DETERMINATION OF ANTIMICROBIAL RESISTANT OF *SALMONELLA SPECIES* AND *ESCHERICHIA COLI* IN BROILER CHICKENS SLAUGHTERED IN COMMERCIAL ABATTOIRS IN LUSAKA PROVINCE, ZAMBIA

BY

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A Dissertation submitted to The University of Zambia in partial fulfillment of the requirements for the award of the degree of Masters of Public Health (MPH)

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DECLARATION

I **Nelson Phiri** declare that this dissertation titled: "**Determination of antimicrobial resistant *Salmonella species* and *Escherichia coli* in broiler chickens slaughtered in commercial abattoirs in Lusaka Province, Zambia**" is my original work and has not been submitted for a degree, diploma or other qualifications at this or another University. It has been prepared in accordance with the prescribed Guidelines for Post-graduate Studies Dissertations of the University of Zambia.

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APPROVAL

The University of Zambia, School of Public Health approves this Dissertation of Nelson Phiri, as partial fulfilment of the requirements for the award of the degree of Master of Public Health.

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Signature …… Date…….

Supervisor: ……..

Signature: …… Date……

DEDICATION

I dedicate this research to my Parents Mr. Nelson C. Phiri and Mrs. Edith Sakala Phiri for their continued moral support and encouragement rendered to me during my study. I also dedicate this study to my beautiful daughter Mayamiko Amanda Phiri, for always being my motivation to work hard.

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LIST OF ABBREVIATIONS

AB	Abattoir
API	Analytical Profile Index
AMR	Antimicrobial Resistance
AST	Antimicrobial/antibiotic Sensitivity Testing
CCPs	Critical Control Points
CSO	Central Statistics Office
E. coli	Escherichia coli
FDBs	Foodborne Diseases
EMB	Eosin Methylene Blue
DHMT	District Health Management Team
DST	Drug Sensitivity Testing
HACCP	Hazard Analysis and Critical Control Points
LCC	Lusaka City Council
LDHMB	Lusaka District Health Management Board
LDHMT	Lusaka District Health Management Team
NA	Nutrient Agar
NaCl	Sodium Chloride
RV	Rappaport Vasiliadis
Sp.	Species
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

DEFINITION OF TERMS

Abattoir: An abattoir is a slaughterhouse or a building where animals are slaughtered for meat.

Antimicrobial: A substance, such as an antibiotic, that kills or stops the growth of microbes, including bacteria, fungi, or viruses. Antimicrobials are grouped according to the microbes they act against (antibiotics, antifungals, and antivirals).

Antimicrobial resistance: the ability of microbes to grow in the presence of a chemical (drug) that would normally kill them or limit their growth

Antimicrobial susceptibility testing (AST): Laboratory testing performed on microbes to find out if they are susceptible or resistant to one or more drugs.

Bacteriological contamination: The invasion of substances by bacteria.

Bacteria: Any group of microscopic single-celled organisms that live in enormous numbers in almost every environment on earth.

Food borne disease: is any illness resulting from the food spoilage of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food or water that is meant for human consumption.

Hazard: A biological, chemical or physical agent in, or condition of food with the potential to cause harm i.e. an adverse health effect to the consumer.

Isolates: Bacteria isolated from a specimen (e.g., stool, blood, food).

Microbial contamination: Inclusion or growth of harmful microorganisms such as *Clostridium botulinum* in an item used for food making it unfit for consumption.

Microbiological contamination: The non-intended or accidental introduction of infectious material like bacteria, yeast, mould, fungi, virus, prions, protozoa or their toxins and by-products.

Multidrug resistance: is antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs

Resistance pattern: A description of the antibiotic resistance testing results for an isolate.

Resistance profile: A description of the resistance patterns for all isolates in an investigation. A resistance profile differs from a resistance pattern, which refers to the characteristics of a single isolate.

ABSTRACT

Food-borne diseases (FBDs) are a threat to public health and are among the top five causes of illness and death worldwide. Increased demand for food globally has led to an increase in poultry production. Previous studies done in Zambia found *Salmonella sp*. and *E. coli* to be major bacterial contaminants in poultry. Most of these FBDs pathogens are known to be resistant to antimicrobials, making their management challenging. Irrational antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms. Drug resistance leads to treatment failures and mortality in animals and humans. This study's aim was to determine the occurrence of antimicrobial resistance of foodborne pathogens namely; *Salmonella sp*. and *E. coli* in broiler chickens slaughtered from commercial abattoirs in Lusaka province, Zambia. The study employed a cross-sectional study in Lusaka and Chilanga districts. Samples were processed for salmonella isolation, phenotyped using the API® and speciated using 16S RNA PCR.

One hundred and fifty (150) swabs were collected (75 cloaca and 75 carcass swabs). Two *Salmonella sp*. and 118 *E. coli* were isolated from cloaca and carcass swabs, respectively. One of the *Salmonella sp.* isolated exhibited resistance to ampicillin (50%), amoxicillin/clavulanic acid (50%) and cefotaxime (50%). Resistance in *E.coli* was observed to ampicillin (72.9%), tetracycline (71.2%), trimethoprim/sulfamethoxazole (60.2%), nalidixic acid (53.4%), chloramphenicol (39%), ciprofloxacin 28%, cefotaxime (27.1%) (10.2%). No resistance (100%) susceptibility was observed to colistin sulphate and imipenem. 107 *E. coli* isolates were resistant to at least one antibiotic (90.7%), while 62 isolates (53.2%) exhibited multiple drug resistance (MDR). There was no statistical association between chicken batch AMR status of and investigated predictor variables ($p>0.05$). The study shows presence of multidrug resistance of *Salmonella sp*. and *E. coli* in broiler chickens and may largely contribute to the wider and broad challenge of antimicrobial resistance

Key words: Foodborne diseases, Antimicrobial resistance, *Salmonella sp*., *E. coli.*

CHAPTER ONE

INTRODUCTION

1.1 Background information

Food-borne diseases are a threat to public health worldwide (Sofos, 2008b) and contribute substantially to global morbidity and mortality rates worldwide (Kariuki et al., 2013). The deleterious impact of these diseases on human health, concurrent with the associated socioeconomic loses has led to an increased demand for the production of safe food globally (WHO 2015). The repercussions are not only health related but also have economic ramifications from the loss of business over food safety issues (Phillips, 2007). The most serious meat safety issues resulting in immediate consumer health problems and recalls from the market place of potentially contaminated products are associated with microbial, and especially bacterial pathogens (Sofos, 2008b).

In recent years, some highly publicized outbreaks of foodborne diseases in the United States, caused by pathogenic bacteria such as *Escherichia coli* O157: H7 and *Listeria monocytogenes*, have brought meat safety and associated issues at the forefront of societal concerns (WHO 2015). Such challenges will continue and in some cases may be intensified in the future. Major causes of concern and product recalls associated with fresh meat products are *E. coli O157: H7* and related enteric pathogens such as *Salmonella sp.* while the gram-positive *Listeria monocytogenes* is the pathogen of concern in ready-to-eat meat and poultry products that allow growth of the organism during storage (Kariuki et al., 2013).

Meat safety challenges associated with microbial pathogens may be divided into those dealing with problems caused by pathogens of current concern, pathogens of potential concern in the future, pathogen changes and adaptations, and the involvement of the environment in microbial pathogen concerns (Sofos, 2008b). Microbial pathogens of current concern that need to be controlled in fresh meat include *Salmonella sp.*,

Campylobacter sp. and enterohaemorrhagic *E. coli.* Even though progress is being made in their control, some of these pathogens will continue being of concern well into the future, considering that some of them (e.g., *Salmonella sp.*) have been the target of control efforts for many decades and are still involved in large numbers of illnesses. A number of new emerging diseases evolving pathogenic microorganisms have been associated with documented foodborne illness episodes in the past 20–30 years and their number appears to be increasing (Aarestrup et al., 2008).

In the United States of America (USA), 60% of cases requiring hospitalization are caused by the food-borne bacteria, but in developing countries, related data is not available because of a lack of precise health-care infrastructure, surveillance and poor data management (Bhandare et al., 2010). Foodborne diseases occur commonly in developing countries, particularly in Africa, because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment and lack of education for food-handlers (Haileselassie et al., 2013).

Most human foodborne diseases are a result of zoonotic infections (Petrovic et al., 2010). Zoonoses are diseases or infections, which are naturally transmissible from animals to humans and vice versa (Petrovic et al., 2010). Zoonoses which occur most frequently in the developed world today are food-borne infections caused by *Salmonella sp., Campylobacter.sp., Viro-cytotoxic E. coli, Yersinia, Listeria* (Hermans et al., 2012). Although various foods can serve as sources of foodborne illness, meat and meat products are important sources of *Salmonella sp.* and *Campylobacter sp*. These bacteria are reported in all meat producing animals and are widespread in poultry production (Sofos, 2008b). The World Health Organization (WHO) has identified non-typhoid *Salmonella sp, E. coli, Staphylococcus aureus* and thermophilic *Campylobacter sp.* as some of the zoonotic food-borne pathogens of importance. These pathogens can be transmitted to humans through the consumption of chicken meat (WHO, 2004).

