PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *SALMONELLA* AND *CAMPYLOBACTER* SPECIES IN CHICKEN WASTE, BUNGOMA COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a Degree in any other University.

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DEDICATION

To my parents, Mr. and Mrs. Kaburia and Hon. Hedwig Ong'udi for beholding the dream, believing in me and walking with me the journey to its fulfilment.

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LIST OF ABBREVIATIONS AND ACCRONYMS

AGPs	Antimicrobial Growth Promoters
AMR	Antimicrobial Resistance
AST	Antimicrobial susceptibility testing
DHPS	Deoxyhypusine synthase enzyme
DNA	Deoxyribonucleic acid molecule
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
GDP	Gross Domestic Product
MKTTn	Muller-Kauffmann Tetrathionate-Novobiocin Broth
MLST	Multilocus sequence typing
NACOSTI	National Commission for Science, Technology and Innovation
NCEZID	National Center for Emerging and Zoonotic Infectious Diseases
OIE	World Organization for Animal Health
RNA	Ribonucleic acid polymeric molecule
RVS	Rappaport-Vassiliadis soya peptone broth
SPSS	Statistics software package for interactive/ batched analysis
WHO	World Health Organization

DEFINITION OF OPERATIONAL TERMS

Antimicrobial resistance (AMR): The ability of microorganisms such as viruses and bacteria to stop an antimicrobial (antibiotics, antivirals and antimalarial) from working against it. World Health Organization (WHO, 2016)

BS EN ISO 6579 -1 – 2017: Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* species.

Chicken waste: chicken droppings/ feces of chicken.

ISO 10272-2:2017: Horizontal method for detection and enumeration of *Campylobacter* species.

Kienyeji: traditional indigenous breed of chicken

Traditional medicine: the knowledge skills and practices based on experience and beliefs in different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of infections.

ABSTRACT

Antimicrobial resistance is a growing threat to public health and is driven by various factors including the overuse or misuse of antibiotics in poultry production which could lead to development of resistant bacteria that can be transferred to humans and hence compromise human medicine. The use of antibiotics in poultry production could be for vaccination/prophylaxis, treatment or growth promotion. This study was a cross-sectional study in Bungoma county that sought to establish the prevalence and antibiotic sensitivity of Salmonella and Campylobacter species, by sampling of chicken waste then analyzing it for presence of Salmonella & Campylobacter bacteria species and testing their sensitivity to 4 antibiotics. The aim of the study was to determine prevalence of Salmonella and Campylobacter species in chicken waste in Bungoma county, to determine the sensitivity of Salmonella and Campylobacter species to Tetracycline, Ampycillin, Imipinem and Co-trimazole antibiotics and to determine risk factors for Salmonella & Campylobacter infection and spread of resistant bacteria among chicken keeping households. Random sampling design was used to recruit 169 households where a duplicate of chicken waste samples was collected and a questionnaire issued to the farmers. The BS EN ISO 6579 -1-2017 technique was used in the identification of Salmonella species and ISO 10272-2:2017 technique used for the detection and enumeration of Campylobacter species. The disk diffusion test was used for antibiotic sensitivity testing of the bacteria. SPSS was used for analysis; Logistic regression was used to measure sensitivity of the bacteria to the specific antibiotics and Pearson's r analysis used to measure correlation among variables. The prevalence for campylobacter was higher in the county of Bungoma at 4.32% compared to that of salmonella at 1.44%. The use of traditional medicine was found less likely to cause presence of resistant bacteria. Risk factors identified for the development and spread of AMR associated with chicken production include; use of antibiotics for growth promotion, frequency of use of antibiotics and use of chicken waste as manure in farming. The study recommended that farmers should be encouraged to obtain already vaccinated chicks and the use of traditional medicine be promoted during chicken production. Further research should be conducted on the possibility of transfer of resistance through food crops.

CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

The development and spread of antimicrobial resistance (AMR) is a threat to public health and sustainable development. According to the Center for Disease control (CDC) a one health approach should be encouraged; a strategy that appreciates the role of animal health and the environment in human health. Some of the drivers include; infection prevention and control, antibiotic use, sharing and tracking of data and environment and sanitation (McEwen & Collignon, 2018)

In many parts of the world, poultry production has continued to develop as a result of urbanization, population growth and economic development. The Food and Agriculture Organization Food Outlook (2014) also projected that global poultry production was expected to rise by 0.9 percent in 2017 with general growth in most parts of the world in the decade. In Africa, the modern poultry industry has been taking shape in the recent years, this is mostly attributed to rapid urbanization and a rising middle class who are changing their consumption patterns from vegetable-based to protein-rich diets, thus, poultry meat and eggs have proven a relatively available and affordable source of protein. Availability of poultry is supported by the short pay back times for poultry meat and egg production, making poultry production easier to start up and expand (Mulder, 2017)

Animal production is both an economic and social activity for Kenyan communities, ranging from simple zero grazing, herding and backyard production to intensive poultry farming mostly for economic purposes. Agriculture contributes about 25% of Kenya's GDP with poultry representing about 30% of the agricultural contribution to GDP ((FAO), Poultry sector country review- Kenya, 2008). Kenya has an estimated poultry population of 31 million birds. Of these, 75% consist of indigenous chicken, 22% of broilers and layers and 1% of breeding stock. Other poultry species like ducks, geese, turkeys, pigeons, ostriches, guinea fowls and quails make up 2 % of the poultry production (Zootecnica International, 2016)

Ensuring food safety is critical in public health, and is a challenge for both producers and consumers. This is partly because there are loopholes in the production cycle that are generally impractical to curb with the existing policies and laws, coupled with lack of awareness among producers and consumers. Improper dumping of animal waste leads to environmental pollution. The waste provides a nutritional source for growth of pathogenic bacteria which cause disease outbreaks and also transfers microorganisms to humans and the environment. Warm blooded animals like chicken can contain bacteria in their gut and the main route of transmission to human is foodborne (WHO, 2018). The transmission of *Salmonella* and *Campylobacter* bacteria to man is mainly through animal products which could be contaminated from the source or during handling.

Antimicrobials are used in chicken production for prophylaxis, treatment and for growth promotion. Worldwide, the bulk of antimicrobials administered are not consumed by patients, but rather, they are given to animals, including cattle, sheep, chicken, pig and fish. The use of immense quantities of antimicrobials in food production and the unintended wide release of antimicrobials into the environment through animal and human sewage and runoff water from agricultural sites has great public health consequences. This is most clearly seen in the development of resistant zoonotic bacteria and associated food-borne diseases in humans (WHO, 2015).

Drug-resistant bacteria can circulate in populations of human beings and animals; through food, water and the environment. The transmission is influenced by trade, travel and both human and animal migration. Resistant bacteria can be found in food animals and food products destined for consumption by humans (FDA, 2015). The human and animal gut can serve as reservoirs for drug resistant genes and contact between companion animals and humans presents opportunities for inter-species transmission of AMR (Marks *et al*, 2011). Spread of antimicrobial resistance leads to standard treatments becoming ineffective, infections persisting and the possibility of spreading to others. The misuse and/or overuse of antimicrobials drives the progress of drug resistance.

1.2 Problem Statement

Transmission of bacteria in chicken could occur from one infected flock to another during their interaction either through contaminated droppings, feed/waters or passed through their feather dander.

The use of antibiotics in chicken has shown to boost its weight hence explosive growth in the markets, however, this comes at a cost of antibiotic resistant foodborne outbreaks with significant effects on human health (McKenna, 2017). Chicken meat has been identified as one of the key vehicles in the transmission of Salmonellosis and campylobateriosis (WHO, 2009). These diseases are major foodborne illnesses affecting humans.

A range of antibiotics are used in the treatment of *Salmonella* and *Campylobacter* in chicken; Amoxycillin, Streptomycin, Sulphonamides, Tetracylines and Fluoroquinolones. The same antibiotics are also used in humans for treatment, thus the development of antibiotic resistance and spread of resistant bacteria to humans compromises public health and human medicine.

In the recent years, various reports have emerged in Kenya on the risk of consuming antibiotics in beef and poultry meat (Otieno, 2017) . Farmers are using antibiotics to boost chicken weight by up to 3% with the antibiotics being administered in water or with poultry feeds (Miyumo, 2015).

A study conducted on the analysis of demand for antibiotics in poultry production in Kiambu county found that antibiotics were widely used in poultry. The antibiotics were mostly acquired from agro-vets and administered without the assistance of a trained veterinary (Wanjiru 2014).

Poultry farming is one universal farming venture, some keeping chicken for subsistence use but most have turned poultry keeping into a business. Use of antibiotics in poultry production is a major concern. Agriculture and livestock keeping is the key economic activity in Bungoma. Intensive poultry farming and demand due to growing economies is often associated with increased use of antibiotics.

This exploratory study in Bungoma county intended to highlight the risk factors in the spread of drug-resistant bacteria associated with chicken keeping. The study sought to determine the extent of bacterial contamination in chicken waste and their susceptibility to specific antibiotics namely; Ampicillin, Tetracycline, Imipinem and Co-Trimoxazole.

1.3 Justification of the Study

Over the years, poultry production has industrialized due to increased population, developing economies and demand for ready protein. There has been growing concern globally on the need to regulate the use of antibiotics in poultry production. Some countries such as the United States and the EU have developed policies regulating the use of antibiotics in poultry production such as the ban on in-feed antibiotics, antimicrobial growth promoters (AGP) in the European Union in 2006.

Public concern on development of AMR with available data on history and current status of antibiotic use in poultry production and effects on public health have been major drivers in the design of policy. Kenya does not have legislation in place to control use of antibiotics in animals but has a national action plan that is in line with the World Health Organization (Kariuki, 2017).

This study sought to highlight status of bacterial infection among chicken in Bungoma county by sampling of the chicken waste. Policy regulating the use of antibiotics in chicken production needs to take into consideration common behavior among farmers that cause and progress antibiotic resistance. This study analyzed various practices among farmers rearing chicken, which was meant to inform the role of the environment in the spread of resistant bacteria. The results will work to advice farmers on good practices in the use of antibiotics during chicken production and the risks involved in chicken keeping, associated with AMR.

1.4 Research Questions

1. What is the prevalence of *Salmonella* and *Campylobacter* species in the sampled chicken waste, Bungoma County?
2. What is the sensitivity of *Salmonella* and *Campylobacter* species found in chicken waste to the specific antibiotics; Ampicillin, Tetracycline, Imipinem and Co-Trimoxazole?
3. What are the practices that may predispose households to the spread of drug resistant *Salmonella* and *Campylobacter* associated with chicken production?

1.5 Objectives of the Study

1.5.1 Broad Objective

To determine the prevalence and antibiotic sensitivity of *Salmonella* and *Campylobacter* species in chicken waste in Bungoma county, Kenya.

1.5.2 Specific Objectives

i. To determine prevalence of *Salmonella* and *Campylobacter* species in chicken waste, Bungoma county.

ii. To determine the antimicrobial susceptibility patterns of *Salmonella* and *Campylobacter* species found in chicken waste to Ampicillin, Tetracycline and Co-trimoxazole antibiotics.

iii. To determine risk factors associated to the spread of drug resistant bacteria through the environment in chicken production.

