent as per the following previous studies (Ahmed *et al*., 2013; Kariuki *et al*., 2006; Ombui *et al.*, 2000; Kikuvi *et al*., 2007b). These results could be supported owing to the empirical widespread usage of these drugs, being that they are widely available and economical to administer in the management of poultry diseases for prophylaxis and as growth promoters.

Over the twenty years, ESBL producing entero-pathogens, especially *E. coli spp.*, *Salmonella spp.*, *Shigella spp.*, and *K. pneumonia spp*., have occurred as severe pathogens, both in society, and hospital-acquired ailments, globally. The manifestation of these ESBLs amongst livestock and human isolates greatly varies worldwide and geologically and is promptly varying over time (Ibrahim and Nihad, 2015; Tofteland *et al*., 2007). In our current study, ESBL producers were found to be positive in 78 isolates (13.2%) out of 591 isolates. We have demonstrated that most of the ESBLs producers were gotten from improved Kienyeji (n=25) poultry production system with the least number of isolates gotten from humans/farmers fecal samples (n=11). We further found that most of the isolates came from the Juja sub-county (n=22). We further found that majority of the ESBLs genes were identified from both broiler (n=24) and improved Kienyeji (n=23) poultry systems with blaOXA (n=20), and blaTEM (n=16) genes being most present. According to Frère *et al*. (1992), antimicrobial resistance mechanisms in *Enterobacteriaceae* is founded on plasmid-mediated production of extended-spectrum β-lactamases (ESBLs) which inactivate β-lactam-antibiotics including Cephalosporins and monobactams by hydrolyzing their β-lactam ring. Bush and Jacoby (2010), further explains that ESBLs can be classified according to their primary sequences and substrate profiles into different families such as the TEM-, the SHV-, the OXA-, and the CTX-M –family.

In the current study, out of 78 isolates that showed potential ESBLs production, only 66 were positive for the tested genes. Additionally, there was a high occurrence of ESBL production by E. coli (54%) and K. pneumonia (31%) isolates, in commercial poultry production systems of Kiambu County. Similarly, this has also been found in Kenya (Ajak Deng *et al*., 2017; Mutsami *et al.*, 2011; Njagi 2003), Syria (Ibrahim AL-Subol and Nihad Youssef (2015), Sudan (Ahmed *et al*., 2013); Jordan (Batarseh *et al*., 2013), India (Goyal *et al*., 2009) and Nihad Youssef (2015). Moreover, in countries like Nigeria (Alo *et al*., 2012) and China (Wang *et al*., 2012) high prevalence of ESBLs producers has been described. This high prevalence of ESBLs producers in our study could be connected with the widespread use of third-generation cephalosporins in poultry production systems in Kenya.

In this study, blaOXA (26%) and blaTEM (21%) we the most identified, this disagrees with a previous studies by Naseer and Sundsfjord (2011) in Brazil who found blaCTX to be the most predominant. Additionally, blaSHV and blaTEM remained the predominant variants of ESBL in year 2000; however, CTX-M enzymes have taken over their place in past decades. Maybe, this could be linked to the hypothesis that ESBL genes are located on a plasmid that can be transferred from one organism to another, rather easily, and can be incorporate in the genetic material coding for other resistance genes. From our study, we also noted both *E.coli* and *Salmonella* isolates have developed resistant to fluoroquinolones as confirmed by the presence of the Qnrs gene. This can be linked with due to the increased irrational use of fluoroquinolones in commercial poultry production as witnessed by Zahraei and Farashi (2006).