Poultry production is one of the most important sectors in agricultural production in many countries including Zambia. The high consumption of chicken meat requires great care to provide the safety of the industry against menacing factors. Along with the development of poultry farms and intensive culture, the occurrence of bacterial diseases and, consequently, overusing antibiotics have increased in recent years (Talebiyan et al., 2014).

Antibiotic usage is considered the most important factor in promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine. Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations (Van den Bogaard et al., 2001). Oral medication of large groups of animals is particularly likely to favour emergence and selection for resistant microorganisms. Also, in animal production, conditions exist that facilitate the spread of bacteria, such as high density and/or poor infection control such as vaccinations and biosecurity (Petrovic et al., 2010).

According to WHO the resistance to antimicrobials is an ability of the bacterial population to survive the effect of inhibitory concentration of antimicrobial agents (Van den Bogaard et al., 2001). When antimicrobials are applied in poultry farming, the drug eliminates the sensitive bacterial strains, leaving behind or selecting those variants with unusual traits that can resist it. These resistant bacteria then multiply, increasing their numbers a million fold a day, becoming the predominant microorganism in the population. Such bacteria transmit their genetically defined resistance characteristics to the subsequent progeny of the strains and to other bacterial species via mutation or plasmid-mediated (Apata, 2009).

Zambia is increasingly experiencing problems associated with human and animal bacterial pathogens that are resistant to antimicrobials (Chishimba et al., 2016, Chiyangi et al., 2017, Mainda et al., 2015a). Drug resistance leads to treatment failures and mortality due to infections with these resistant bacteria (Mubita et al., 2008). Zambian

abattoirs are thus still facing challenges in processing meat that is wholesome and safe for human consumption due to contamination by these resistant bacteria. This comes as a result of a lot of poultry farmers employing antibiotics as growth promoters which are perceived as an inexpensive management practice. Use of antibiotics is employed by farmers in disease prevention as a mitigation measure against the highly prevalent unhygienic conditions and absence of biosecurity. Due to the uncontrolled sale and misuse of antimicrobials, these drugs, subsequently end up in meat consumed by humans (Mainda et al., 2015b). Consequently, numerous resistance genes in human pathogens have been linked to the consumption of food of animal origins (Castillo Neyra et al., 2014).

This study's aim was to determine the occurrence of antimicrobial resistant profiles of *Salmonella sp.* and *E. coli* in broiler chickens slaughtered from commercial abattoirs in Lusaka province, Zambia. The study went a step further to analyze the antimicrobial patterns of the isolated bacterial pathogens.

This research was done in Lusaka and Chilanga districts where the commercial abattoirs are located. The abattoirs included in this study were purposively selected because most of the birds dressed from these are consumed in Lusaka district which is the most populated district in Zambia.

1.2 Problem Statement

In developing countries, small-scale poultry farming is being advanced as an inexpensive source of protein and income. However, the majority of small scale farmers misuse or abuse antimicrobials either as growth promoters and/or disease prevention remedies to compensate for poor hygiene. Antimicrobial resistance (AMR) is a growing public health concern globally, including developing countries such as Zambia (Mainda et al., 2015b). AMR has largely been contributed by uncontrolled or irrational antimicrobial use in poultry as growth promoters and therapeutic purposes. The overuse and misuse of antibiotics in human medicine and in animal agriculture, where the vast majority of antimicrobials are used contribute to the evolution and spread of antibiotic-

resistant pathogens (Koluman and Dikici, 2013). Farmed animals and the broader environment can serve as reservoirs of AMR genes that can be exchanged across species (Braykov et al., 2016).

Zambia is increasingly experiencing problems associated with human and animal bacterial pathogens that are resistant to antimicrobials (Chiyangi et al., 2017, Mainda et al., 2015a). Drug resistance leads to treatment failures and mortality due to infections with these resistant bacteria (Mubita et al., 2008). In a human study done in Democratic Republic of Congo, Mozambique, Tanzania, and Zambia, high levels of antibiotic-resistant microbes associated with acute respiratory and diarrheal diseases were observed including *Salmonella sp.* and *E.coli* (Mshana et al., 2013).

Zambian abattoirs are thus still facing challenges in offloading onto the market meat that is wholesome and safe for human consumption due to the presence of resistant bacteria, in chicken presented for slaughter. Moreover, due to the absence of official surveillance systems for antibiotic residues in meat products, a number of farmers do not adhere to recommended withdraw periods of these drugs and subsequently end up in meat consumed by humans (Mainda et al., 2015b). As a result of these practices, numerous resistance genes in human pathogens are linked to the consumption of food of animal origins (Castillo Neyra et al., 2014). Multiple pathways link AMR in these reservoirs to human health. Epidemiological studies going back to the 1970s show an association between antibiotic use on farms and colonization with livestock-associated strains in workers and surrounding communities (Silliker, 1980).

In the recent past, Lusaka has been recording diarrheal and food-borne illnesses as some of the highest causes of morbidity both among the adults and children (Chiyangi et al., 2017, Hang'ombe, 2017). Diarrhoea is ranked to be the third most cause of morbidity for all age groups in the Lusaka District (LDHMB, 2010). The statistics from the Lusaka District Health Management Team (LDHMT) reflected the incidence of diarrhoea in 2015 to be around 97/1000 and 82/1000 in 2014 for all age groups in Lusaka. Currently, typhoid, a food borne disease caused by a bacterium *Salmonella typhii* has become a

common cause of mortality in the infected people. Since the majority of Zambians consume chicken, it is likely that pathogens in dressed chickens are responsible for some of the observed diarrhoeal cases affecting people in Lusaka and surrounding areas. Diarrheal diseases account for approximately 25% of all deaths in under 5-year-old children in developing countries and common pathogens (*Campylobacter sp*., *Salmonella sp., Shigella sp*. and diarrheagenic *E. coli*) causing diarrhoea in human have been found in animals (WHO, 2014)

The study is important to determine the occurrence of these bacteria and their resistance patterns in chickens as they can be transmitted to humans and vice versa. It is also important in ascertaining if the trend of AMR is similar to those documented in human studies and hence helps to implement effective monitoring and surveillance of antimicrobials using a "One Health" approach.

1.3 Study Justification

It is undeniable that AMR is a global public health problem. This study is important for establishing the current state of AMR profiles of *Salmonella sp.* and *E. coli* in Zambian Poultry (broiler chickens). The study will provide valuable insights in identifying resistant strains of pathogens associated with foodborne infections in chickens which will ultimately result in improved knowledge needed for implementation of food safety measures. Research into the conditions, factors and practices that bring about antimicrobial resistance is very important because it can help to identify areas that require intervention in order to improve the quality and safety of poultry meat coming from these slaughterhouses. Few studies have so far been conducted on AMR food safety-related zoonotic pathogens in Zambia and causal pathogens linked to AMR bacteria and the also the contribution of irrational antimicrobial usage to this rising problem (Chishimba et al., 2016).

AMR data generated from this study may influence implementation strategies for official AMR control. This is so because, data on the state of antimicrobial resistant pathogens are required for risk-based application of official controls, whereby control

efforts are targeted towards productions processes and products posing a higher risk with respect to AMR. The results from this study will provide baseline information for effective monitoring and surveillance of antimicrobial levels in poultry hence control strategies as well as generating hypothesis for further research in antimicrobial resistance. Furthermore, information generated from this research will be shared with various stakeholders and policymakers such as the Ministry of Health (MOH), Ministry of Fisheries and Livestock and Lusaka City Council (LCC) and can probably be used if need be to develop appropriate interventions and mitigation measures to improve the safety of poultry meat sold to the public.

1.4 Research Question

Are antimicrobial resistant *Salmonella sp.* and *E.coli* present in broiler chickens slaughtered in commercial abattoirs in Lusaka Province, Zambia?

1.5 General Objective

To determine the occurrence of antimicrobial resistance *Salmonella sp*. and *E. coli* in broiler chickens slaughtered in commercial abattoirs in Lusaka Province.

1.5.1 Specific Objectives

1. To isolate and identify *Salmonella sp.* and *E. coli* in broiler chickens slaughtered in commercial abattoirs in Lusaka Province.
2. To determine the antimicrobial resistance patterns of *Salmonella sp.* and *E. coli* isolates in broiler chickens slaughtered in commercial abattoirs in Lusaka Province.
3. To identify factors associated with the prevalence of isolated AMR *Salmonella sp.* and *E. coli* in broiler chickens slaughtered in commercial abattoirs in Lusaka Province.