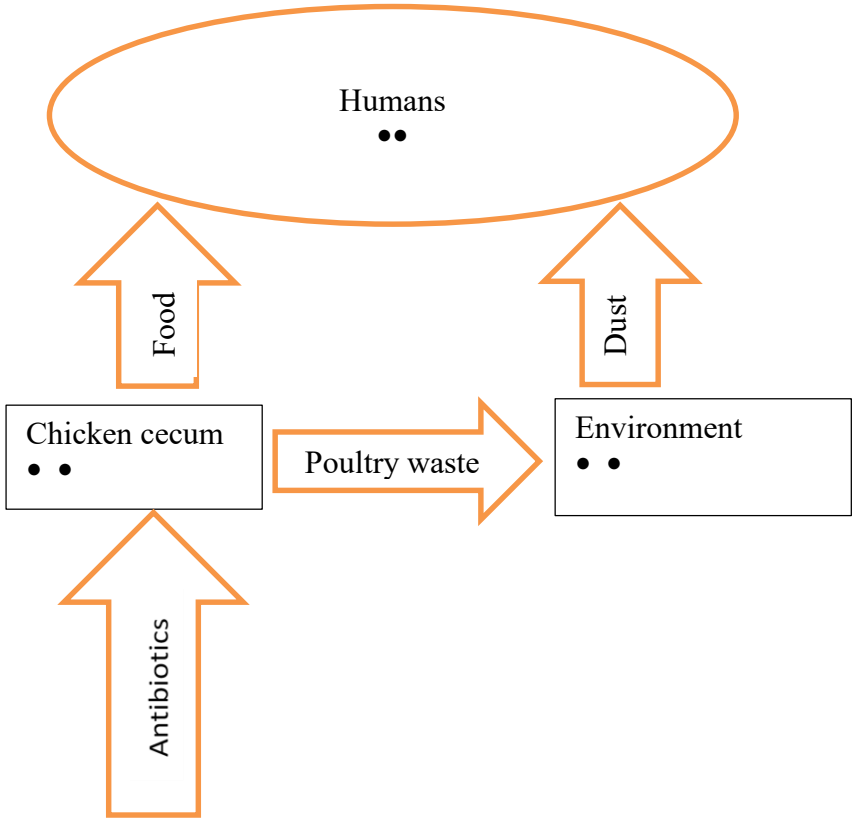
1.6 Significance of the Study

The results and findings have identified potential risk factors and hotspots for bacterial contamination and infection. It will therefore form a basis for areas of intervention and hence influence policy, on the rational use of antibiotics especially in chicken production, to control the rise of AMR in Kenya.

1.7 Conceptual and Theoretical Framework

Live chicken may have bacteria on their bodies or in their gastrointestinal tract that end up in their droppings. The bacteria may be transmitted to humans through contact with contaminated soil, water, plants and other objects including food products. According to Chlebicz and Śliżewska (2018), Campylobateriosis and Salmonellosis are major zoonotic diseases with great public health effects coupled with great economic effects.

Bacterial infection both in chicken and humans is treated by use of antibiotics, some of which are common to both chicken and humans. Chicken excessively exposed to antibiotics can develop resistant bacteria which can be transmitted to humans through contaminated chicken waste containing resistant bacteria or improperly cooked and handled chicken products such as meat and eggs. There are various mechanisms in the transfer of zoonoses, such as food, animal waste, soil/environment or direct contact. The use of contaminated chicken droppings/waste as garden manure also poses a potential risk of transfer of resistant bacteria to humans through the environment and food.



● Gene for antibiotic resistance

Figure 1. 1: Conceptual framework on the spread of antimicrobial resistant

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter presents the review of relevant literature on chicken production, trends in antibiotic use, the use of traditional medicine, development of resistant bacteria and the role of the environment in the spread of antimicrobial resistance.

2.2 Poultry Production

In many parts of the world, poultry production continues to industrialize and grow. This growth has been associated with various factors including increased population, economic growth and greater purchasing power. World poultry meat production increased from 114,898 thousand million tons in 2015 to 116,208 thousand million tons in 2016. For developing countries, there was an increase of 198,000 million tons produced in 2016 compared to 2015 according to FAO Food Outlook (2016). Meat consumption is projected to grow by nearly 35 percent in the next decade especially in the sub-Saharan Africa due to the population growth forecast from 0.96 billion to 1.22 billion with increasing income growth and GDP growth rates (Conway, 2016). In this, poultry meat is seen as the meat of choice to feed the growing population.

For most Kenyan communities, animal production including poultry rearing is both a social and economic activity. Poultry production ranges from backyard production to intensive poultry production mostly for economic purposes. Poultry production is supported by quick and short payback times, relatively easy production systems and availability of ready protein food sources in form of eggs and meat for families.

2.3 Risk factors in Poultry Production

Public health and food safety are major concerns in the production of poultry. Two major risk factors identified are; microbiological and chemical. Microbiological includes bacteria such as *E. coli, Campylobacter spp* , *Salmonella spp* and parasites. Chemical factors which lead to contamination of poultry products may result from antibiotic and pesticides residues.

Chicken, are affected by various viral, bacterial and parasitic diseases during their growth. They include the Newcastle disease, fowl cholera, coccidiosis, fowl typhoid, influenza, pullorum, salmonellosis and ectoparasites such as fleas, mites and lice. (Ogada , 2016)

2.3.1 Bacterial infections and Chicken

Bacterial infection in poultry is affected by various factors such as the age; with newly hatched chicks being most susceptible, animal health status which determines survival of bacteria within the gastric system and physiological and environmental stressors including their food and water. Different Salmonella serovars lead to different diseases in poultry i.e. fowl typhoid is caused by *Salmonella gallinarum*, pullorum disease caused by *Salmonella pullorum* and Salmonellosis. Antibiotics such as amoxicillin, sulphoamide, tetracyclines, penicillin and fluoroquinolones have been used in the treatment of such diseases in poultry (Ogada, 2016). *Campylobacter jejuni* is the most common in poultry with *Campylobacter coli* being a rare strain. Antibiotics such as Azithromycin, Ciprofloxacin, tetracycline and fluoroquinolones have been used in the treatment and management of *Campylobacter* infections in chicken.

Antibiotics have been used in the treatment and management of bacterial infections both in humans and poultry. The United States FDA in a national strategy to combat Antibiotic-resistant bacteria, limited and restricted the use of two classes of antibiotics in chicken production because of the relative importance of those drugs to human medicine – Cephalosporins and Fluoroquinolones (Landers *et al*, 2012)

One of the major factors that has led to and aggravated the misuse of antibiotics in animal production is their use in growth promotion and availability of these drugs over the counter without the necessary prescription or consultation with a veterinary doctor. Good hygienic practices during poultry production and proper periodic vaccination of chicks would improve the health of the poultry by limiting incidences of disease outbreaks and therefore minimize the use of antibiotics for treatment. Lack of vaccination could be linked to scarcity, high cost and lack of knowledge on their use by farmers.

A study in Korea by Kidie *et al* (2013) that looked at the prevalence and antimicrobial resistance of *Salmonella* isolated from poultry slaughterhouses, found that 68.2% of the samples were contaminated with Salmonella. *Salmonella enteritidis* was established to be the most dominant serovars with 12.5% of the total samples. Among them, 52.3% were resistant to one antibiotic and 21.6% showed resistance to more than two antibiotics.

A most recent study in Malaysia aimed at determining the prevalence of various *Salmonella* serotypes in chickens, environmental samples and carcass contact surfaces, found 88.46% of the samples showing presence of Salmonella (161 out of 182 samples) with 17 serotypes isolated. (Nidaullah *et al*. 2017).

2.3.2 Salmonella and Campylobacter Bacteria

Studies have identified *Campylobacter/salmonella* in humans and animal meat (Osano and Arimi, 1999). (Turkson *et al* 1988) reported the highest isolation of *Campylobacter*s: from pigs with diarrhoea (55.1%), chicken (51.5%), dogs with diarrhoea (47.2%), pigs (44.0%), ducks (29.4%), goats (6.3%), cattle (5.8%) and humans with diarrhoea (3.1%), and sheep (2.0%) respectively. Out of 317 isolates in Turkson's study, the results indicate that domestic animals could serve as reservoirs potentially being of epidemiological significance in human Campylobacter cases (Turkson *et al*., 1988).

In a study to identify *Campylobacter* species isolated from human in Nairobi, 96% were *Campylobacter jejuni* and *salmonella* (Osano and Arimi, 1999). Among patients with diarrhea in Bungoma, out of the isolated samples. *Shigella* was found most frequent, followed by *Campylobacter* species and *V. cholerae*. Among <5 years old children, *Campylobacter* was isolated from the majority (Shapiro *et al*., 2001). This was in agreement with a rural western Kenya study (Brooks *et al*., 2006) and another done in Ethiopia (Mitike *et al*., 2009), which reported *Campylobacter* isolation rates twice that of salmonella and shigella species from under 15 years old children.

2.4 Zoonotic and Public Health Importance of Salmonella and Campylobacter Bacteria

Salmonella and Campylobacteriosis are zoonotic. The causative bacteria are commonly found in food animals and in pets in this case chicken (Heredia & García, 2018). This makes contact with any infected member of these groups of animals a potential risk for transmitting the pathogen to humans.

Campylobacter gastroenteritis instigated by *Campylobacter jejuni* and *Campylobacter coli* is of major public health importance among all infections caused by Campylobacter (Heredia & Gracia, 2018). Growing antimicrobial resistance observed in *Campylobacter*, in both medicine and agriculture is acknowledged by many global experts as an important emerging community health concern (Moore *et al*., 2006).

Poor hygiene, sanitation and interaction with animals in growing economies leads to frequent contracting of enteric pathogens. Persons working with farm animals, laboratory technicians/ personnel and those handling human excreta have increased risk of contracting *salmonella and Campylobacter* enteritis.

2.4.1 Role of livestock in transmission of Campylobacter to humans

More recent studies using Multi Locus Sequence Testing (MLST) technique compared wild and farmed animal *Campylobacter* genotypes with human isolates and attributed animals as a source of human Campylobacteriosis (Wimalarathna *et al*., 2013). Sheppard *et al*., (2009) linked chicken isolates to human disease causing isolates. The infection is mainly transmitted from animals through eating undercooked meat from diseased livestock in this case poultry, contact with infected animals and handling of contaminated manure with bare hands and failure to clean hands properly afterwards (Lupindu *et al*., 2012).

2.5 Global Antimicrobial Consumption Trends

According to Landers *et al*, 2012, the bulk of antimicrobials administered worldwide are not consumed by patients, but rather, are given to animals including cattle, sheep, pigs, chicken, and fish, for purposes of health and food production. More than 70 percent of the antibiotics deemed medically important for human health by FDA sold in the United States (and over 50 percent in most countries in the world) are used in livestock; animal consumption figure of 8,893,103kg and human consumption of 3,379,226kg based on calculations by IMS Health.

In 1998, the European Union banned the use of antibiotics as growth promoters, only allowing their use for the treatment of sick animals in 2006. In some developing and emerging countries, antimicrobials are freely available to anyone, with the risk of availability of adulterated antimicrobials circulating as normal goods worldwide for use in animals. According to the World Organization for Animal Health, some countries especially in Europe, have relevant legislation concerning appropriate conditions for the importation, manufacture, distribution and use of veterinary products, including antimicrobials whereas in other countries, mostly developing and emergent countries, legislation is totally non-existent. In other areas, where legislation exists, it is very often not properly applied because of lack of public funds for the implementation of controls (WHO, 2001)

Global consumption of antimicrobials in food animal production was estimated at 63,151 (±1,560) tons in 2010 and was projected to rise by 67%, to 105,596(±3,605) tons, by 2030. Two thirds (66%) of the global increase (67%) in antimicrobial consumption is due to the growing number of animals raised for food production. In 2010, the five countries with the largest shares of global antimicrobial consumption in food animal production were China (23%), the United States (13%), Brazil (9%), India (3%), and Germany (3%). By 2030, this ranking is projected to be China (30%), the United States (10%), Brazil (8%), India (4%), and Mexico (2%) (Thomas *et al*. 2015).