Furthermore, the study found a high co-carriage of AMR resilient genetic factors among *E.coli* and *Salmonella* isolates with blaTEM+OXA, blaTEM+QnrS, and blaTEM+OXA+QnrS being the most occurring. This is due to the fact that these inheritable traits are situated on plasmids and therefore can be transmitted from one micro-organism to another and hence coding resistance to other antimicrobial classes. This agrees with a study carried out by White, McIver, and Rawlinson (2001), who established integron and cassettes in *Enterobacteriaceae* species. A previous study by Spanu *et al.*, (2002) expressed that majority of microbes that carry bla genes also carry integron which is commonly harbored in mobile genetic elements such plasmids, therefore, suggesting spread and attainment in microbial Populations. This genomic study on 78 confirmed ESBL producing phenotype strains by PCR revealed that 85% (n=66) of the isolates were positive for at least one of the studied genes of resistances. This indicates, chances of occurrence of multidrug resistance strains due to strong selection pressure expressed by Enterobacteriaceae in the environment for their survivability against current therapeutic options.

## 6.2 CONCLUSIONS

1. It was concluded that both fecal and stool materials from commercial poultry and humans can be a reservoir of multi-drug resistance enterics. This is a potential route of spreading out resistance genes amongst livestock and Humans, which pose a great danger to public health of Kiambu County residence.
2. There is still low level of knowledge on importance of prudence use of antimicrobial agents and observation of withdraw period in commercial poultry production

3. There is also need to strengthen antimicrobial surveillance and monitoring strategies at national and county government level in Kenya.
4. There is need to advocate for the medical significance of ESBL-generating *E. coli, Salmonella, Shigella,* and *K. pneumoniae*, as common causes of infectious diseases among the commercial poultry production systems of Kiambu County Kenya.
5. More studies, intended at unraveling the molecular mechanisms of resistance are necessary for this will offer a better understanding of the epidemiology associated with ESBL-producing microbes of *Enterobacteriaceae* in commercial poultry production systems in Kenya.

## 6.3 RECOMMENDATIONS OF THE STUDY

- There is need for continuous education awareness campaigns to commercial poultry farmers towards rational use of antibiotic drugs in Kiambu County, through the County Director of Livestock, fisheries and Veterinary services.
- Strict government regulatory framework measures on antibiotic agents use need to be put in place for implementation with continuous monitoring of their application.
- Furthermore, there is need for an alternating schedule or Zoning for the antibiotics classes used in each region in order to help halt multi-drug resistance occurrence.
- In our study $bla_{TEM}$, $bla_{OXA}$, $bla_{SHV}$ and $bla_{CTX-}$ (Beta-lactams) and QNRs (Flouroquinolones) genes we detected. Therefore, were also not able to determine the mechanisms of resistance to other classes of antimicrobial agents such as Macrolides, and Antimetabolites such Trimethoprim and Sulfamethoxazole. Hence need, for more extensive study to get the full picture of antimicrobial resistance patterns to other classes of drugs.
- There is need for a establishment of a comprehensive surveillance systems to monitor antimicrobial resistance in order to reduce the selective pressure downstream on humans.
- Further and future studies need to use high resolution methods such as whole genome sequencing and SNP typing which can give a better understanding of resistance gene content and bacteria evolution in the environmental compartments.
- Further studies need to be carried to ascertain whether if there is any relationship that exist between plasmid carried in *E.coli*, *Salmonella spp*, *Shigella spp* and *Kebsiella spps* from Poultry, Humans and environment.

- Lastly, the alternative herbal remedies mentioned by the farmers need to be testing for efficacy among the isolates that expressed multi-drug resistance to ascertain their efficacy.

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## APPENDICES

### Appendix 1: NACOSTI Research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 317283

Date of Issue: 13/April/2021

**RESEARCH LICENSE**

**This is to Certify that Mr.. James Gakunga Ndukui of University of Nairobi, has been licensed to conduct research in Kiambu on the topic: ANTIMICROBIAL USE, THEIR RESIDUES AND DRUG RESISTANCE PATTERNS IN COMMERCIAL POULTRY PRODUCTION SYSTEMS IN KIAMBU COUNTY, KENYA. for the period ending : 13/April/2022.**

License No: NACOSTI/P/21/8761

317283

Applicant Identification Number

Director General

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

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**Appendix 2: Approval from County Director of Livestock, Fisheries & Vet. Services-Kiambu County**