1.6 Organization of Dissertation

The organization of the dissertation is in such a way that, chapter one talks about the introduction in which the terms are defined such as foodborne diseases, antimicrobial resistance and related pathogens across the globe, Africa as well as our country. In Chapter two, we present the literature on prevailing conditions of *Salmonella sp.* and *E.coli* in food animals and the factors that have contributed to the increase in the incidence of AMR. The research question and objectives were also addressed in this chapter. Chapter three further goes on to present the methodology that was used to identify the AMR profiles of the two important bacteria: *Salmonella sp*. and *E.coli*. This chapter also includes study area, target population, type of study employed, and the sample size as well as sampling methods employed, laboratory methods up to data analysis. In Chapter four we present the results as generated by Stata and WHONET analytical software. The results were generated and presented in tables and figures to clearly understand the results. In chapter five, the findings were discussed with reference to other studies done by other researchers and interpretation of what could have led to the finding in this study. In chapter six, we give conclusions of the study and offer recommendations to the Zambian government and other stakeholders concerned with AMR.

1.7 Conceptual Framework

The Conceptual Framework in (Figure 1) can be explained in the following factors:

- In Figure 1, there are two overlapping pools of bacteria (animal and human). The emergence or entry of an antimicrobial-resistant bacterium carrying a particular antibiotic-resistance gene into the animal pool may be a rare event but antibiotic use in animals will amplify the resistant bacteria or genetic determinant. The chance of the resistant bacteria spreading to humans, or of an antibiotic-resistance gene transferring to the human bacterial population, increases with every increase in the size of the pool of resistant bacteria or genetic determinants

in the animal or human environment. Once such transfer occurs, the establishment of a significant pool of resistant organisms in the human bacterial population requires further selection with the same antibiotic or one that corselets for the antibiotic-resistance gene.

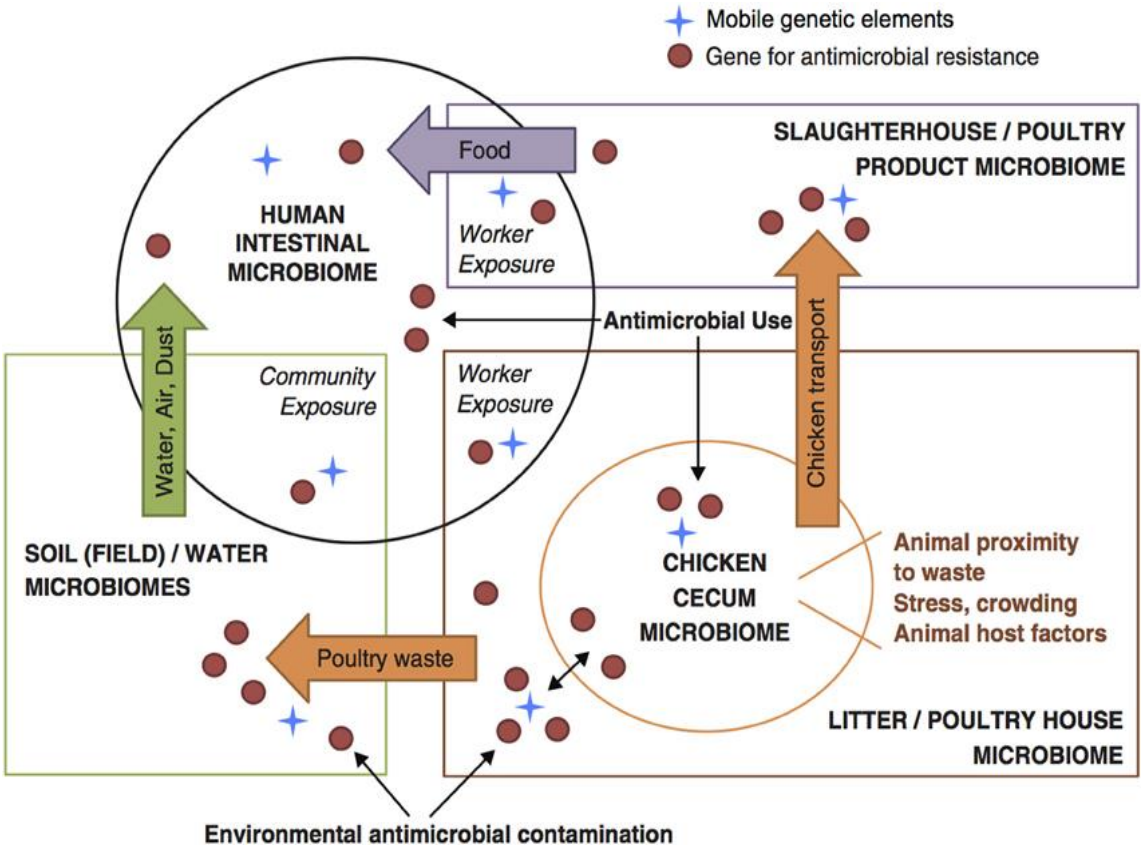

Figure 1: Conceptual framework for factors influencing AMR bacteria in poultry and humans.

Source: Adapted from (Salisbury et al., 2002) - A risk analysis framework for the long-term management of antibiotic resistance in food-producing animals.

- If animals and humans are both exposed to a particular antibiotic and there is a connection between the bacterial pools through a contact such as food, then there is potential for amplification of antibiotic-resistant bacteria to occur in both pools simultaneously. However, if the human population is not exposed to the same antibiotic or one that co-selects for resistance to that antibiotic, then the potential

for amplification in humans is low. The reverse is also true for antibiotic spread from humans to animals.

- The greatest concern at this stage is that a rare antibiotic-resistant strain of bacteria may emerge in animals as a result of antibiotic use. This resistance may not be detected immediately in animals, be spread to humans and then amplified by human use of antibiotics that occurs in the human healthcare environment.

CHAPTER TWO

LITERATURE REVIEW

2.1 Food borne diseases and infections

The safety of meat has been at the forefront of societal concerns in recent years, and indications exist that challenge the safety of the meat (Sofos, 2008a). According to WHO, highly publicized outbreaks of foodborne diseases, worldwide, are caused by pathogenic bacteria such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella sp.* and Campylobacter Of more concern are antibiotic-resistant strains of thermos-tolerant *Campylobacter sp., Salmonella sp.*, Vero-cytoxigenic *Escherichia coli* (VTEC) in food microbiology (Organization, 2015).

Recently in South Africa, an outbreak of listeriosis, a serious foodborne disease, was reported between January 2017 to March 2018. In this outbreak 978 laboratory-confirmed listeriosis cases were reported to the National Institute for Communicable Diseases (NICD) from all provinces. The outcome of this illness is known for 674 patients, of whom 183 (27%) of them died; a case fatality rate which is comparable to other recorded listeriosis outbreaks worldwide (WHO, 2018). Most of the cases of Listeriosis involved persons who have higher risks for a severe disease outcome, such as neonates, pregnant women, the elderly and immunocompromised persons. In this outbreak, 42% of the cases were neonates who were infected during pregnancy or delivery. The same strain was also found in the processing environment of the manufacturer of the implicated product and it was later discovered that this product was believed to be the source of the outbreak (WHO, 2018).

The food processing company and three of its retailers exported to 15 countries in the African region. All of these countries issued recalls for the implicated products. Environmental samples from other food production companies in South Africa also tested positive for Listeria but with strains different from the outbreak strain. After this outbreak, WHO recommended countries to strengthen national food safety and disease

surveillance systems as a pre-requisite to prevent similar events in the future and to ensure a safe food supply for their populations. Additionally, countries were urged to pay closer attention to common foodborne pathogens such as *Salmonella sp.*, *Campylobacter jejuni, E. coli* and *Listeria monocytogenes* (WHO, 2018).

Previous studies have shown that food-borne pathogens, such as *Salmonella sp.* and *E. coli*., are highly prevalent, and have been isolated in stool samples from humans affected by food-borne illnesses, as well as in the meat and poultry products processed for human consumption (Mshana et al., 2013). Two of the most common etiologic bacterial organisms responsible for causing gastroenteritis, a major public health concern in most regions of Zambia are Salmonella spp. and *E. coli* (Chishimba et al., 2016, Hang'ombe et al., 1999, Mainda et al., 2015b). In poultry, these have been isolated in studies done by Hang'ombe et al., (1999), William et al., (2012) and Shamaila et al. (2018) in market ready broiler chickens.