2.5.1 Antimicrobial Consumption Trends in Kenya

Data on antibiotic use in livestock and chicken production is scarce, due to lack of publicly funded surveillance systems and the disinclination by food animal producers, animal feed producers, and veterinary pharmaceutical companies to provide comprehensive reports of antimicrobial consumption or sales.

Antibiotic usage data is scarce in Kenya since very limited survey or data on usage is collected, and animal producers and pharmaceuticals have little incentive to report such information. Where usage data is available, it usually takes the form of volume sales data, rather than actual usage of the products.

The major disadvantage of antimicrobial sales or distribution data is that, it fails to adequately indicate how and/or if antimicrobials were used. Farmers have resulted to the use of traditional/ herbal medicine for the treatment of diseases in chicken due to various reasons such as financial constraints, ease of access and the belief that traditional medicine has less effects as compared to conventional treatment.

2.6 Major Diseases Affecting Poultry and antibiotics Used

i) Penicillin's are effective in the treatment of sinusitis and chronic respiratory disease in poultry.

ii) Coccidiosis is a disease in poultry worldwide and is caused by a protozoan parasite (Eimeria) that invades the cells of poultry intestines. Ionophores are used primarily as an anti-microbial but can control some bacteria so is often grouped with antibiotics.

iii) Lincomycin is effective against bone and joint infections, as well as necrotic enteritis caused by Clostridium perfringens.

iv) Macrolides are effective against Mycoplasma and Orhnithobacterium rhinotracheale and can be used to treat necrotic enteritis.

v) Doxycycline is a semisynthetic tetracycline. Tetracyclines are effective against Mycoplasma, Chlamydia, Pasteurella, Clostridium, Ornithobacterium rhinotracheale, and some protozoa (Jacob, 2015).

2.7 Antimicrobial Resistance – mechanisms and patterns

The three fundamental mechanisms of antimicrobial resistance are (1) enzymatic degradation of antibacterial drugs, (2) alteration of bacterial proteins that are antimicrobial targets, and (3) changes in membrane permeability to antibiotics (Giedraitiene *et al*, 2011). Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome. The most important mechanism of resistance to the penicillins and cephalosporins is antibiotic hydrolysis mediated by the bacterial enzyme beta-lactamase. The expression of chromosomal beta-lactamase can either be induced or stably depressed by exposure to beta-lactam drugs. Methods to overcome

resistance to beta-lactam antibiotics include the development of new antibiotics that are stable to beta-lactamase attack and the co-administration of beta-lactamase inhibitors with beta-lactam drugs. Resistance to methicillin, which is stable to gram-positive beta-lactamase, occurs through the alteration of an antibiotic target protein, penicillin-binding protein 2. Production of antibiotic-modifying enzymes and synthesis of antibiotic-insensitive bacterial targets are the primary resistance mechanisms for the other classes of antibiotics, including trimethoprim, the sulfonamides, the aminoglycosides, chloramphenicol, and the quinolone drugs. Reduced antibiotic penetration is also a resistance mechanism for several classes of antibiotics, including the beta-lactam drugs, the aminoglycosides, chloramphenicol, and the quinolones (Dever , 1991)

2.7.1 Resistance to Quinolones

The acting mechanism of quinolones is through hindrance of bacterial DNA synthesis causing cell death. Quinolones exert their action by targeting the enzymes topoisomerase IV and DNA gyrase found in bacteria which are involved in DNA duplication, transcription, repair and recombination (Jacoby, 2005). The products of the enzymes are large structures which have two sub units each i.e. ParC and ParE, GyrA and GyrB respectively (Wieczorek and Osek, 2013a). Resistance mainly occurs through replacement of amino acids in a segment known as the quinolone resistance-determining region (QRDR) within DNA attachment area on the enzymes. In *Campylobacter*, resistance to fluoroquinolones has been reported to be mainly a result of gyrA gene mutations (Engberg *et al*., 2001). A Thr86Ile point mutation in the gyrA gene is reported to be responsible for high resistance to ciprofloxacin. This mutation is similar to Ser83Leu mutation in *Escherichia coli* (Piddock, 1999). There exist other mutations affecting the gyrA gene of *C.jejuni* that are attributed to increased resistance to nalidixic acid and the inverse for ciprofloxacin (Beckmann *et al*., 2004). More than one-point mutation can also occur (Piddock,1999).

Since *C. jejuni* and *C. coli* lack an alternative area that can be responsible for quinolone resistance, a unique alteration of the Gyr A is thus enough to result in resistance to fluoroquinolones (Engberg *et al*., 2001). The cmeABC efflux system responsible for multiple antimicrobial resistance also works in tandem with the gyrA mutations resulting in resistance (Lin, *et al*, 2002).

2.7.2 Resistance to Tetracycline

Tetracyclines act by attaching to ribosomes and hampering elongation of protein production (Gibreel *et al*., 2004). They use their attachment to Mg+2 cations to go through outer membrane porins (Chopra and Roberts, 2001).

Ribosomal protection proteins such as the *tetO* and the *tetM* genes, facilitate tetracycline resistance (Connell *et al*., 2003). The tetO is liable for tetracycline resistance in *Campylobacter and salmonella* (Connell, *et al*, 2003). *Tet M* is the only other gene that has been identified in *Campylobacter* isolates (Abdi-Hachesoo *et al*., 2014).

The *tetO* gene, is plasmid mediated and is associated with very elevated tetracycline resistance levels (Gibreel *et al*., 2004). It has however been reported to be found on the chromosome in some isolates (Gibreel *et al*., 2004). It is likely that other mobile extra chromosomal genetic elements may be involved in the attainment and distribution of *tetO.* (Wieczorek and Osek, 2013a). Examples of these could be integrons and transposons. Studies show the likelihood of *Campylobacter tetO* having been obtained from *Streptomyces, Streptococcus,* or *Enterococcus species* through horizontal genetic transmission (Batchelor, *et al* 2004).

2.7.3 Resistance to Macrolides

Macrolides act by targeting the 50S subunit and interrupting production of proteins (Wieczorek and Osek, 2013a). Studies show the 23S rRNA nucleotides 2058 and 2059 to be of key importance in the attachment of macrolides. Changes in the attachment area of macrolides on the ribosome are what mediate their resistance (Batchelor *et al*., 2004). Replacement of nucleotides at positions 2074 and 2075 of the adenine residues in the 23S rRNA gene in *Campylobacter* frequently occur in erythromycin resistance (Luangtongkum *et al*., 2009). The A2074C, A2074G, and A2075G mutations result in increased macrolide resistance in *C. jejuni* and *C. coli*, with erythromycin resistance corresponding with resistance to all other macrolides, lincosamides and streptogramin antimicrobials (Avrain, *et al* 2004).

Other mechanisms include:

L4 and L22 protein modification could result in low resistance levels. However, theprecise of these alterations is still not clear (Cagliero, *et al*, 2006).

There are approximately eight efflux systems recognized with the CmeABC multiple drug efflux pump which combined with target mutations works to facilitate resistance (Cagliero *et al*., 2006). This is an energy dependent efflux pump which is chromosomally encoded by three genes cmeA, cmeB and cmeC. These three genes are a “periplasmic protein, an inner membrane drug transporter, an outer membrane protein respectively”. These work together to remove antimicrobials among other substances from a *Campylobacter* cell (Lin *et al*., 2003)

2.7.4 Resistance to Aminoglycosides

Aminoglycosides act through the 30S ribosomal subunit, preventing precise codon-anticodon identification and in disturbance of protein longation by impeding the movement of Trna from the A-site to the P-site (Jana and Deb, 2006).

Enzyme changes which weaken the aminoglycoside attachment to the rRNA are what effect their resistance (Llano-Sotelo, *et al* 2002). These aminoglycoside deactivating enzymes are classified as: “aminoglycoside adenyltransferases, acetyltransferases and phosphotransferases”, all with their own specific alteration areas and products (Wieczorek and Osek, 2013). They act by compromising attachment of aminoglycosides to their targets through shifting a substrate functional group to the antimicrobial (Toth *et al*, 2010). The acetyltransferases use acetyl-coA to acetylate the amino groups of these antibiotics, the adenylyltransferases, modify hydroxyl groups of aminoglycosides by transferring the nucleoside moiety and the phosphotransferases modify the antibiotics by phosphorylation of their hydroxyl groups (Toth *et al*., 2010).

A gene: apha-3 responsible for kanamycin-resistance among others, are recognized as part of a resistance cluster in *C. jejuni* plasmid (Gibreel *et al*., 2004) This gene is suggested to have been transferred to *Campylobacter* from gram positive bacteria. The apha-3 gene is also present on plasmids mediating tetracycline resistance in *Campylobacter/salmonella* genus (Gibreel *et al*., 2004).

2.7.5 Resistance to Other Antimicrobial Agents.

Efflux pumps are involved in this resistance (Lin *et al*., 2002).

Chloramphenicol acts by inhibition of protein elongation in bacteria (Wieczorek and Osek, 2013a). resistance to chloramphenicol is via an acetyltransferase encoding gene that is plasmid mediated (Wieczorek and Osek, 2013a) this has been shown in *C.coli* although this resistance is rarely seen phenotypically (Wieczorek and Osek, 2013a).

Sulphonamide resistance in *C. jejuni* is a chromosome mutation with substitutions of various amino acids in the dihydropteroate synthetase (DHPS). Competition for DHPS between sulphonamides and PABA (4-aminobenzoic acid) prevents the latter from assimilation into folic acid (Engberg *et al*., 2001).

Another mechanism by which both *Campylobacter/salmonella* has been reported to develop resistance to multiple drugs is the CmeABC multidrug efflux pump (Pumbwe, *et al*, 2004). The three fragments of the pump i.e. membrane fusion proteins inner drug transporter and outer membrane protein act to enable the transportation of substrates from outside the cell into the cell matrix (Krishnamoorthy, *et al* 2008).

2.8 Use of traditional medicine in chicken production

Herbal medicines have always been a form of therapy for livestock among resource poor smallholder farmers. There is, however, little documentation of the use of traditional medicines, as many researchers and health practitioners view these practices as backward.

Use of herbs/ traditional medicine in chicken has proven efficient in the treatment and management of diseases. A research on Traditional herbal preparations for indigenous poultry health management in Western Kenya showed that herbal extracts play an important role in antimicrobial activity against disease causing bacteria in poultry and that farmers believe the use of traditional medicine to manage and treat infections in chicken (Okitoi, *et al*, 2007).

Some farmers use traditional medicine to complement conventional medicine whereas some solely use traditional medicine. Some of the commonly used herbs include Aloe Vera, Capsicum and Neem. Different parts of the plants are used; leaves, fruit, roots or bark, mostly administered to the chicken through drinking water.

Aloe species is arguably the most important, as it is found in many geographical regions and is believed to be effective against a wide range of diseases and ailments i.e. coccidiosis, fowl typhoid and Newcastle disease.