COUNTY GOVERNMENT OF KIAMBU
LIVESTOCK, FISHERIES AND VETERINARY SERVICES

COUNTY DIRECTOR OF LIVESTOCK,FISHERIES&VET.SERVICES

Address
*Tel: 067 20374*
*E-mail:kiambucountygovernment@gmail.com*lifivedept@gmail.com

P.O BOX 2344-00900
KIAMBU, KENYA

September 22, 2020

*When replying please quote our Ref No. & Date*
*Website:www.kiambu.go.ke*
*When replying please quote*
Ref.KCG/ALF/RESEARCH/VOL.1/49
**TO**

**Subcounty Veterinary officers**

Ruiru
Gatundu South
Gatundu North
Thika
Juja
Kikuyu

**RE: REQUEST TO COLLECT FAECAL SAMPLES FROM POULTRY FARMERS IN KIAMBU COUNTY**

Permission is hereby granted to James Gakunga Ndukui ID. NO.24627417 to collect faecal and meat samples for microbial and antibiotic residual analysis from your Sub counties;

Please accord him the necessary assistance.

**Dr.Tabitha Gathecha**

**For: County Director of Livestock, Fisheries & Veterinary services**

**Kiambu County**

**Appendix 3: Published manuscript for Specific Objective 1**

Open Journal of Animal Sciences, 2021, 11, 658-681
https://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

# Antimicrobial Use in Commercial Poultry Production Systems in Kiambu County, Kenya: A Cross-Sectional Survey on Knowledge, Attitudes and Practices

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## Abstract

**Background:** Antibiotic resistance is putting the commercial poultry production systems across the globe at risk of losses due to the failure of treatments for animals and human health which has been associated with overuse and misuse of antibiotics in poultry farming. One possible approach to the resistance problem is ensuring adequate knowledge, attitudes, and practices on antimicrobial use by farmers on commercial production systems across the globe are applied. Therefore, the current study was carried out to determine factors that could promote antimicrobial use in commercial poultry production systems of Kiambu County, Kenya. **Materials and Methods:** A descriptive cross-sectional survey was conducted on commercial poultry production systems of Kiambu County to determine the level of knowledge, attitudes, and practices on antimicrobial use. One hundred and fifty-six (n = 156) semi-structured questionnaires imprinted in a mobile Open data kit were administered to capture the level of knowledge, attitudes, and practices on antimicrobial usage in commercial poultry production systems of Kiambu County. **Results:** Out of the 156 commercial poultry farmers who participated in this study, females accounted for (64%) with 51% of them keeping layers between 50 - 500 birds as medium scale farmers. In the study, most of the farmers were 45 years and above (61%) and they had obtained a secondary level of education (34%). We further noted that 67% of the farmers obtain antibiotics through self-prescription/personal experience. Additional lack of veterinary

**Appendix 4: Published manuscript for Specific objective II**

Scientific Research Publishing

Pharmacology & Pharmacy, 2021, 12, 219-236
https://www.scirp.org/journal/pp
ISSN Online: 2157-9431
ISSN Print: 2157-9423

# Antimicrobial Resistance Patterns of Selected *Enterobacteriaceae* Isolated from Commercial Poultry Production Systems in Kiambu County, Kenya

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## Abstract

**Introduction:** In the last two decades, the treatment of enteric infections has been complicated by the emergence of antimicrobial resistant strains. Occurrence of multidrug resistant Extended Spectrum Beta Lactamase (ESBL) producing *Enterobacteriaceae* poses the greatest risk to public health by raising morbidity and mortality by six folds in developing countries. The present study aims to determine the antibiotics resistance patterns of selected *Enterobacteriaceae* isolated from commercial poultry production systems in Kiambu County. **Methods:** A laboratory based cross-sectional study was conducted in six purposively selected Sub-Counties of Kiambu County from October 2020, to February 2021. A total of 437 fecal samples were collected from each household. The antibiotic susceptibility testing using disk diffusion method was used against *E. coli*; *Salmonella spps.*; *Shigella spps.*; and *Klebsiella spps.* which were isolated and identified through standard biochemical. **Results:** Out of 437 fecal and stool samples collected, 591 isolates were recovered with *E. coli* (48.9%) being the most frequently identified, followed by *Shigella spps.* (18.8%), *Salmonella spps* (18.3%), and *Klebsiella spps.* (14.0%). The study shows there was high prevalence of multiple resistance among isolates especially to Sulfamethoxazole (79%), Trimethoprim (71%), and Tetracyclines (59%), correspondingly. Additionally, the isolates showed the highest rate of susceptibility against Cefuroxime (94%), Gentamicin (93%), Ceftriaxone (91%),