2.2 Antimicrobial resistance of food-borne bacteria

2.2.1 The Concept of antimicrobial resistance

Antimicrobial resistance can be defined as a natural consequence of infectious agents' adaptation to exposure to antimicrobials used in medicine, food animals, crop production and use of disinfectants in farms and household (Byarugaba, 2004). Bacterial infections, which contribute most to human and animal diseases in developing countries, are also those in which emerging antimicrobial resistance is most evident (Shears, 2001). The frequent administering of antibiotics in the treatment of livestock diseases may contribute to the emergence of antimicrobial resistant strains (Talebiyan et al., 2014). AMR is now a global problem that threatens the return to the pre-antimicrobial era. There are no new antimicrobials being developed (Organization, 2014). The development of new antibiotics by the pharmaceutical industry, a strategy that had been effective at combating resistant bacteria in the past, had essentially stalled due to economic and regulatory obstacles (Ventola, 2015). The number of new antibiotics developed and approved has decreased steadily over the past three decades (although

four new drugs were approved in 2014), leaving fewer options to treat resistant bacteria (Control and Prevention, 2015).

2.2.2 Antimicrobial resistance in *Salmonella sp.* and *E. coli*

In numerous studies done in Asia, tetracycline resistance to *E. coli* has been reported (Chiu and Ou, 1996). Partly is because tetracyclines are inexpensive antibiotics and have been used extensively in the prophylaxis and therapy of human and animal infections and at sub-therapeutic levels in animal feed as growth promoters. The presence of tetracycline-resistant pathogens now limits the use of these agents in the treatment of diseases (Braykov et al., 2016). However, little attention has been paid to the relationship between resistance and virulence genes in specific chicken bacterial isolates (Chiu and Ou, 1996). In a study by (Diarrassouba et al., 2007) both *Salmonella sp.* and *E. coli* isolates were observed to be resistant to penicillin, erythromycin, tylosin, clindamycin, and novobiocin and exhibited different resistance levels to other antibiotics. All the *E. coli* strains were susceptible to enrofloxacin. Tetracycline resistance was found in 56 of the 74 *E. coli* isolate and more than 20% of *E. coli* isolates were multi-resistant to 12 antibiotics. However, all the *Salmonella sp.* isolates were susceptible to gentamicin, enrofloxacin, and only one isolate was intermediately susceptible to sarafloxacin. Almost all (90.3%) of the *Salmonella sp.* isolates were resistant to spectinomycin, but resistance to the в-lactams amoxicillin and ceftiofur was 41.9% and 43.6%, respectively. *Salmonella spp.* isolates were less multi-resistant than were the *E. coli* isolates. More than 90% of the *Salmonella spp.* isolates were multi-resistant to six antibiotics, with one isolate resistant to 13 of the 18 antibiotics tested.

In Zambia, studies done by (Chishimba et al., 2016) on market-ready chickens revealed that overall 20.1%, of total samples analyzed in his study, contained Extended-Spectrum Beta-Lactamases (ESBL) producing *E. coli*. The antimicrobial sensitivity test revealed that 85.7% of ESBL-producing *E. coli* isolates conferred resistance to beta-lactam and other antimicrobial agents. These results indicate that poultry is a potential reservoir for ESBL-producing *E. coli*. They further indicated that control of the presence of ESBL-

producing *E. coli* in poultry destined for human consumption requires strengthening of the antibiotic administering policy because antibiotic administration in food animals was gaining momentum for improved animal productivity in developing countries such as Zambia.

2.3 Modes of transmission and mechanism of resistance of *Salmonella sp.* and *E. coli to* antibiotics

There are a number of mechanisms in which enterobacteria such as *Salmonella sp.* and *E. coli* may present resistance to antibacterial agents. These are outlined below;

2.3.1 Clonal expansion

Some species of bacteria are innately resistant to at least one class of antimicrobial agents. In such instances, all strains of that bacterial species will also be resistant to all the members of those antibacterial classes. The most important of these is the acquired resistance, where an initially susceptible group of bacteria become resistant to an antibacterial agent and multiply and spread under the selective pressure of use of that agent. Several mechanisms of antimicrobial resistance readily spread to a variety of bacterial genera (Tenover, 2006). Clonal expansion is a mechanism of resistance where a few resistant isolates (colony) multiplies and become many. A study by Wimalarathna et al., (2013), found that antimicrobial resistance in *Campylobacter sp*. isolated from chicken meat was widespread and the pattern of resistance suggests that horizontal gene transfer has a role in the acquisition of resistance. The study also gave evidence for the proliferation of resistant lineage clusters that indicate that conditions occur that favour resistant strains potentially on poultry farms through cloacal expansion (Wimalarathna et al., 2013)

2.3.2 Transfer of genetic elements

The first mechanism of resistance is through the organisms acquiring genes encoding enzymes, such as b-lactamases, that destroy the antibacterial agent before it can have an effect on the bacteria. The second method is that the bacteria may acquire a gene that encodes for efflux pumps. Acquisition of efflux pumps gives new bacterial ability to extrude the antibacterial agent from the cell before it can reach its target site and exert its

effect. Lastly, bacteria may acquire several genes for a metabolic pathway which ultimately produce altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent, or bacteria may acquire mutations that limit access of antimicrobial agents to the intracellular target site through down-regulation of porin genes. Normally susceptible populations of bacteria may become resistant to antimicrobial agents through either, mutation and selection, or by acquiring from other bacteria the genetic information that encodes resistance. Resistance through bacteria acquiring genetic information that encodes resistance may occur through mechanisms which include transformation, conjugation or transduction.

Transduction

In the transduction process, a bacteriophage transfers, from a resistant to a sensitive bacterium, extrachromosomal bacterial DNA incorporated in its protein. The previously sensitive bacterium, then, will acquire resistance and transfer it to its daughter cells. This mechanism is easily observed in *Staphylococcus aureus* strains that acquired resistance to penicillins.

Transformation

Transformation occurs when bacteria that are sensitive to one substance incorporate the DNA with genes that encode resistance that is found in the environment. These bacteria, then, become resistant to one or more antimicrobials. Some bacteria, in certain growth phases, are able to excrete DNA to the environment.

Conjugation

Conjugation is caused by passage of genes (R factors) from a resistant to a sensitive bacterium by attachment to a sex pilus. The R factor may contain resistance information against several antimicrobials. Conjugation and production of the sex pilus require the intervention of another group of genes, called transference factor. Without them, the process is not carried out. The R determinant complexes, plus the resistance transfer factor, are known as the R factor. R factor is important to Gram-negative bacteria, especially enterobacteria. *E. coli*, *Salmonella sp*. *Shigella*, *Klebsiella* and *Pseudomonas*

aeruginosa are among microorganisms capable of transferring this type of resistance to sensitive bacteria. This resistance mechanism has been observed in relation to tetracyclines, chloramphenicol, sulphonamides, penicillin's and aminoglycosides (Tessari et al., 2012).

2.4 Contamination of poultry products

In chickens, the main route of transmission of Salmonella is vertical through eggs. Infection of breeder flocks with *Salmonella* leads to rapid dissemination of the organism to progeny broiler and commercial egg-laying flocks. Salmonella is also spread between birds horizontally by the faecal-oral route. The bacterium survives for long periods in the environment and has been isolated from litter and dust in poultry houses. While muscles are sterile in healthy living birds, various microbiotas are hosted in the digestive tract, lungs, skin as well as feathers (Rouger et al., 2017).

In slaughterhouses, the carcass contact surfaces, air (aerosols), and liquids (wash water) also may harbour bacteria. Bacterial contamination may occur from equipment surfaces, water, and animal microbiota. The skin of poultry carcasses and cuts is directly in contact with air and equipment surfaces and is therefore easily contaminated. In fresh meat, bacteria are present on the surface rather than in the meat. However, in processed products such as those which have been marinated, bacteria can migrate into the muscles (Vihavainen et al., 2007). Bacterial contamination by equipment surfaces can take place early in the process through the rubber fingers used for feather removal or conveyor belts. During the subsequent processing steps (deboning, cutting, mincing, and mixing) for meat-based foodstuff production, manipulators, air and equipment surfaces are the main sources of contamination (Álvarez-Astorga et al., 2002).

The water baths used during the process have a washing effect that diminishes the bacterial loads but can also promote cross-contamination between carcasses (Goksoy et al., 2004). The evisceration step, because of the microbiota present at high counts in the digestive tract, is a critical point of carcass contamination. The gastrointestinal tract of birds hosts many bacteria, including some that can be potentially dangerous for the consumer such as *Campylobacter sp*. or *Salmonella sp*. (Hinton Jr et al., 2004). After

initial contamination, some bacteria can persist during meat product storage and can be recovered from killed animals before the scalding step, as well as after 10 days of the storage of carcasses at refrigerated temperature (Al-Nehlawi et al., 2013). Figure 2 below summarises the different stages of slaughter and the CCPs where contamination is likely to take place.