The use of traditional medicine presents a number of advantages such as; ease of availability and use without complications on dose format, less costly/ affordable, ease of administration & storage and negligible side effects to the chicken. In addition, with the development of resistance of pathogens to drugs, traditional medicine might be the route to take since herbs tend to be broad spectrum (Okitoi, *et al*, 2007).

2.9 Antimicrobial Pollution on the Environment and its effects to the Food Chain

The ecological impact resulting from use of large volumes of antibiotics in food production and the resultant release into the environment through animal and human sewage and runoff water from agricultural processes is an important aspect to be considered in the study of AMR. This poses a health hazard with public health consequences, most clearly seen in resistant zoonotic bacteria associated with food-borne disease in humans.

According to a study on food animals and antimicrobials with their effect to human health, 93 percent of medically-important antibiotics were administered via feed or water in agriculture in the US. Scientific studies also suggest that 75-90 percent of tested antibiotics are excreted from animals un-metabolized (Marshall & Levy 2011)

Animal waste which could not only contain resistant bacteria, but also antibiotic residues may enter soil and water sources including groundwater, leading to emergence and spread of resistant bacteria. This waste from chicken is often composted used on crops as manure, posing a health hazard to consumers due to the risk of transmission of resistant bacteria.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

This chapter will explain in detail the equipment and scientific procedures that were used in the research which includes data collection; both chicken waste samples and farmers' information collected using questionnaires.

3.2 Research Design

This study was an exploratory descriptive, cross-sectional study. The purpose of the study was to study the characteristics of a representative sample of the farmers and chicken populations in Bungoma county at one point in time, without a comparison group. It examined the possibility of presence of resistant bacteria and their transfer to humans through the environment.

3.3 Variables

The dependent variable were the species of bacteria – *Salmonella* and *Campylobacter*. The independent variable for the laboratory analysis were the antibiotics; Ampicillin, Tetracycline, Imipinem and Co-Trimoxazole.

For the questionnaire, the dependent variable was the presence of bacteria, with the independent variables; the frequency and purpose of use of antibiotics, level of education or training of the farmer on the use of antimicrobials and the levels of sanitation and hygiene.

3.4 Location of the Study

The study was conducted in Bungoma County. This is a region in the Western of Kenya with Bungoma town as its capital. It has a population of about 1.3 million in an area of approximately 2.1 km^2. Bungoma county is made up of six sub-counties namely; Kimilili, Webuye, Sirisia, Kanduyi, Bumula and Kabuchai.

The economy of Bungoma county is mainly agricultural with the people practicing both livestock and crop farming. Agriculture is the backbone of Bungoma county since many families rely on animal keeping and crop production. The main crops produced include; maize, sweet potatoes, finger millet, bananas and vegetables, mostly done in small scale and the excess sold or traded for other family needs. The main livestock reared in Bungoma county are; cattle, goats, pigs, sheep and poultry.

Most farmers practice backyard production as compared to intensive production which is practiced by farmers who engage in poultry keeping solely for commercial purposes. Poultry keeping and especially chicken rearing is one tradition among the communities in Western Kenya. This is because of their value for chicken meat as a delicacy and in the recent times, it being an economic activity. Poultry production has presented the possibility of relatively easy production and returns in the trade of its products; chicken meat and eggs.

3.5 Study Population

The study population was the farmers in Bungoma county and the target population being farmers practicing chicken production in Bungoma county.

The unit of sampling for this study was the household where chicken waste samples were collected using swabs and a questionnaire administered to the farmer.

3.5.1 Inclusion Criteria

Farmers in Bungoma county practicing chicken production who consented to the study.

Farmer considered to be of sound mind, 18 years of age or older

All types and breeds of chicken i.e. mixed, indigenous and graded chicken were included in the study.

3.5.2 Exclusion Criteria

Farmers rearing chicken who did not consent to the study and do not meet any of the above inclusion criteria.

3.6 Sampling Techniques and Sample size

Random sampling was used in the identification of households, where the chicken waste was sampled and a questionnaire administered to the farmer. The first household was identified by the guidance of random distribution geo codes generated using Google maps or the arc GIS application.

3.6.1 Sample Size Determination

Cochran (1977) was used to calculate the sample size, by assuming the expected prevalence at 88.46% applied from a study on the prevalence of Salmonella in poultry processing environments in wet markets, Malaysia (Nidaullah *et al*, 2017), 95% confidence interval and desired absolute precision of 5%.

$$n_o = \frac{Z^2 \times P\,(1-P)}{d^2}$$

where, n_0 is the sample size, z is the selected critical value of desired confidence level (1.96), p is the estimated proportion in the population which is ˆp= 0.8846 q is 1-p.

$$n_0 = \frac{1.96^2 \times 0.8846\ (1- 0.8846)}{0.052^2}$$

157 chicken households

A total of 169 chicken households were randomly identified where a representative chicken waste sample was obtained in duplicates and a questionnaire administered. 12 samples were added in order to cover for non-respondence.

Table 3. 1 Representation of number of households i.e. samples collected per sub-county and the totals

Sub-county	**No. of samples collected**
Webuye East	24
Kanduyi	29
Kabuchai (Mt Elgon)	28
Kimilili	30
Bumula	29
Sirisia	29
TOTAL	169

3.7 Data collection tools/ instruments

The questionnaire (appendix II) was administered to the farmers. The questionnaire is divided into 4 sections namely; demographic profile, chicken production, antibiotic use & therapy and hygienic practices in chicken production. This was meant to inform various factors including the culture of use of antibiotics in chicken production and the measures put in place during chicken production to curb bacterial infection in chicken among the chicken keeping households.

3.7.1 Pilot Study or Pre-Testing

A pilot study and pre-testing was done in Webuye east sub-county. 24 households were sampled. This was ideal in provision of credible facts on the study population.

3.7.2 Validity and Reliability

Validity was ensured by pretesting of the research tools prior to the data collection exercise.

Reliability was ensured by collection of the chicken waste samples in duplicates and proper training of all research assistants on administration of the questionnaires and proper sample collection.

3.8 Data Collection Techniques

Chicken waste samples were collected using swabs in duplicates per household. On identification of a fresh chicken waste sample, gloves were worn and seal broken to open the vial. The chicken waste was swabbed to ensure the bud was soaked and chicken waste attached to it. The swab was carefully inserted back into the Amies transport media and closed. The vial was clearly labelled with sample number, sub-county, time and date of collection. Samples were transported in a cool box at -20^{o}C to the Laboratory for analysis within 24 hours – detection of *Salmonella* & *Campylobacter* species and sensitivity testing as per the procedures described below.

3.9 Microbiological Tests

3.9.1 Protocol for qualitative determination of Salmonella

Detection, enumeration and serotyping of Salmonella was done in accordance to BS EN ISO 6579-1:2017 – Microbiology of the food chain – horizontal method for the detection, enumeration and serotyping of Salmonella. It provided guidelines on the detection of Salmonella species from environmental samples.

Pre-enrichment in non-selective liquid medium - about 25g of the sample was inoculated in 225ml of buffered peptone water at ambient temperature and incubated for 24 hours at 37^{o}C.

Selective enrichment - 0.1ml of the culture obtained was then inoculated with Rappaport-Vassiliadis medium (RVS) and Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth. The RVS was incubated at 35^{o}C for 24 hours and the MKTTn broth at 37^{o}C for 24 hours.

Isolation - Cultures obtained were inoculated in selective and differential media. Cultures obtained from the RVS broth were inoculated in Xylose Lysine Deoxycholate agar (XLD agar), incubated at 37^{o}C for 24hours, whereas cultures obtained from MKTTn broth were inoculated in Mackonkey agar at 37^{0}C for 24 hours (BS EN ISO 6579-1:2017)

Biochemical Confirmation

TSI Agar- Sub-cultured suspect colonies grew and gave Alkaline-Acid slant (Pink-Orange) and Black color from Hydrogen Sulphide, proving positive for Salmonella.

SIM Agar- Indole reaction- On adding kovac's reagent the pink ring would appear, showing presence of motility (BS EN ISO 6579-1:2017).

3.9.2 Laboratory protocols for Campylobacter

Campylobacter enrichment

The swab was soaked in 10ml of the Bolton Broth, and allowed to stand for 10 minutes. 10ml of the labelled Universal tube was taken and incubated at 37°C under micro aerobic conditions for 4-6 hours. Then at 41.5°C under micro aerobic conditions for 44±4 hours.

Campylobacter culture isolation

The broth was further sub-cultured onto Campylobacter agar and incubated at 41.5°C under micro aerobic conditions for 42 hours.

Plates were then examined for the presence of *Campylobacter* spp. after incubation.

Campylobacter confirmation

For each sample (or initial mCCDA plate) that showed growth, four suspect colonies were taken and sub-cultured on Columbia blood agar plates. The blood agar plates were then incubated under micro-aerobic conditions for 48 hours at 41^{o}C and observed for any hemolysis.

Identification

For each sample that showed growth, suspect colonies were subjected to Oxidase discs and Catalase test. They were observed for purple color formation on oxidase discs and bubbles in catalase test after addition of hydrogen peroxide.

3.9.3 Laboratory Protocol for Antimicrobial Sensitivity Testing

Kirby Bauer disc diffusion technic was used for sensitivity testing. Briefly, Mueller Hinton agar was prepared and sterilized using an autoclave at 121^{o}C, 15 psi for 15 minutes. The agar was then dispensed in sterile petri-dishes and allowed to settle. Using McFarland's standard, the colonies were standardized to 0.5 by distilled water.

Using a dry swab, an inoculum of the sample was picked and streaked on the plate to form a lawn. The antibiotics (Tetracycline, Cotrimoxazole, Ampicillin, Imipinem) were dispensed and incubated at 35^{o}C for 24 hours and the observed for zone of inhibition and the diameter measured using an mm ruler (CLSI guidelines for AST).

Table 3. 2 Clinical and Laboratory Standards Institute (CLSI) M100 Performance Standards for Antimicrobial Susceptibility Testing

Antibiotic	**Sensitive**	**Intermediate**	**Resistant**
Tetracycline	≥ 15	12 – 14	≤ 11
Ampicillin	≥ 17	14 -16	≤ 13
Co-trimoxazole	≥ 26	26 – 32	≤ 32
Imipinem	≥ 23	20-22	≤ 19

3.10 Data Analysis

Prevalence was calculated by dividing the number of positive samples by the total number of samples. Data from the questionnaires was managed using Microsoft Excel 2010 i.e. data entry, coding, cleaning and summarization. SPSS version 23 software was used in data analysis, with Pearson's r correlation used to analyze the relationship between variables. Table 3.3 below shows ranges used to explain the degree of correlation among the variables.

Table 3. 3: Values showing degree of correlation among variables

Sign of correlation coefficient	**Strong**	**Moderate**	**Weak**	**Very weak**
+ values **Positive relationship**	0.5 to 1.0	0.3 to 0.49	0.1 to 0.29	0 to 0.09
- Values **Negative relationship**	-1.0 to -0.5	-0.49 to -0.3	-0.29 to -0.1	-0.09 to 0

Logistic regression modelling was used in the analysis of bacterial sensitivity i.e the probability of having bacteria present for every antibiotic administered and for every traditional medicine used. The dependent variable was taken to be the presence or absence of bacteria with the types of antibiotics and traditional medicine as independent variables.