## Appendix 5: Accepted manuscript for Specific objective III

SAGE track

Select journal: Microbiology Insights

Microbiology Insights (MBI)

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Complete submission draft (1)

Manuscripts with decisions (2)

Manuscripts with Decisions

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| Manuscript ID | Manuscript Title | Date Submitted | Date Decisioned | Status | Due Date | Actions |
|---|---|---|---|---|---|---|
| MBI-2021-0049 | Molecular characterization of ESBLs and QnrS producers from selected Enterobactaeraceae strains isolated from commercial poultry production systems in Kiambu County, Kenya | 18-Jul-2021 | 14-Oct-2021 | Minor Revision | 13-Nov-2021 | ...... |
| MBI-2021-0049.RV1 | Molecular characterization of ESBLs and QnrS producers from selected Enterobactaeraceae strains isolated from commercial poultry production systems in Kiambu County, Kenya | 16-Oct-2021 | 31-Oct-2021 | Accept | | ...... |

Showing 1 to 2 of 2 entries Previous 1 Next

## Appendix 6: Research questionnaire

## A QUESTIONNAIRE ON KNOWLEDGE, ATTITUDES, PRACTICES AND GENDER ON USE OF ANTIMICROBIAL IN POULTRY PRODUCTION SYSTEM IN KIAMBU COUNTY, KENYA

Dear respondent;

Am **Ndukui James Gakunga** a PhD student at the Department of Public Health, pharmacology and toxicology, university of Nairobi carrying out a study on " **Determination of antimicrobial use, and their resistance patterns of selected *Enterobacteraceae* isolated from commercial poultry production systems in Kiambu county, Kenya"**, as part of my special research project.

As one of the stake holder in poultry production systems, you have been selected to participate immensely to this call voluntarily. The information you will generate is purely for academic purposes and will be treated with utmost confidentiality and will not be used whatsoever to incriminate / hurt your business enterprise. This study will help to improve the marketability of your poultry product (Eggs and meat) nationally and internationally as per the EU and FDA requirements. This will also help to improve and avoids public health risks associated with consumption of drug residues from food animals.

Kindly participate with right thoughts in order to help generate valid, correct, plausible and accurate data to foster policy changes/ implementation in the community.

### Consent Form

**I ,………………………………..**the undersigned farmer has been explained about the objectives of the study. However, the information generated here will be kept confidential and used solely in this study. I assume that the information generated here within will help greatly to promote the rationale use of antimicrobial in poultry farms.

Signature…………………………………………

Date……………………………………………

The following statements below constitute a wide array of issues experienced by poultry farmers. Therefore, each issue is rated according to the burden of the dilemma using the following scale 1, 2, 3, 4, 5  6………..12.

Part A: It consists of close- ended questions (**Please circle where necessary**).

| S/No: | Category | Variables | Code (Options) |
|---|---|---|---|
| **1.** | Demographic data | a. Sex | 1. Male;   2. female |
| | | b. Age | 1. 15-25<br>2. 26-35<br>3. 36-45<br>4. Above 45 |
| | | c. Education level | 1. Primary; 2. Secondary; 3. Certificate; 4. Diploma; 5.Degree Holder; 6.MSc; 7. Others and 8.Non |
| | | d. Marital status | 1. Single<br>2. Married |
| | | e. How long have you been in poultry farming? | 1. Less than 1 year/<1 yr; (2). One year; (3). Two years; (4). Three years; (5). Four years; (6) Five years;  More than 5 years (>5yrs). |
| | | f. What average number of Birds do you keep? | 1. Free range (<50); 2). Small scale (51-500); 3). Medium scale (501-1,000); 4). Large scale (1001-5000);  5). No idea. |
| | | g. What production type of birds do you have? | 1. Native for multipurpose; 2). Layers for egg production; 3). Broiler for meat production; 4). Cocks for meat; 5). Other birds; 6). No idea |
| | | h. Do you have any management | 1. Yes;<br>2. No |