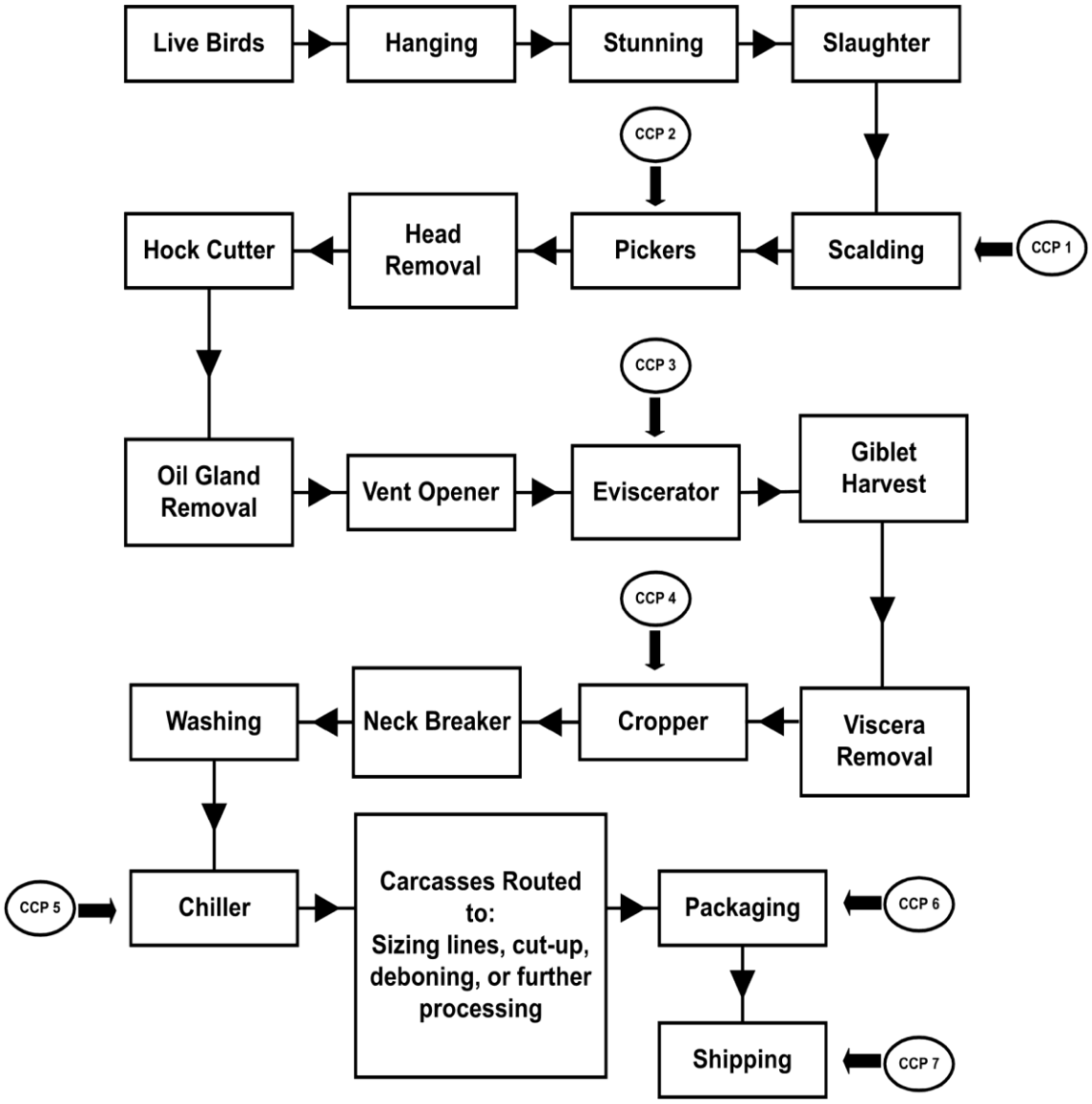

Figure 2: HACCP flow diagram for poultry processing indicating contamination points and Critical Control Points (CCPs).

2.5 Poultry processing and microorganism associated with fresh poultry meat carcasses

Foods of animal origin are important vehicles for the transmission of several zoonoses (Ramchandani et al., 2005). Conditions existing in slaughterhouses and the current meat handling practices in many developing countries have been found to contribute significantly to the spread of zoonotic diseases (Sofos, 2008b). The microbiological quality of poultry is largely dependent on the numbers and types of bacteria, in particular, psychrophilic spoilage bacteria present on the product at the end of processing. In the slaughterhouse, resistant strains from the gastrointestinal tract may contaminate chicken carcasses and, as a result, chicken meats are often related to multi-resistant *E. coli* (Talebiyan et al., 2014). Studies have shown that in the slaughterhouses several factors pose as sources of contamination and some of these include the surfaces, air (aerosols), liquids and the food handlers. However, bacteria from the air and the environment can contaminate broiler meat as well (Vihavainen et al., 2007). The skin of poultry carcasses and cuts are directly in contact with air and equipment surfaces and are therefore easily contaminated (Rouger et al., 2017).

Prevention of carcass contamination during slaughter and subsequent steps in food preparation is difficult, therefore antimicrobial resistant bacteria derived from the intestinal tract of food animals may be transmitted to humans through food (Carraminana et al., 2004). Carraminana et al., (2004), stated that one control strategy is to reduce carcass contamination through improvements in on-farm management, transport, abattoir lairage management, killing floor practices and chiller practices. Although veterinary antimicrobial use may constitute a threat to human health, the impact of resistance among zoonotic bacteria and the risk of transfer of resistance determinants between animal and human pathogens remains unquantifiable (Duffy et al., 2008). Currently, limited information about the antibiotic resistance properties of *Salmonella sp.*, in the poultry processing environmental or on raw broiler meat is available in Zambia. Therefore, it would be beneficial to have some data about *Salmonella sp.* and *E. coli* resistance to antibiotics used in poultry production, since the

consumption of poultry products is often associated with salmonellosis and *E. coli* infection.

A study was carried out at a poultry processing plants in Lusaka, Zambia to identify and describe bacteria found in chicken carcasses leaving the processing plant for retail outlets (Hang'ombe et al., 1999). In this study, thirteen different bacteria were found which included *Escherichia coli* with a prevalence of 41.7%, and *Salmonella sp.*, with a prevalence of 20.53%. Others include *Staphylococcus sp., Pseudomonas sp., Klebsiella spp., Citrobacter sp., Acinetobacter ssp., Proteus sp., Flavobacterium spp., Streptococci sp., Alcaligenes sp., Micrococcus sp.,* and *Bacillus sp.,* with the prevalence of 2.49%, 6.71%, 1.91%, 6.71%, 0.58%, 9.02%, 1.15%, 1.72%, 0.77%, 3.84%, and 2.88%, respectively. These results showed that the chicken carcasses entering the Zambian market are a potential source of bacterial pathogens to consumers (Hang'ombe et al., 1999). It was suggested after this study that much more attention should be paid to hygiene in the processing plants in order to control the bacterial contamination of poultry meat. Other studies were done by (Goksoy et al., 2004), also reviewed that coliforms, *Enterobacteriaceae, and Staphylococci/Micrococci* were isolated in high amounts from poultry in the processing plants. Humphrey and Jorgensen, 2006 also documented the microorganisms that are potentially pathogenic serotypes of *E. coli* such as *0157; H7, Salmonella sp.* and *Campylobacter spp.* may be internally contained or may be found on the animal surfaces such as the cloaca and other animal surfaces (Humphrey and Jorgensen, 2006).

2.6 Poultry Production in Zambia

In Zambia, two types of poultry producers are identifiable. These are classified as primary and secondary producers. Primary producers are mainly corporate firms who dominate this group by a handful of integrated companies currently amounting to about ten (10) in totals across the country. Most of these companies are neither locally owned nor managed. They import their inputs from neighbouring countries. Many corporate companies are interested in the further vertical integration of their poultry businesses.

Next, to expanding their production capacities (e.g. Feed production, broiler and egg production), they are interested in investing in other activities like production of day-old chicks, slaughtering and further processing of broilers (PAoZ, 2015).

Small and medium size poultry farmers occupy the secondary production segment. They produce the bulk of the poultry meat and eggs in Zambia. Poultry is kept in simple, open houses and manual feeding is employed. Simple water bowls are used while heating is undertaken through wood or charcoal. Some small farms produce maize and soy for stock feed. To reach the pace set by primary producers, small-scale farms must optimize their production in terms of feed efficiency to lower production costs. They can form production clusters to reap benefits accruing from the scale. Small and medium scale farms offer the most scope for improvement. They use simple manual equipment, small amounts of feed additives, drugs and vaccines and do purchase small amounts day-old chicks. These inputs are purchased from local suppliers (PAoZ, 2015).

2.7 Risk factors associated with AMR *Salmonella sp.* and *E. coli*

The presence of AMR bacteria in primary animal production represents a high risk for humans since AMR bacteria of animal origin can be transmitted from animals to humans through the food supply (foodborne pathogens), water or direct contact with animals (Ramchandani et al., 2005). In farms, factors that can influence bacterial resistance vary depending on flock health status, farm management and environment. These practices include over-prescription of broad-spectrum drugs by veterinarians instead of narrow-spectrum drugs, feeding of low doses of antibiotics for growth promotion (Sarkar and Gould, 2006) and use of non-approved drugs or drugs used in an extra-label manner are believed to contribute to the development of antimicrobial resistance (Phillips, 2007). Although widespread use of antimicrobials in the primary sector has benefits for producers, it also contributes to the increasing emergence of AMR bacteria (Sharma et al., 2005).