3.11 Logistical and Ethical Considerations

Ethical approval was sought from the Kenyatta University Research Ethics Committee (Appendix III). Permission was obtained from the National Commission for Science Technology and Innovation (NACOSTI) to conduct the study (Appendix V). Thereafter, the Bungoma county – Ministry of Agriculture, Livestock and Fisheries was informed on the study and granted access by informing the respective chiefs on the intention of the study and the agricultural extension officers in the area informed. Informed consent (Appendix I) was further sought from the respondents (farmers) during sampling and the intention of the study clarified to encourage voluntary participation. Confidentiality was ensured by coding of the questionnaires other than using the farmers' names.

CHAPTER FOUR: RESULTS

Introduction

This chapter presents detailed data analysis and findings of the study, depicted in graphs, tables and pie-charts. A total of 169 households were sampled and a questionnaire administered to a farmer. The response rate was 100%.

Analysis within this study was mainly based on 3 research objectives which were to establish; 1. Prevalence of salmonella and campylobacter in Bungoma county 2. Sensitivity of Salmonella and Campylobacter species found in chicken waste to Ampicillin, Tetracycline, Imipinem and CoTrimoxazole antibiotics 3. identifying risk factors associated to the spread of drug resistant bacteria through the environment in chicken production**.**

4.1 Demographic and socio-economic characteristics of the respondents

Results indicate that a majority 74.6% (n= 126) of the respondents were female with only 25.4% (n=43) being male.

Table 4. 1: Gender of the respondents

Gender		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	male	43	25.4	25.4	25.4
	female	126	74.6	74.6	100.0
	Total	**169**	**100.0**	**100.0**	

Most of the respondents attained primary and secondary education 33.7% and 34.9% respectively, with the minority having attained tertiary education 12.4% (n=21).

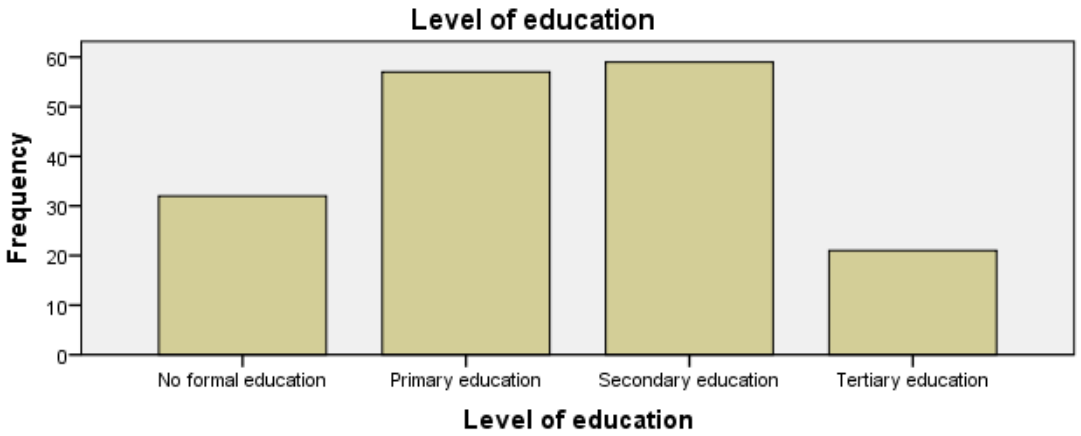


Figure 4. 1: Level of education of the respondents

On the level of income, the number of respondents who had an income of less than ksh 10,000 per month 47.9% (n=81) was compared to those who had an income of more than ksh 10,000 in a month 52.1% (n=88).

Table 4. 2: Level of income

Level of income		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Less than 10000 per month	81	47.9	47.9	47.9
	More than 10000 per month	88	52.1	52.1	100.0
	Total	**169**	**100.0**	**100.0**	

The findings of the study indicated that majority of the farmers in Bungoma county practiced backyard/free-range production 97% (n=164) where chicken interact with other livestock as opposed to intensive production at 3% (n=5) as shown in Table 4.3 below;

Table 4. 3: Type of production practiced

Type of production		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Backyard production	164	97.0	97.0	97.0
	Intensive production	5	3.0	3.0	100.0
	Total	**169**	**100.0**	**100.0**	

Farmers attributed this to the relative ease in care and the method being less costly. The study also showed that 85.2% of the farmers (n=144) reared indigenous (kienyeji) chicken, 8.3% (n=14) rearing layers or broilers and 6.5% (n=11) rearing mixed breed also referred to as the improved indigenous (kienyeji) breed. Many respondents were small scale farmers with a majority at 93.49% (n=158) rearing less than 100 chicken and only 6.51% (n=11) keeping a flock of more than 100 chicken.

A significant majority of the farmers solely used homemade feeds on their flock 70.4% (n=119), with 5.3% (n=9) depending on commercial feeds and 24.3% (n=41) using both commercial and homemade feeds.

Table 4. 4: Types of feed used

Types of feed used		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Commercial feeds	9	5.3	5.3	5.3
	Homemade feeds	119	70.4	70.4	75.7
	Both	41	24.3	24.3	100.0
	Total	**169**	**100.0**	**100.0**	

4.2 Establishing prevalence of *Salmonella* and *Campylobacter* species in chicken waste Bungoma county

The prevalence for Campylobacter was higher in the county of Bungoma at 4.32% compared to that of *Salmonella* at 1.44% (figure 4.1)

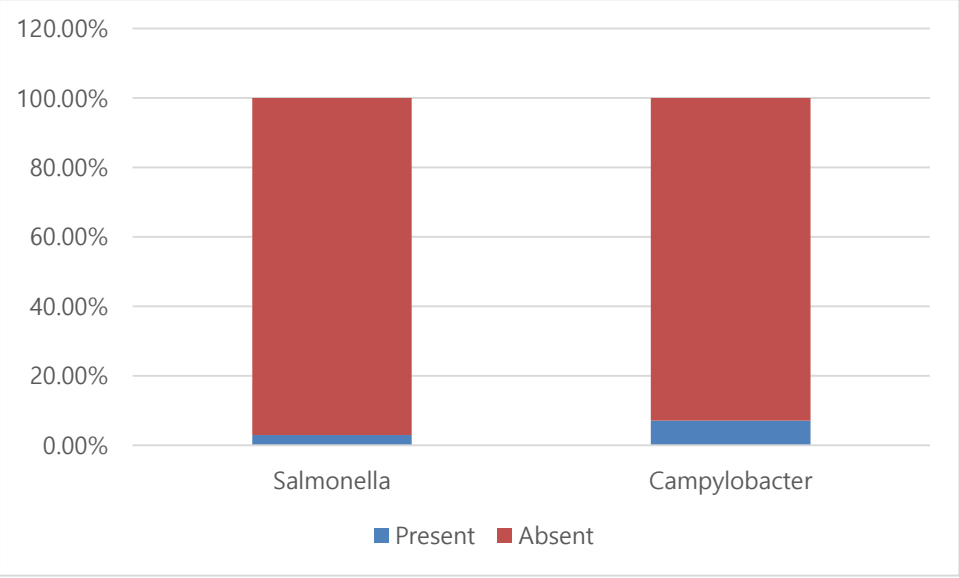


Figure 4. 2: Prevalence of Salmonella and Campylobacter species in chicken waste, Bungoma county

4.3 Sensitivity of *Salmonella* and *Campylobacter* species found in chicken waste to antibiotics

From the questionnaire, data submitted by the farmers on antimicrobial use and therapy, indicated below diseases common in those frequencies;

Table 4. 5: What types of diseases are common to their ages?

Types of disease		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Newcastle disease	20	11.8	11.8	11.8
	Fowl typhoid	35	20.7	20.7	32.5
	Influenza and Brochitis	31	18.3	18.3	50.9
	Newcastle disease & Fowl typhoid	46	27.2	27.2	78.1
	Fowl typhoid & Influenza and Brochitis	19	11.2	11.2	89.3
	Newcastle disease & Influenza and Brochitis	7	4.1	4.1	93.5
	Others e.g. coccidiosis, diahohea, swelling eye	6	3.6	3.6	97.0
	None	5	3.0	3.0	100.0
	Total	**169**	**100.0**	**100.0**	

Results also indicated that 54.5% (n=92) farmers used antibiotics in the treatment or management of diseases, 18.9% (n=32) used traditional medicine, 26% (n=44) used both antibiotics and traditional medicine whereas only 0.6% (n=1) did not use any form of therapy during chicken production.

Table 4. 6: Types of medicine used during chicken production

Kind of medicine		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Antibiotics	92	54.4	54.4	54.4
	Traditional medicine (Aloe vera, e.t.c)	32	18.9	18.9	73.4
	Both	44	26.0	26.0	99.4
	None	1	.6	.6	100.0
	Total	**169**	**100.0**	**100.0**	

Farmers were also interviewed on the types of antibiotics frequently used on their flock. This was to guide on the types that are common in poultry production and human medicine hence the attendant risk of development in resistance and the threat it poses to public health. Table 4.7 below indicates results obtained;

Table 4. 7: Types of antibiotics used on the flock

Types of antibiotics		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Esb3 30%	75	44.4	44.4	44.4
	Chick Start	13	7.7	7.7	52.1
	Biotrim	13	7.7	7.7	59.8
	Others	25	14.8	14.8	74.6
	None	31	18.3	18.3	92.9
	Esb3 30% & Chick Start	12	7.1	7.1	100.0
	Total	**169**	**100.0**	**100.0**	

*Others include; biosol, Biotrim, Chick Start, Amoxillin, Panadol, syringe, Egocin, Trimovet, Tylodoxy

Frequency of use of antibiotics is a factor that plays a role in the development of resist ant bacteria. Below data indicates the frequency of use of antibiotics by farmers;

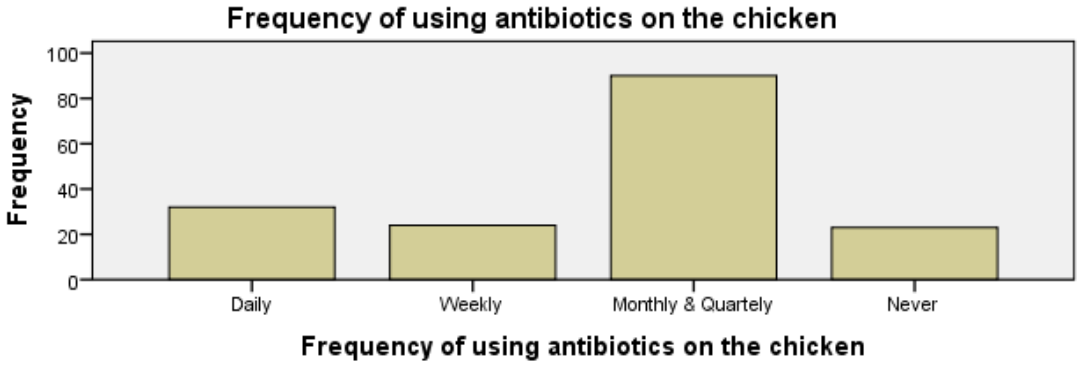


Figure 4. 3: Frequency of use of antibiotics on the flock

Results indicate that a majority 28.4% (n=48) of the farmers, administered antibiotics through drinking water.