| | | | |
|---|---|---|---|
| | | records of your farm? | |
| | | i. What type of records do you keep? | 1. Health care (Drug & treatment record); 2). Feed records; 3). Income record; 4). All of the above ; 5).No records |
| 2. | Role played by farmers on the use of veterinary antibiotics in poultry systems | a. Who reports disease/ sickness in birds? | 1. Yourself,<br>2. The workers<br>3. No idea |
| | | b. Who carries diagnosis of sick birds/ tells bird are sick? | 1. Veterinarian; 2). CBAHW (Community based animal health worker); 3). Yourself; 4). Fellow farmers; 5). Farm workers; 6). No idea |
| | | c. How do you tell that the birds are sick? | 1. Through clinical signs; 2). Physical examinations; 3). Laboratory examination; 4) Personal experience; 5) All the above; 6). No idea |
| | | d. Who does the treatment of your sick birds? | 1. Veterinarian; 2). CBAHW(Community based animal health worker); 3).Yourself; 4).Fellow farmers; 5). Farm workers; 6). All of the above |
| | | e. Do you have any contacts of a Vet/ CBAHW that treats your animals? | 1.Yes<br>2. No<br>If yes, what are their contacts…………………? |
| 3. | Access of antibiotics to farmers and data on their use in poultry | a. What is your source of antimicrobial? | 1. Veterinary drug shop; 2). Livestock markets; 3). Veterinarian; 4). NGO's; 5). Hawkers<br>6). Others (Fellow farmers, workers, Human clinics) |
| | | b. How were the antimicrobial obtained? | 1. Through prescription by a veterinarian; 2). Personal experience; 3). Fellow farmer; 4).Through hawking; 5). No idea |

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| | | c. Do you get advice on how to use the antimicrobial? | 1. Yes<br>2. No |
| | | d. If yes, From who? | 1. Drug shop seller; 2). Veterinarian; 3). From a CAHW in the area; 4). From a fellow Farmer; 5). Others (workers, Human clinics) |
| | | e. Do you follow the advice? | 1. Yes<br>2. No |
| | | f. Were you satisfied with the quality of advice provided? | 1. Yes<br>2. No |
| | | g. What is the nearest from where you get your veterinary services? | 1. Short distance (1-2km); 2). Medium distance (3-4km); 3). Long distance (Above 5km); 4). No idea |
| 4. | Scrutinizing and assessing challenges facing Kenyan poultry farmers on antibiotic use | a. What are the major challenges in accessing antibiotics? | 1. Drug not available in the market; 2). Long distances from the source; 3). Lack of money to buy the drugs; 4).They is expensive; 5). All of the above; 6). No challenges |
| | | b. What challenges are there regarding the use of antibiotics? | 1. Lack of knowledge on antibiotic drug use; 2). Unfamiliar language used in the inserts on their use; 3). No challenges |
| | | c. What are the challenges regarding dosage formulation? | 1. Dosage calculation and estimation difficult; 2). Hard to follow dosage guideline on the leaflet; 3). Lack of specific measuring containers; 4). No challenges |
| | | d. What are challenges | 1. Poor packaging; 2). Some drugs are fake; 3). Some drugs are very expensive; 4). No challenges |