Research on the poultry food value chain in Zambia such as those done by (Hang'ombe, 2017) and (Chishimba et al., 2016) in the past has mostly concentrated on the hygiene

practices in the abattoirs and very little attention has been paid to antimicrobial resistance patterns of enterobacteria specifically *Salmonella sp*. and *E. coli*.

CHAPTER THREE

METHODOLOGY

3.1 Study Design

The study employed a quantitative cross-sectional study, designed to investigate the occurrence of antimicrobial resistant *Salmonella sp.* and *E. coli* bacteria in broiler chickens sampled from commercial abattoirs of Lusaka and Chilanga districts. The study was done in such a way as to investigate the causes relating to the antimicrobial use and resistance in poultry meat and, the actual levels of resistant bacteria's present in chickens.

3.2. Study Area

This study was done in Lusaka Province, Zambia. Lusaka district, which is the capital of Zambia, is the largest city in Zambia and Chongwe districts were the two districts where research was conducted. Lusaka is one of the fastest-developing cities in Southern Africa with an estimated population of about 2.9 million as of the last population census projection in the year 2016 (CSO, 2010). It is located at -15.41 latitude and 28.29 longitudes and situated at elevation 1277 meters above sea level (Google Earth, 2018). There are three major abattoirs involved in the processing of poultry meat in Lusaka Province. Of the three abattoirs, two were recruited in the study, as the third abattoir did not give consent.

3.3. Study Population

The reference populations in this study were broiler farms that supplied chickens to the abattoirs in the study areas.

Inclusion Criteria: Farms with market-ready broiler chickens received at commercial abattoirs in the study area.

Exclusion Criteria: Farmers with chickens brought in dead upon receipt at the abattoirs and hence not processed as well as those who did not consent to the study.

3.4 Sample size determination

Since there were only two abattoirs and only a few supplied these abattoirs, we included all suppliers to get a reasonable sample for analysis (complete enumeration). Hence from each batch of chickens, 3 cloaca swabs and 3 carcass swabs were collected from the 25 batches. Based on the above, assumptions, we planned to collect 150 samples, as summarised in table 1. Two abattoirs were recruited in the study and twenty-five chicken batches were sampled from the 17 farmers who supplied chickens to these abattoirs; Samples collected included 75 cloaca swabs and 75 carcass swabs bringing the total sample size to 150 samples.

Table 1: Summary of samples collected

Abattoir	Number of farmers	Number of batches	Cloaca swabs	Carcass swabs	Total
A	10	10	30	30	60
B	7	15	45	45	90
Total	17	25	75	75	150

Twenty-five batches of broiler chickens were sampled belonging to 17 farmers, each batch averaging about 4500 chickens. Samples collected included 75 cloaca swabs and 75 carcass swabs. Hence the total sample size came to 150 (**n=150**).

3.5. Data collection techniques and tools

3.5.1 Sampling technique and Sample size

Samples were collected from the two abattoirs classified as strata. The complete enumeration was done where chickens were sampled from each farmer who supplied chickens to a respective abattoir. Convenience sampling technique was employed were

chickens from farmers (batches) were sampled as they deliver chicken until everyone who supplies to the respective abattoir has been sampled. More than one batch of chickens was sampled from a farmer depending on the number of farms a farmer had.

From each batch of chickens, sampling units (chickens sampled) were selected using guidelines from the food and drug manual (Food and Administration, 2009). According to the inspection manual guideline 2009, the recommended sample size using the range of 500 to 1000 slaughter capacity would be three chickens per batch.

Samples were collected at two main points; cloaca swabs in the receiving bay before hosting the birds on the hackles. At this point, three cloacal swabs were collected from each batch using a sterile cotton swab and put in amies transport media (Oxoid). Carcass swabs were collected at packaging after processing before the carcasses were chilled. Similarly, three carcass swabs were collected from each batch. Swabs were collected from under the wing were bacteria population is thought to concentrate during processing (Logue et al., 2003). These were immediately put in a cool box with ice packs and processed for further analysis within twenty-four hours. Random "blind" sampling method was used to select the 3 chickens and cloacal swabs. This method was used as it yields information about the average composition of the lot. It is employed when you have no information or method of determining which units are violated. Usually, the violation is concealed and must be found by laboratory methods (Food and Administration, 2009). The samples were transported to the University Of Zambia, School of Veterinary Medicine, Department of Disease Control, Public Health laboratory in a cool box with ice packs and processed within twenty-four hours.

3.6.2 Laboratory analysis

In the laboratory, all media were prepared according to the manufacturer's instructions. Quality control of the media was ensured by incubating two plates for 24 hours; one plain plate without sample and another plate with sample inoculated containing *E. coli* ATCC 25922 strain and *Salmonella typhimurium* and observed if there was growth or

not on the un-inoculated plate. If there was no growth on the un-inoculated media, that batch was passed to be clean.

3.6.2 Isolation and identification of *Salmonella sp.*

Swabs containing samples were placed in 10 mL of buffered peptone (oxoid) water as a pre-enrichment media and incubated at 37°C for 24 hours. Aliquots from pre-enrichment were inoculated into selective enrichment liquid media at a ratio of 1:10 of Rappaport Vassiliadis (RV) broth for a period of 48 hours. A loop full of broth was streaked on plates of xylose lysine deoxycholate agar (Oxoid) and MacConkey agar (Oxoid) (Manual, 1982)). The plates were incubated at 37 ºC for 24 hours. Suspected colonies of *Salmonella sp*. from each plate were collected for presumptive identification based on their morphological characteristics and various biochemical tests that included oxidase, motility, Triple Sugar Iron agar (TSI), Indole, Methyl red, Urease and Citrate utilization test. The colonies identified on the basis of biochemical tests were subjected for serological tests using polyvalent serum against O and H *Salmonella sp.* antigens. The colonies that agglutinated within one to two minutes were considered as positive for *Salmonella sp.*, and were preserved in Nutrient agar (Oxoid) at 4°C. Suspected colonies from each plate were confirmed by 16S PCR sequencing.

3.6.3 Isolation and identification of *Escherichia coli*

Swabs containing samples were placed in 10 mL of buffered peptone water as a pre-enrichment media and incubated at 37°C for 24 hours. For isolation of *E. coli*, the samples collected were streaked onto MacConkey (Oxoid) agar and incubated for 24 hours at 37˚C in an aerobic environment. MacConkey (MAC) agar is a selective media for Enterobacteriaceae organisms, which are lactose fermenting and produce a pink hue on the media, and three colonies from each sample that matched this description were subjected to biochemical testing. *E. coli* was isolated on Eosin methylene blue agar (EMB) by plating followed by aerobic incubation at 37°C for 24hrs. After incubation *E. coli* was observed to have a distinct green metallic sheen. For each batch of samples

analyzed, the reference strain (*Escherichia coli* ATCC 25922) was also cultured at the same time with the sample for quality control purposes.

Colonies were further subjected to biochemical tests that exhibited an acid slant, an acid butt, and no hydrogen sulfide (H_2S) production on TSI was subjected to further biochemical testing for Citrate agar to differentiate from *Citrobacter*. *Salmonella sp.* isolates and some *E.coli* were also confirmed through API 20 E Biochemical Test Strips. The plastic strip holds twenty mini-test chambers containing dehydrated media having chemically-defined compositions for each test.

Some pure suspected colonies were randomly selected from each plate and confirmed by 16S PCR. Isolates were stored in 10% glycerol and stored at -20°C.

3.6.4 Identification of bacteria using the 16S RNA sequencing

The use of 16S rRNA gene sequencing to study bacterial phylogeny and taxonomy has been by far the most common housekeeping genetic marker. To confirm for the presence of *Salmonella sp.* and some *E.coli,* the 16S rRNA sequencing was performed. The sequencing procedure was used as outlined by the Clinical Laboratory Standard Institute (CLSI). All the two Salmonella suspects and some selected *E.coli* isolates were subjected to 16S RNA sequencing. Briefly, the procedure involves firstly isolation of the bacteria (*Salmonella sp. and E.coli*) followed by culture on brain-heart-infusion broth (Nissui, Tokyo, Japan). After incubation, DNA was extracted by boiling methods (Reich and Klein 2013). The *Salmonella sp.* and *E.coli* isolates were subjected to PCR for confirmation using 16S RNA primers. The PCR was performed in a total reaction volume of 10µl consisting of 5µl Phusion master mix, 2µl sterile distilled water, 2µl primers (forward and reverse) and 1µl bacterial DNA template. The PCR was performed using the rapid cycle DNA amplification method comprising of an initial denaturation step at 98ºC for 30 seconds, followed by 35 cycles of template denaturation at 98ºC for 1 second, primer annealing at 60ºC for 5 seconds and 72ºC for 1 second with a final extension at 72ºC for 10 seconds. The PCR products were later viewed with ethidium bromide after electrophoresis through 1.5% agarose gel (Clinical and Laboratory

Standards Institute, 2009). After successful sequencing of the gene, the sequenced gene was compared with GenBank to obtain a match of the interested bacteria through PubMed Blast.