Table 4. 8: Mode of administration of the antibiotics

How the drug is administered		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Drinking water	48	28.4	28.4	28.4
	Injection	9	5.3	5.3	33.7
	Nosal drops	48	28.4	28.4	62.1
	Others	1	.6	.6	62.7
	Drinking water & Nosal drops	32	18.9	18.9	81.7
	Drinking water, Injection & Nosal drops	10	5.9	5.9	87.6
	None	16	9.5	9.5	97.0
	Injection & Nosal drops	5	3.0	3.0	100.0
	Total	**169**	**100.0**	**100.0**	

Framers were also interviewed on whether they use antibiotics for growth promotion besides management of infections/ diseases. Results indicated that 13% (n=22) of the farmers used antibiotics for growth promotion whereas 87% (n=147) did not use antibiotics for growth promotion as shown in table 4.9 below;

Table 4. 9: Use antibiotics for growth promotion

Response		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	22	13.0	13.0	13.0
	No	147	87.0	87.0	100.0
	Total	**169**	**100.0**	**100.0**	

Results further indicated that only 7.1% (n=12) of the farmers, consulted with a veterinary officer before acquiring antibiotics with majority, 81.7% (n=138) acquiring the drugs over the counter.

Table 4. 10: Mode of acquiring the antibiotics

Source		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Referral from a veterinary doctor	12	7.1	7.1	7.1
	Over the counter	138	81.7	81.7	88.8
	None	19	11.2	11.2	100.0
	Total	**169**	**100.0**	**100.0**	

To measure the sensitivity of *Salmonella* and *Campylobacter* species in chicken waste specific to the 4 antibiotics, logistic regression was used with presence or absence of the two bacterial species taken as the dependent variable while the antibiotics type was taken as the independent variable. Sensitivity analysis was then done to the model so as to determine its capability to identify true positives i.e. correctly identify households with presence of any of the two bacteria. Results are as given below;

Table 4. 11: Logistic regression results for Salmonella

Variable	**Min**	**IQ**	**Median**	**Max**	**Intercept**	**Drugnum**
Tetracycline	-0.186	-0.186	-0.1865	2.850	-4.043	-16.523
Ampicillin	0	0.001	0	0.05	2.713	3697.038
Imipinem	-0.186	-0.186	-0.186	-0.186	-5.668	-0.004
Cotrimoxazole	-0.186	-0.186	-0.186	-0.186	1.44	0.996
Null deviance	20.936 on 138 degree of freedom					
Residual deviance	20.207 on 137 degree of freedom					
Number of fis her scoring ite rations	19					

From the logistic regression modeling results traditional medicine is 16.5 times less likely to be associated with the presence of a Salmonella bacteria compared to the use of antibiotics. Sensitivity obtained for the model is 1 which represents a high sensitivity proportion showing that the model is good for the prediction of presence of the salmonella bacteria.

Table 4. 12: Antimicrobial susceptibility profiles of Salmonella

Antibiotic	**Susceptible No(%)**	**Intermediate No(%)**	**Resistant No(%)**
Tetracycline	0 (0)	0 (0)	5(100)
Ampicillin	0 (0)	1(20)	4(80)
Co-trimoxazole	0 (0)	0 (0)	5(100)
Imipinem	0 (0)	0 (0)	5(100)

Biochemical confirmation was done in SIM agar (Sulphur, Indole and Motility) Blackening of the medium along the line of inoculation proves a positive test for Hydrogen Sulphide (H_2S), with the diffuse zone of growth showing positive for motility. Positive test for Indole is denoted by pink color after addition of Kovacs reagent. Plates below show samples that tested positive for *Salmonella*.

Plate 4. 1: Discs showing samples positive for Salmonella specie

Campylobacter

The results indicate that traditional medicine is 25.66 times less likely to cause the presence of Campylobacter bacteria compared to antibiotics. Sensitivity of the model is 0 meaning that the model is not a good model for the prediction of presence of campylobacter bacteria

Table 4. 13: Logistic regression results for Campylobacter

Variable	**Min**	**IQ**	**Median**	**Max**	**Intercept**	**Drugnum**
Tetracycline	-0.298	0.00002	-0.187	0.00002	22.57	-25.66
Ampicillin	-0.298	0.00002	0	0.00002	4474.90	4474.90
Imipinem	-0.298	0.00002	-0.187	0.00002	0.005	0.006
Cotrimoxazole	-0.298	0.00002	-0.187	0.00002	0.996	0.995
Null deviance	121.4292 on 138 degree of freedom					
Residual deviance	8.2269 on 137 degree of freedom					
Number of fis her scoring ite rations	21					

The results of antimicrobial susceptibility testing indicated that all bacteria were resistant to the 4 types of antibiotics tested against as shown in table 4.14 below;

Table 4. 14: Antimicrobial susceptibility profiles of Campylobacter

Antibiotic	**Susceptible No(%)**	**Intermediate No(%)**	**Resistant No(%)**
Tetracycline	0 (0)	0 (0)	12(100)
Ampicillin	0 (0)	0 (0)	12(100)
Co-trimoxazole	0 (0)	0 (0)	12(100)
Imipinem	0 (0)	0 (0)	12(100)

Campylobacter confirmation was done through hemolysis and oxidase test. Production of bubbles proves positive for catalase test with production of Hydrogen peroxide, positive for oxidase test is shown by the purple color

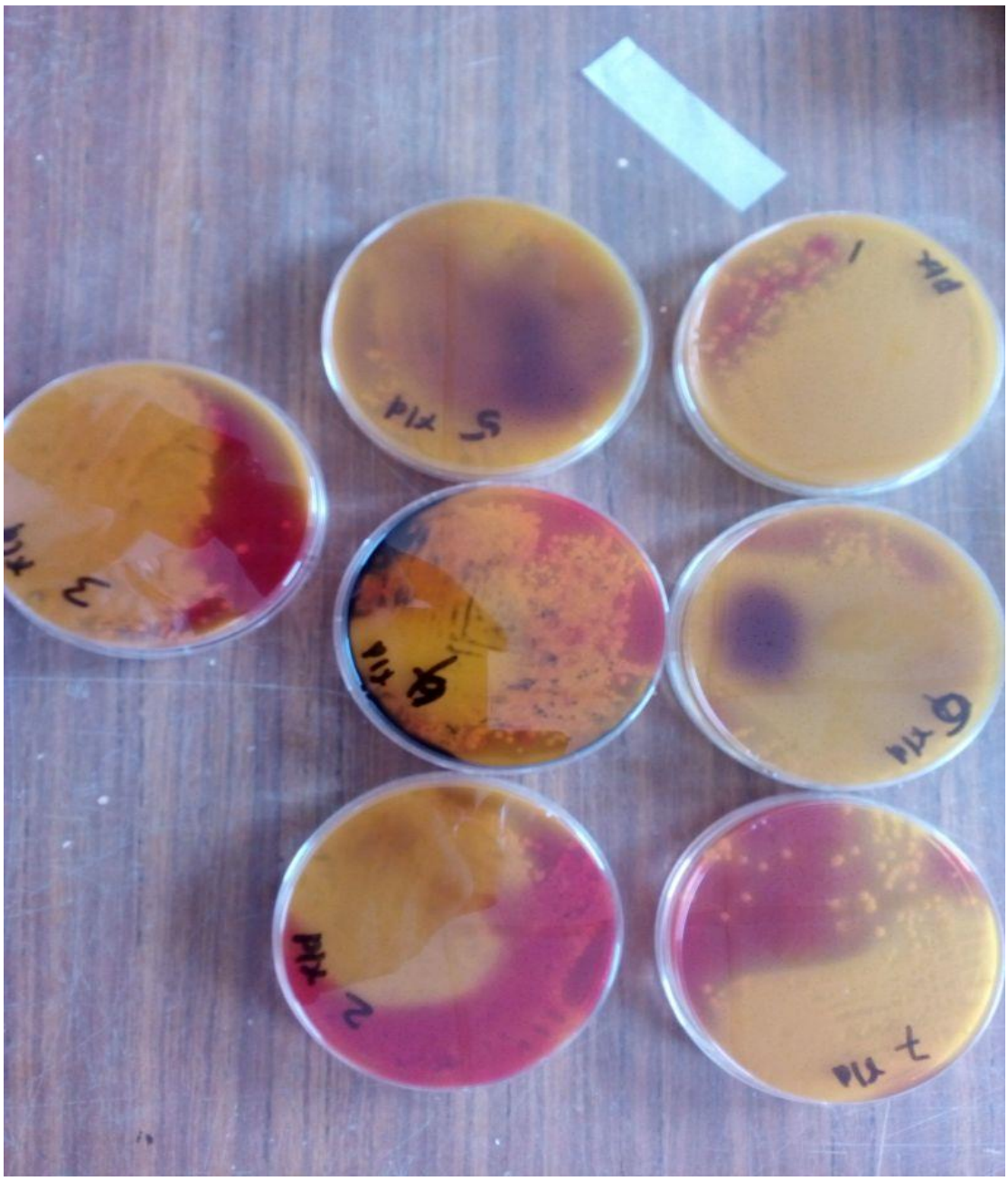


Plate 4. 2: Discs showing samples tested positive for *Campylobacter* species

The zones of inhibition around the disc shows the degree of sensitivity of the bacteria to the antibiotic.

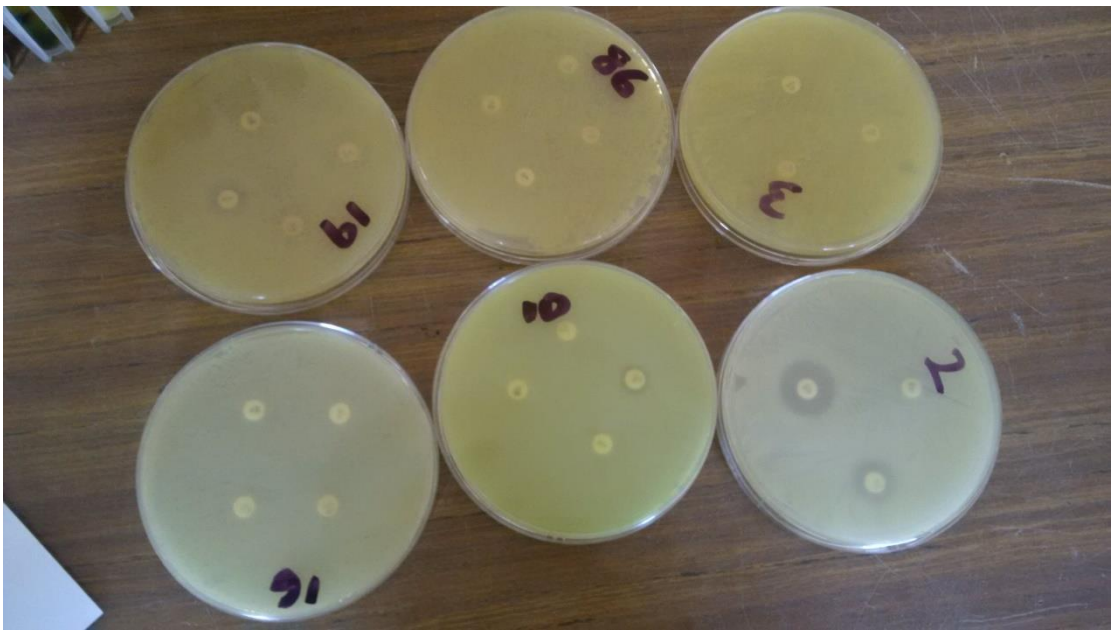


Plate 4. 3: Discs showing samples resistant after antimicrobial sensitivity testing

4.4 Perceived risk factors for the spread of drug resistant *Salmonella* and *Campylobacter* species.

Perceived risk factors were associated with various hygienic practices conducted by farmers during poultry production that would influence infection of chicken by bacteria and development of resistance against antibiotics by the bacteria. These practices include management of infections which affect the transfer of resistant bacteria from chicken flock to humans. Practices analyzed include; availability of proper and separate housing for the chicken, availability of water-bath at the entrance and how often the chicken pen are cleaned respectively.