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| | | regarding Quality of the drug? | |
| | | e. What are some of the challenges regarding service provider? | 1. Lack of veterinary officers; 2). Lack of CBAHW; 3). Lack of consultation services; 4). No challenges |
| 5. | Types of antimicrobial used in poultry farming | a. Do you stock some of this classes /types of antimicrobial listed below;<br>1. Tetracyclines (1a. Tetracylines, 1b. oxytetracyline 1c. doxycycline<br>2. Amoxicillin (2a. Hipramox p® powder, 2b. Amoxi, 2c. Sacox, 2d, Panax,)<br>3. Sulfadimidine (3a. sulfadimethoxine)<br>4. Enrofloxacine (4a. Hipralona enro.S®)<br>5. Amprolium ®powder (5a. Amprolium hydrochloride)<br>6. Diaziprim ®powder<br>7. Poltricin ®powder | 1. Yes<br>2. No<br>If yes which one do you stock or buy…..? |

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| | | 8. Penicillin G/Benzylpenicillin<br>9. Erythromycin/Tylosin<br>10. Neomycin sulfate/ Streptomycin/ Spectinomycin<br>11. Metronidazole<br>12. None of the above | |
| | | b. Which class/type of antimicrobial mentioned above do you currently use? | 1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11 and 12 |
| | | c. Which class/ type of antimicrobial did you previously use for the last 1year? | 1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11 and 12 |
| | | d. How frequent do you give this antimicrobial? | 1. Daily in their feeds/water; 2). Once in before dispatchment; 3). Twice before dispatchment; 4). As recommended by the manufacturer; 5). None of the above |
| | | e. Of which purpose do you use these antimicrobial for? | 1. Prophylaxis/ Prevention; 2). Treatment; 3). As growth promoters; 4). 2 and 3; 5). All of the above; 6). None of the above |

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| | | f. Do you know withdraw periods of antimicrobial used in the farm? | 1.Yes<br>2. No |
| | | f. How soon do you sell eggs/ meats after treating the birds (**withdraw period**)? | 1. After 1 day; 2).After 1 week, 3).After 2 weeks; 4). As soon as possible, 5). None of the above |
| | | g. Which drugs do you use to treat the following clinical signs;<br>1. Coughing<br>2. Diarrhea (Profuse, acute and Chronic)<br>3. Sudden death<br>4. Emaciation<br>5. Swelling of joint and footpad<br>6. Head, wing and neck paralysis<br>7. Swelling of eye with watery discharge | 1. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>2. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>3. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>4. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>5. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>6. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>7. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>8. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>9. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>10. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>11. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>12. 1, 2, 3, 4, 5 , 6, 7, 8, 9 ,10, 11 and 12<br>13. If not included in the above list indicate it here? .................................................................... |
| 6 | Alternative remedies to conventional medicine used in poultry disease management | a. Do you use other alternative remedies/treatment apart from these antibiotics? | 1. Yes<br>2. No<br>If yes please state their names here………………………………….................. ................................................................................ |
| | | b. What purpose do you use them in poultry production? | 1. For treatment<br>2. Prophylaxis/ prevention of diseases |

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| | | | 3. To promote growth<br>4. All of the above<br>5. None of the above |
| | | c. Which diseases (5g) are they applied to if they are used for treatment/ prophylaxis? | Please list here……………………………………… ………………………………………………… …. |
| | | d. Why do you prefer this alternative remedies? | 1. They are cheap and safe<br>2. Readily and locally available<br>3. They are natural<br>4. They are efficacious<br>5. All of the above<br>6. None of the above |

7. Do you think it's important to observe withdraw period? ………………….. If **yes**, why do you think so…………………………………………………………………………… ……………………………………………………………………………
8. Do you know any health implication of associated with consuming animal products contaminated with antimicrobial residues? ....................................If **yes,** which ones? …………………………………………………………………………… ……………………………………………………………………………
9. Do you know of any regulatory body involved in control and monitoring of veterinary drugs (Antimicrobials)? ..............................If **yes,** please name it……………………………………………………………………………
10. What are the possible solutions to the above mentioned challenges facing poultry farmers on the use of antibiotics in Kenya?............................................................................................................... ...........................................................................................................................

Date ………………….. Signature of the interviewee…………………………

Area of residence………………. Designation…………………………………..

Date ……………………. Name of the interviewer …………………………

Signature ………………. Areas of study …………………………………