3.6.5 Antimicrobial Susceptibility Testing (AST)

The AST was done using Disc Diffusion Testing as proposed by the National Committee for Clinical Laboratory Standards (NCCLS, 1990, MA-A4) as elaborated in this account by the Kirby-Bauer disc diffusion method (Bauer,1966) in accordance with the World Health Organization guidelines using Mueller Hinton (Oxoid) Agar plates.

The procedure involves preparation of inoculums by the direct saline suspension of nutrient broth culture made of the isolated colonies from a pure culture of a non-selective media of Nutrient agar plates which have been incubated for 18 to 24 hours. A sterile swab from incubation plate was dipped in the normal saline (NaCl) inoculums and then streaked on the surface of the Mueller-Hinton Agar (Oxoid) plate. The pure colonies of the organism suspended in sterile normal saline were compared to a turbidity equivalent to that of a 0.5 McFarland turbidity standard as shown in Figure 3.

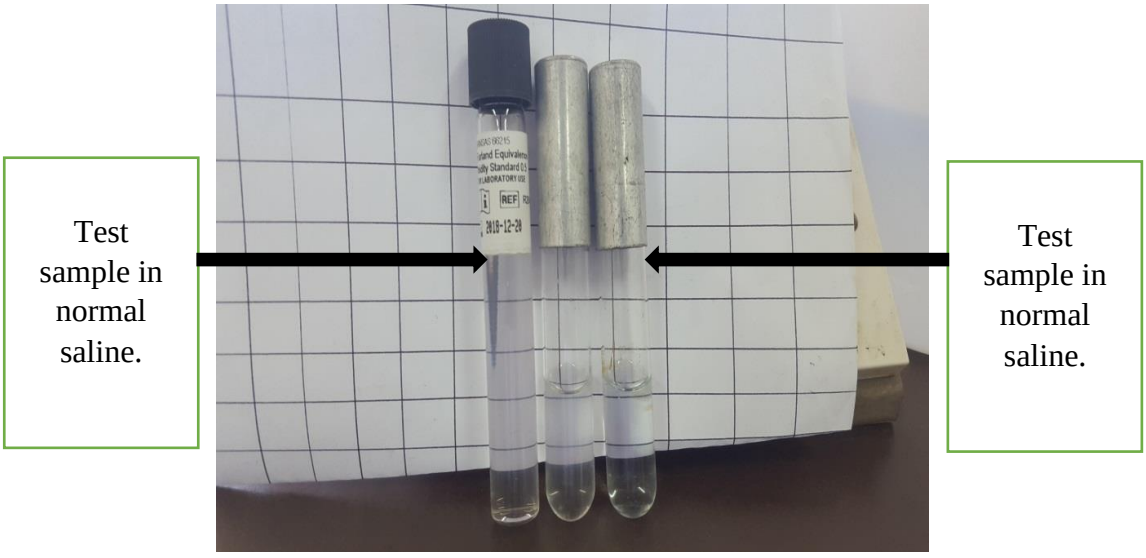

Figure 3: Comparing the turbidity of the pure colonies streak on normal saline to the equivalent of 0.5 McFarland turbidity standards.

The antimicrobial discs contained the following drugs of both veterinary and human importance Amoxicillin-clavulanic acid, Ampicillin, Tetracycline, Chloramphenicol, Cefotaxime, Ciprofloxacin, Nalidixic acid, Colistin Sulphate, Imenepem and Trimethoprim-sulphamethoxazole. Selection of these antibiotics was based on literature review, a list of essential drugs for enterobacteria as recommended by WHO and through pilot on commonly used drugs in veterinary practice for food animals.

These antibiotics were dispensed onto the surface of the Mueller Hinton (Oxoid) containing about 4mm of the Agar plates at least 24 mm apart from the centre of each other using an oxoid antibiotic disc dispenser as shown in Figure 3.

Figure 4: Oxoid antibiotic disc dispenser.

The plates were put in an incubator at 35^0C for 16 to 18 hours. The inhibition zone diameters were measured using a digital Vernier calliper (figure 5) and recorded in millimetre and compared to that adapted from the antimicrobial usage chart from the National.

Committee for Clinical Laboratory Standards (NCCLS) approved standard. Results were recorded to the nearest whole millimetre and the 3 outcomes determined which include Resistant, Intermediate and Susceptible. Quality control standard laboratory procedures

were strictly adhered to in order to avoid contamination. *Escherichia coli ATCC 25922* strain and *Salmonella typhimurium ATCC 14028* were used as control organisms.

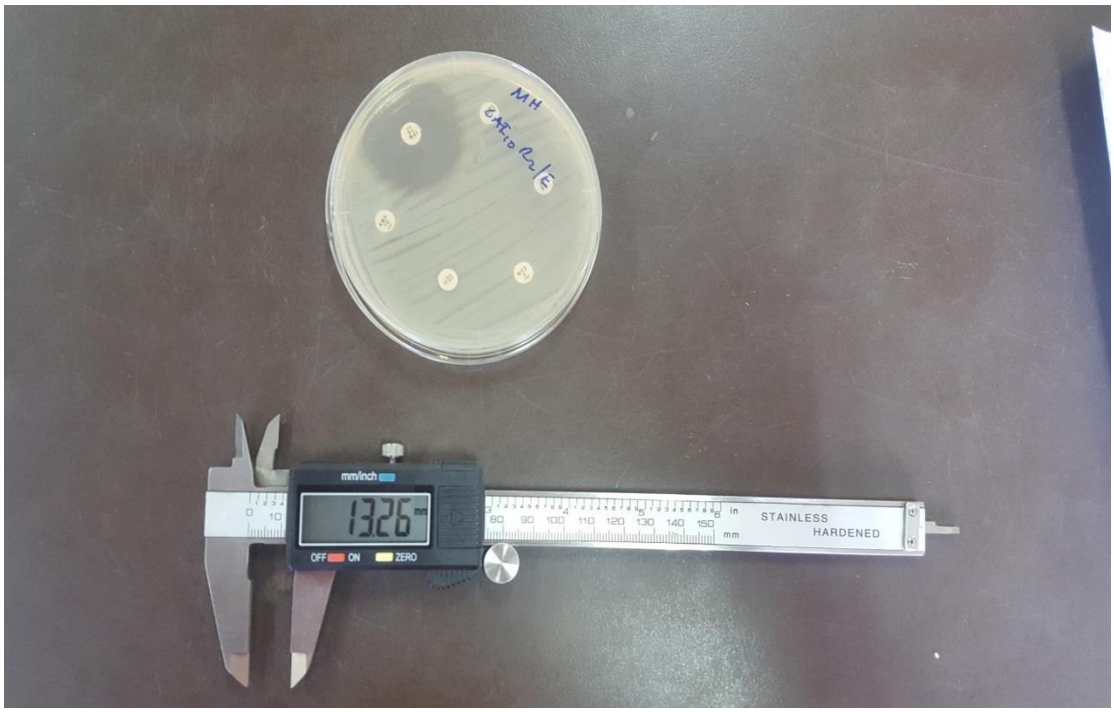

Figure 5: Disc showing zone of inhibition to a sample cultured on Mueller Hinton Agar vernier calliper.

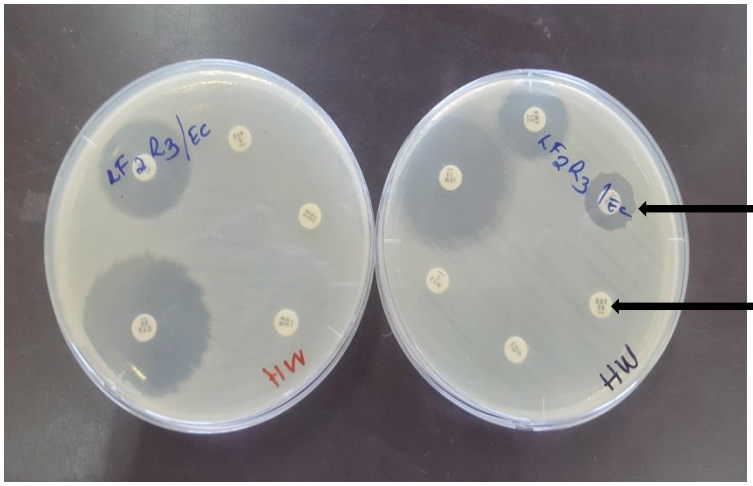

Figure 6: Antibiotic discs indicating the interpretation of the bacterial antibiotic sensitivity results (resistance and susceptible).