Majority of the interviewed farmers 83.4% (n=141) shared a house with the chicken flock as opposed to 16.6% (n=28) who had a separate housing for the chicken as indicated by figures on table 4.15 below;

Table 4. 15: Availability of Proper & separate housing for the chicken

Response		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	28	16.6	16.6	16.6
	No	141	83.4	83.4	100.0
	Total	**169**	**100.0**	**100.0**	

Results further indicated that only 1.8% of the farmers had a water bath at the entrance of the chicken pens. This was only available with farmers who practiced intensive production to minimize transfer of bacteria from the environment into the chicken pen and vice versa. Table 4.16 below shows that most farmers 98.2% (n=166) did not have a water bath.

Table 4. 16: Availability of Water – bath at the entrance of chicken pen

Response		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	3	1.8	1.8	1.8
	No	166	98.2	98.2	100.0
	Total	**169**	**100.0**	**100.0**	

Of the farmers interviewed, 85.8% cleaned the chicken pens daily compared to 10.1% who cleaned the pens weekly and a minority 7.1% of the interviewed farmers who cleaned the pens occasionally, as depicted on table 4.17 below. This factor infers to the possibility of the chicken getting infections and transfer of infections among the flock.

Table 4. 17: Frequency of cleaning the chicken pens

Frequency		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Daily	145	85.8	85.8	85.8
	Weekly	17	10.1	10.1	95.9
	Not often	7	4.1	4.1	100.0
	Total	**169**	**100.0**	**100.0**	

Results indicated that a majority of the respondents 90.5% (n=153) did not receive any training on handling and dispensation of antibiotics during chicken production as opposed to 9.5% (n=16) of the farmers who received training on proper dispensation of antibiotics during chicken production as shown below;

Table 4.18: Have you and any other workers handling chicken received any training on antimicrobial dispensation?

Response		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	16	9.5	9.5	9.5
	No	153	90.5	90.5	100.0
	Total	**169**	**100.0**	**100.0**	

Results further indicated that 85.2% of the farmers interviewed used chicken manure in the garden while 14.8% composted it as waste.

Table 4.18: Disposal of chicken manure

Disposal method		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Use in the farm/manure	144	85.2	85.2	85.2
	Compost as garbage	25	14.8	14.8	100.0
	Total	**169**	**100.0**	**100.0**	

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter presents discussion of the results of the study "Prevalence and Antibiotic Susceptibility patterns of *Salmonella* and *Campylobacter* species in Chicken Waste, Bungoma County" conclusions of the study and recommendations based on the findings.

5.2 DISCUSSSION

5.2.1 Prevalence of *Salmonella* and *Campylobacter* species

The prevalence of *Campylobacter* species was higher at 4.32% compared to *Salmonella* species at 1.44% (Figure 4.1). It is evident from the study that the low prevalence of *Salmonella* species is majorly attributed to the investment by farmers in the control of the pathogen. Agro-vets who are the primary point of consultation and recommendation of drugs to the farmers (Figure 4.2) showed to have proper knowledge on signs and symptoms thus proper reference of drugs for use in cases of Salmonella bacterial infection unlike for the treatment and control of Campylobacter.

A study conducted on the prevalence and antimicrobial resistance of *Salmonella* isolated from livestock and humans in Korogocho and Viwandani slums showed a low prevalence of 0.62% (5 out of 801 samples) for *Salmonella* species (Cianjoka, 2018)

A similar study on antimicrobial resistance genes in *Salmonella* and *Escherichia coli* isolates from chicken droppings in Nairobi also established lower levels of *Salmonella*; 57% and 12% of the analyzed samples contained *Escherichia coli* and *Salmonella* species respectively (Lang'ata *et al,* 2019)

A European study (Goncalves-Tenorio, *et al*, 2018) conducted on the prevalence of pathogens in poultry meat suggested that *Staphylococcus aureus* was the main pathogen detected in poultry meat (38.5%) followed by *Campylobacter* species (33.3%) with *Listeria monocytogenes* and *Salmonella* species presenting lower prevalence of 19.30% and 7.10% respectively.

In chicken meat, compared to other poultry meat, *Campylobacter* species presented the highest prevalence of 48.6% (Goncalves-Tenorio, *et al*, 2018). Poultry has been pointed out as a major source of campylobacteriosis in humans especially *C. jejuni* and *C. coli* because the bacterium is enteric; found in the intestinal tract of chicken (Humphrey *et al*, 2005).

A study in the United states of America on the prevalence of Salmonella in diverse environmental farm samples found a prevalence rate of 4.7% with majority of the positive findings from swine farms followed by dairy, poultry and beef farms respectively. (Rodriguez *et al*, 2006)

Prevalence of 97% (n=5) at layer farms and 93% at broiler farms was established in the study of prevalence and types of *Campylobacter* on poultry farms and in their direct environment. The study also found identical sequence types in surface water at the farm environment with detectable species being; C. *jejuni*, C. *coli* and C. *lari* (Schets *et al*, 2017)

The higher prevalence of *Campylobacter* bacteria could be related to the poor hygienic standards and sanitary conditions in chicken production. Most of the farmers and Agro-vets interviewed did not seem to have knowledge or awareness on the prevention and treatment of Campylobacter bacterial infection.

5.2.2 Sensitivity of *Salmonella* and *Campylobacter* species found in chicken waste to the specific antibiotics

From the logistic regression modeling results, use of traditional medicine is 16.5 times less likely and 25.66 times less likely to be associated with the presence of *Salmonella* bacteria and *Campylobacter* bacteria respectively compared to the use of antibiotics, as shown in Figure 4.3.

Bacteria was found resistant to all the antibiotics tested against; tetracycline, amoxilin, impenem and co-trimoxazole.

Salmonella bacteria is mostly associated with and is widely prevalent in food animals such as chicken and can be transmitted to humans through the environment, food processing/production or through physical contact (Chlebicz & Śliżewska, 2018)

Resistance develops due to various factors such as the overuse/misuse of antibiotics during chicken production. Most farmers 54.4% (n=92) used antibiotics during chicken production while 26% (n= 44) used both antibiotics and traditional medicine (Table 4.6). Of the 169 interviewed farmers, only 7.13% (n=12) consulted with a veterinary officer before acquiring and using antibiotics whereas 81.7% (n=138) bought over the counter (Figure 4.10).

A number of studies have demonstrated increased resistance over time for S*almonella* bacteria. A review on antimicrobial resistance for bacterial poultry pathogens showed that Enterobacteriaceae, displayed considerably higher levels of AMR compared with *Salmonella pullorum/Salmonella gallinarum,* with prevalence of resistance over >80% for ampicillin, amoxicillin, tetracycline across studies. (Nguyen *et al*, 2015)

The use of traditional medicine such as Aloe Vera and Capsicum was observed prominent especially among farmers in Kabuchai, Kimilili and Bumula sub counties. Some farmers solely depended on traditional medicine, however, others used both traditional and conventional medicine for prevention and treatment of diseases. Aloe species was found to be the most commonly used since it served as a broad spectrum remedy in poultry health management among farmers in western Kenya (Okitoi *et al* 2007). The low prevalence of resistant bacteria could be linked to the use of traditional medicine.

Most traditional medicine used are plant based. According to Gupta and Birdi 2017, metabolites found in traditional medicine can serve as potentials for antimicrobials and resistance modifiers due to the presence of a wide variety of secondary metabolites. The pathogen may have reduced ability to develop resistance to botanicals/traditional medicine owing to the fact that they not only act to kill the bacteria but also to disrupt major pathogenic activities (Gupta & Birdi, 2017).

5.2.3 Analysis of risk factors for the spread of resistant bacteria associated with chicken production

The intestinal flora of poultry can provide a reservoir for food-borne pathogens including *Salmonella* and *Campylobacter* bacteria which could develop antibiotic-resistance thus can infect or colonize humans via the food chain.

To find out risk factors for the spread of resistant bacteria correlation analysis was used to analyze various variables i.e. frequency of antibiotics administration, use of antibiotics for growth promotion, how the antibiotics were acquired, type of production practiced and feeds used, how chicken waste was disposed and whether the farmers had received ant training on antimicrobial dispensation.

169 farmers were surveyed about how often they used antibiotics and how they acquired the antibiotics. A Pearson's r data analysis revealed a weak positive correlation (Table 5.1) The prudent use of antibiotics in prophylaxis and treatment is encouraged because it helps prevent the spread of bacteria/infection. The lack of use of antibiotics even in vaccination predisposes the chicken to the danger of being susceptible to infections thus exposure to resistant bacteria. Most farmers interviewed acquired the drugs over the counter without consultation with a veterinary officer (Table 4.10)

Table 5. 1: How often do they use antibiotics compared to how they acquire the a ntibiotics

Correlations

		Frequency of using antibiotics on the chicken	How do you acquire the antibiotics
Frequency of using antibiotics on the chicken	Pearson Correlation	1	.187*
	Sig. (2-tailed)		.015
	N	169	169
How do you acquire the antibiotics	Pearson Correlation	.187*	1
	Sig. (2-tailed)	.015	
	N	169	169

Results indicated a negative moderate relationship between how the antibiotics were administered and whether the farmers had received any training on antimicrobial

dispensation (Table 5.2). Most farmers administered the antibiotics via drinking water, thus spillage of the water would lead to groundwater pollution. Lack of training on proper dispensation of antibiotics would translate to improper dosage and poor handling of antibiotics during chicken production. These practices promote the likelihood of development of resistant bacteria as a result of excessive exposure to ant ibiotics.

Table 5. 2: How do you administer the antibiotics compared to have you and any other workers handling chicken received any training on antimicrobial dispensation

Correlations

		How the drug is administered	Have the workers received training on antimicrobial dispensation
How the drug is administered	Pearson Correlation	1	-.043*
	Sig. (2-tailed)		.579
	N	169	169
Have the workers received training on antimicrobial dispensation	Pearson Correlation	-.043*	1
	Sig. (2-tailed)	.579	
	N	169	169

Of the interviewed farmers, a correlation analysis between frequency of cleaning the chicken pens and frequency of use of antibiotics revealed a strong negative relationship (Table 5.3). Therefore, the study established that the frequency of cleaning the chicken pens did not influence on how much antibiotics were being used during chicken production. However, a review on the effect of management practices on Campylobacter prevalence in poultry farms highlighted factors such as biosecurity, antibiotic usage, cleaning and disinfection, water and seasonality of infections and carriage as risk factors in the spread of campylobacter bacteria among poultry flocks. (Sibanda *et al*, 2018)

Table 5. 3: How often do you clean the chicken pens vs How often do you use antibiotics on the chicken

Correlations

		How often do you clean the chicken pens	Frequency of using antibiotics on the chicken
How often do you clean the chicken pens	Pearson Correlation	1	-.053
	Sig. (2-tailed)		.493
	N	169	169
Frequency of using antibiotics on the chicken	Pearson Correlation	-.053	1
	Sig. (2-tailed)	.493	
	N	169	169

A correlation analysis on the relationship between frequency of use of antibiotics and disposal of chicken waste produced a strong positive relationship (Table 5.4). This means an increase in the use of antibiotics led to an increase in chicken waste disposal. Most farmers interviewed (85.2%) responded to be using chicken waste as manure in the garden for crop farming as opposed to 14.8% who composted it as garbage.

Table 5. 4: How often do you use antibiotics on the chicken vs How do you dispose chicken manure?

Correlations

		Frequency of using antibiotics on the chicken	How do you dispose chicken manure
Frequency of using antibiotics on the chicken	Pearson Correlation	1	.064
	Sig. (2-tailed)		.409
	N	169	169
How do you dispose chicken manure	Pearson Correlation	.064	1
	Sig. (2-tailed)	.409	
	N	169	169

The analysis further established that of the 169 farmers interviewed, there was a strong positive relationship between the type of feed used and whether the farmer was using antibiotics for growth promotion (Table 5.5). Some commercial feeds contain in-feed antibiotics for growth promotion.

Table 5. 5: What type of feed do you use vs Do you use antibiotics for growth promotion apart from treatment

Correlations

		Type of feed used	Use antibiotics for growth promotion apart from treatment
Type of feed used	Pearson Correlation	1	.075
	Sig. (2-tailed)		.335
	N	169	169
Use antibiotics for growth promotion apart from treatment	Pearson Correlation	.075	1
	Sig. (2-tailed)	.335	
	N	169	169

A study in Vietnam on potential risk factors for carriage of antimicrobial-resistant Escherichia coli in households and small scale farmers identified the use of antibiotics and farm management practices as potential factors for AMR (Nguyen *et al*, 2015)

5.3 CONCLUSION

i. The prevalence of *Campylobacter* bacteria was 4.32% and that of *Salmonella* bacteria was found to be 1.44% in Chicken waste, Bungoma county.

ii. All the tested bacteria isolates were found to be resistant to all the four tested antibiotics; Tetracycline, Ampycillin, Imipinem and Co-trimazole.

iii. Use of antibiotics for growth promotion, frequency of use of antibiotics and disposal of chicken waste were found to be risk factors associated with development and spread of antimicrobial resistance in chicken production.

5.4 RECOMMENDATIONS

5.4.1 Recommendations from the study

1. Farmers should be encouraged to obtain already vaccinated chicks and conduct periodic vaccinations which will help reduce disease infections thus reduced incidences of bacterial infection and use of antibiotics for treatment.

2. Use of traditional medicine such as Aloe vera and Capsicum should be encouraged because its less costly, easily available and has proven effective in the prevention and treatment of diseases in chicken. Use of traditional medicine also minimizes the risk of development of resistant bacteria in chicken.

3. It is paramount for farmers to be trained on good practices on the use of antibiotics during chicken production i.e. acquiring of drugs, use, administration, storage and disposal. Farmers should also be trained on proper disposal of chicken waste to reduce the risk of transfer of resistant bacteria through the environment.

4. There is need to develop and enhance regulation on the use and purchase of antibiotics used in poultry production and surveillance/ monitoring of drugs used in food-animal production.

5.4.2 Recommendations for further research

1. With more resources and funding, more farmers should be included in the study in order to get a wide range of data on practices regarding antibiotic use during chicken production.

2. Research should be conducted on the possibility of transfer of resistant bacteria through food crops since 85.2% of the interviewed farmers used chicken waste as compost manure in the gardens.

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APPENDICES

APPENDIX I: PARTICIPANT INFORMATION CONSENT FORM

This consent form is for farmers practicing chicken production in Bungoma county, who are invited to participate in this research study.

Title of Study: Prevalence and antibiotic sensitivity of *Salmonella* and *Campylobacter* species in chicken waste, Bungoma county, Kenya.

Principal Investigator: Kaburia Joan Ntinyari, Kenyatta University

Introduction

I am a student in the school of public health, Kenyatta University undertaking a degree in master of science environmental health. I am doing a research on *Salmonella* and *Campylobacter* prevalence in chicken waste in this area because of the culture of the people in western Kenya making them prominent in chicken keeping. The research will involve sampling of chicken waste and the filling of a questionnaire in a Tablet, which you will be guided through. Your participation in this research is entirely voluntary.

Purpose of the research:

Chicken can be infected with bacteria which can be transferred to humans through the environment via contaminated soil or water, through physical contact and also via improperly handled food. Too much use of antibiotics on chicken results to these bacteria becoming resistant making treatment not effective and infections more severe. You can help us by letting us know about your hygienic practices during chicken production and your use of antibiotics on chicken. This will help us identify the risk factors associated with presence of *Salmonella* and *Campylobacter* species and antimicrobial resistance.

Benefits

There will be no direct benefits to you from this study, however, your participation will help us find out more on the risks involved and how to prevent bacterial infection in chicken and spread of antimicrobial resistance.

Risks

Both the farmer and the chicken will not be exposed to any physical or health risks during and after the research.

Reimbursements

You will also not be provided with any form of incentive in order to participate in this research.

Participant Statement

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Participant Name

……

Signature ……. Date …….

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Researcher's name:

……

Signature: ……. Date

…….

For any other necessary information on the study, please contact:

Investigator: Kaburia Joan Ntinyari Telephone no: 0714342678

APPENDIX II: FARMERS QUESTIONNAIRE

This questionnaire contains 4 sections, designed to help the researcher establish the different types of chicken reared, types of production practiced by the farmers and their practices regarding use of antibiotics.

SECTION A: DEMOGRAPHIC PROFILE

1. Gender □ Male □ Female
2. Respondents highest level of education
   - □No formal education
   - □Primary education
   - □Secondary education
   - □Tertiary education (University/College/Polytechnic)
3. Level of income
   - □Less than ksh 10,000 per month
   - □More than ksh 10, 000 per month
4. Respondents' role in the farm
   - □Owner □Worker

SECTION B: LIVESTOCK PRODUCTION

5. What type of production do you practice?
   - □ Backyard Production □ Intensive production
6. What types/breeds of chicken do you have?
   - □ Kienyeji
   - □ Layers/ Broilers

□ Mixed breeds / Improved kienyeji

7. How many chicken do you have?

□ Less than 10

□ 10-100

□ More than 100

8. How old are the chicken?

□ 1day – 21 days

□ 3weeks – 9weeks

□ Above 10 weeks

9. What type of feed do you use?

□ Commercial feeds

□ Homemade feeds

□ Both

SECTION C: ANTIMICROBIAL USE AND THERAPY

10. What types of diseases are common to their ages?

a. Newcastle disease

b. Fowl typhoid

c. Influenza and Brochitis

d. Others ……

11. What kind of medicine do you use on your chicken?

□Antibiotics

□Traditional medicine (Aloe vera, e.t.c)

□ Both

12. What types of antibiotics have you used on you flock?

a. Esb_3 30%

b. Chick Start

c. Biotrim

d. Others (state)…….

13. How often do you use antibiotics on the chicken?

□ Daily □Weekly □ Monthly or quarterly □ Never

14. How do you administer the antibiotics?

a. Drinking water

b. Injection

c. Nosal drops

d. Other means (state)……

15. Do you use antibiotics for growth promotion apart from treatment?

Yes □ No □

16. How do you acquire the antibiotics?

□ Referral from a veterinary doctor □ Over the counter

SECTION D: HYGIENIC PRACTISES IN POULTRY PRODUCTION

17. What sanitary and hygiene measures available at the homestead/farm?

proper & separate housing available for the chicken Yes □ No □

water – bath available at the entrance Yes □ No □

how often do you clean the chicken pens? Daily □ Weekly □ Not often □

18. Have you and any other workers handling chicken received any training on antimicrobial dispensation?

□ Yes □ No

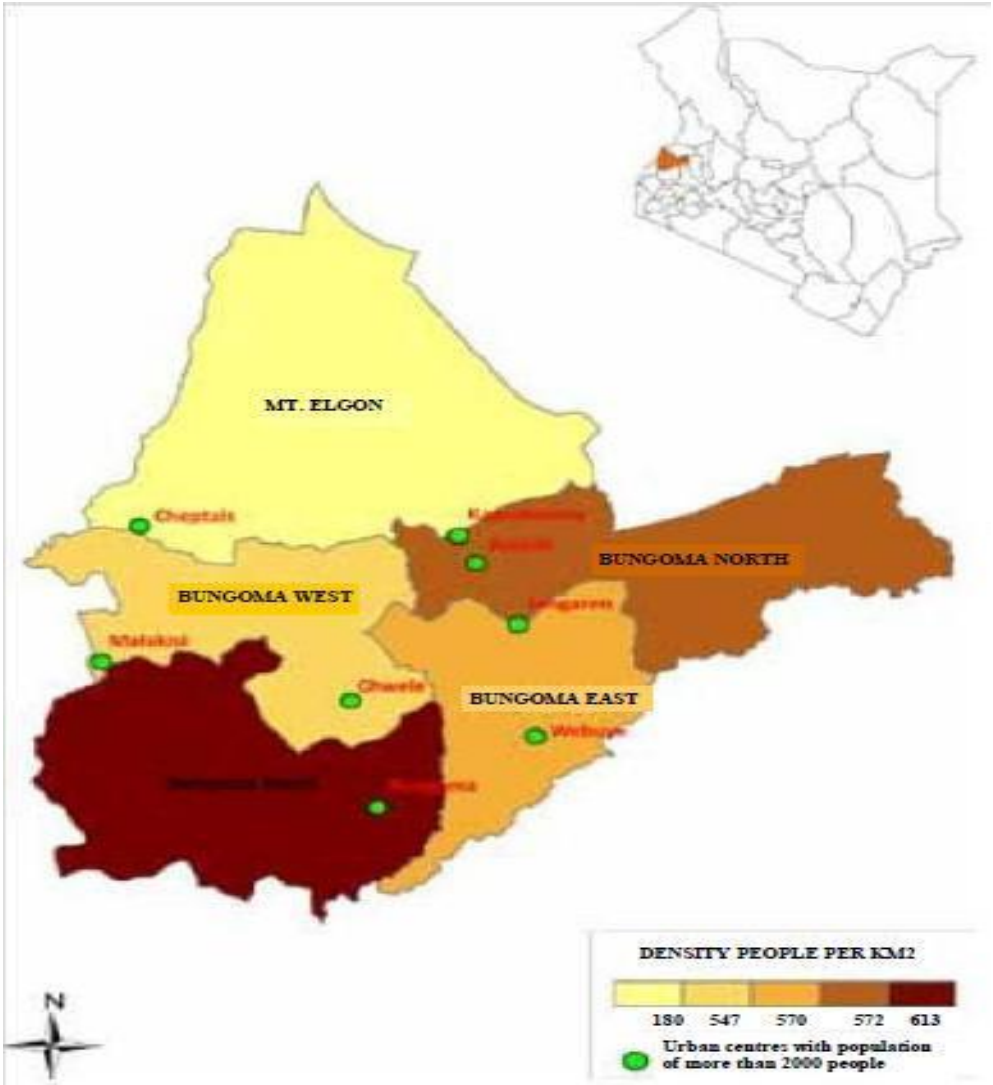
19. How do you dispose chicken manure?

□ Use in the farm / manure □ Compost as garbage

APPENDIX III: LETTER OF AUTHORIZATION- GRADUATE SCHOOL

APPENDIX IV: NACOSTI PERMIT

APPENDIX VI: BUNGOMA COUNTY MAP



Sub-counties

1. Webuye
2. Kimilili
3. Sirisia
4. Mt. Elgon
5. Kanduyi
6. Bumula