3.7. Data Collection

To investigate some factors associated with the resistant *Salmonella sp.* and *E. coli*, a structured questionnaire was administered. This was done by the principle investigator interviewing some farmers upon delivery of chicken batches.

3.8 Data processing and analysis

Before analysis, data from the questionnaires was sorted and checked for completeness. This data was coded and entered into spreadsheet (Excel). The recoded zones of inhibition for AST were entered into WHONET and analysed. Other specific analysis and graphs were done in STATA® statistical package version 13.0 (Stata Corporation, college station, Texas). Frequency distribution was reported for all categorical variables. Fisher's exact test was used to evaluate the relationship between the chicken batch AMR outcome and the hypothesized risk factors (predictor variables) since the cell frequency was less than 5. In this study chicken batch, AMR status was defined as: Resistance of any of the isolates in from a batch to at least 3 different classes of antibiotics. All data was be analysed at 5% (0.05) significance level with 95% confidence interval.

3.9 Ethical Considerations

Ethical approval to undertake the study was obtained from The University of Zambia Biomedical Research Ethics Committee (**UNZABREC**), Reference. **No. 064-06-17**. Permission was also granted by the Local City Councils, Ministry of Fisheries and Livestock and the Managers of the abattoirs where the study was conducted. Before proceeding to obtain the samples, the purpose of the study was explained to respondents and informed consent was sought. Confidentiality and anonymity were maintained. All the information collected from the abattoir during the course of the research was kept strictly confidential, and any information which left the abattoirs had names and address removed so that they cannot be recognised. Guidelines for Good Research Practice according to research ethics were taken into consideration throughout the research

process. Furthermore, the researcher ensured that the proposed research methodology for conducting the study was followed. This meant that there was no doctoring or alteration of the research findings aimed at satisfying (suit) the researcher's views.

CHAPTER FOUR

RESULTS

4.1 Frequency distribution of *Salmonella sp.* and *E. coli* isolates from cloaca and carcass swabs of broiler chickens

A total of 150 samples were collected from two abattoirs (Table 2). From the 150 samples collected, two salmonella isolates were isolated from a cloaca swab and from a dressed carcass. Both were isolated from one abattoir (B) representing 2.6%, and 1.3% overall considering both abattoirs.

For *E. coli,* 118 isolates were isolated from the 150 samples collected comprising 31.4% (37/118) from abattoir A, of which 16.9% (20/118) were from cloacal swabs and 14.4% (17/118) from carcass swabs. Similarly, from abattoir B, 68.6% (81/118) *E.coli* were isolated, of which 34.7% (41/118) were from cloacal swabs and 33.9% (40/118) were from carcass swabs (Table 2).

Table 2: Distribution of *Salmonella spp*. and *E.coli* isolates by slaughterhouse

Abattoir ID	No. of batches	Samples collected	Cloacal Isolates	Carcass Isolates	Total Isolates	Overall Proportion
			Salmonella sp.			
A	10	60	0	0	0	0.0%
B	15	90	1/45 (0.02%)	1/45 (2.6%)	2	2.6%
Total	25	150	1/75 (0.013%)	1/75 (1.3%)	2	1.3%
			E. coli			
A	10	60	20/30 (66.7%)	17/30 (56.7%)	37	61.7%
B	15	90	41/45 (91.1%)	40/45	82	90%

				(88.9%)		
Total	25	150	61/75 (81.3%)	56/75 (74.6%)	118	78.6%

4.2 Antibiotic sensitivity testing results for *Salmonella spp*. and *E.coli* isolates

All the isolates, *Salmonella spp*. (n=2) and *E. coli* (n=118) were subjected to different antimicrobial agents for sensitivity using the disc diffusion method and the results are shown in Tables 3 and 4.

Table 3: Isolates patterns of *Salmonella sp*. and *E. coli* resistance to antibiotics

Antibiotics tested	No. of Salmonella (n=2)	No. % Resistance	No. of *E. coli* (n=118)	% Resistance
Amoxicillin-clavulanic acid (AMC)	1	50	12	10
Ampicillin (AMP)	1	50	86	73
Colistin (COL)	0	0	0	0
Cefotaxime (CTX)	1	50	32	27
Chloramphenicol (CHL)	0	0	46	39
Ciprofloxacin (CIP)	0	0	33	28
Imipenem (IPM)	0	0	0	0
Nalidixic Acid (NAL)	0	0	63	53
Tetracycline (TCT)	0	0	84	71
Trimethoprim/Sulfamethoxazole (SXT)	0	0	71	60

One of the two *Salmonella sp*. isolated exhibited resistance to 3 antibiotics, namely; Amoxicillin-clavulanic acid (50%), Ampicillin (50%) and Cefotaxime (50%) (Table 3). *E. coli* isolates showed different patterns of resistance, the highest was Ampicillin 73% (86/118) while the lowest was Cefotaxime 27% (32/118). However, there was no

resistance seen to Imipenem and Colistin sulphate for both *Salmonella sp.* and *E. coli* isolates.

Table 4: Antibiotic resistance patterns of *Salmonella sp*. for abattoirs A and B to different antibiotics.

Antibiotic name	Breakpoints (mm)	%R	%I	%S	%R. 95% C.I.	Number
Amoxicillin/Clavulanic acid	14 - 17	50.0	0.0	50.0	2.7-97.3	2
Ampicillin	14 - 16	50.0	0.0	50.0	-	2
Chloramphenicol	13 - 17	0.0	0.0	100.0	-	2
Ciprofloxacin	21 - 30	0.0	0.0	100.0	-	2
Colistin	S >= 11	0.0	0.0	100.0		2
Cefotaxime	23 - 25	50.0	0.0	50.0	2.7-97.3	2
Imipenem	20 - 22	0.0	0.0	100.0	-	2
Nalidixic acid	14 - 18	0.0	0.0	100.0	-	2
Trimethoprim/Sulfamethoxazole	11 - 15	0.0	0.0	100.0	-	2
Tetracycline	12 - 14	0.0	0.0	100.0	-	2

Note: Resistance (%R), Intermediate (%I), and Susceptibility (%S) and their respective breakpoints.

Salmonella sp. isolates exhibited resistance to 3 drugs Amoxicillin-clavulanic acid of 50% (2.7-97.3; 95CI), Ampicillin 50% (2.7-97.3; 95CI) and Cefotaxime 50% (2.7-97.3; 95CI). There was no resistance to other drugs which included Chloramphenicol, Ciprofloxacin, Colistin, Imipenem, Nalidixic acid, Trimethoprim/Sulfamethoxazole, and Tetracycline (Table 4).

The antibiogram pattern (Table 5) of the 118 isolates revealed that all the isolates were sensitive to Colistin sulphate (100%) and Imipenem (100%). However, maximum

resistance was observed to Ampicillin 72.9% (95% CI: 63.8 – 80.5%), followed by Tetracycline 71.2% (95% CI: 62.0 – 79.0%), Trimethoprim/sulfamethoxazole 60.2% (95% CI: 62.0 – 69.0%), Nalidixic acid 53.4% (95% CI: 44.0 – 62.6%), Chloramphenicol 29.0% (95% CI: 30.3 – 48.4%), Ciprofloxacin 28% (95% CI: 20.3 – 37.1%) while the lowest was cefotaxime 27% (95% CI: 19.5 – 36.2%).

Table 5: *E. coli* antibiogram resistance patterns for both abattoir A and B.

Antibiotic name	Code	Breakpoints	%R	%I	%S	**%R.** 95% C.I.	No.
Amoxicillin/Clavulanic acid	AMC	14 - 17	10.2	7.6	82.2	5.6-17.5	118
Ampicillin	AMP	14 - 16	72.9	0.8	26.3	63.8-80.5	118
Chloramphenicol	CHL	13 - 17	39.0	6.8	54.2	30.3-48.4	118
Ciprofloxacin	CIP	16 - 20	28.0	6.8	65.3	20.3-37.1	118
Colistin	COL	S >= 11	0.0	0.0	100.0	-	118
Cefotaxime	CTX	23 - 25	27.1	1.7	71.2	19.5-36.2	118
Imipenem	IPM	20 - 22	0.0	0.0	100.0	-	118
Nalidixic acid	NAL	14 - 18	53.4	12.7	33.9	44.0-62.6	118
Trimethoprim/Sulfamethoxazole	SXT	11 - 15	60.2	0.0	39.8	50.8-69.0	118
Tetracycline	TCY	12 - 14	71.2	2.5	26.3	62.0-79.0	118

Note: Resistance (%R), Intermediate (%I), and Susceptibility (%S) and their respective breakpoints. (n=118).

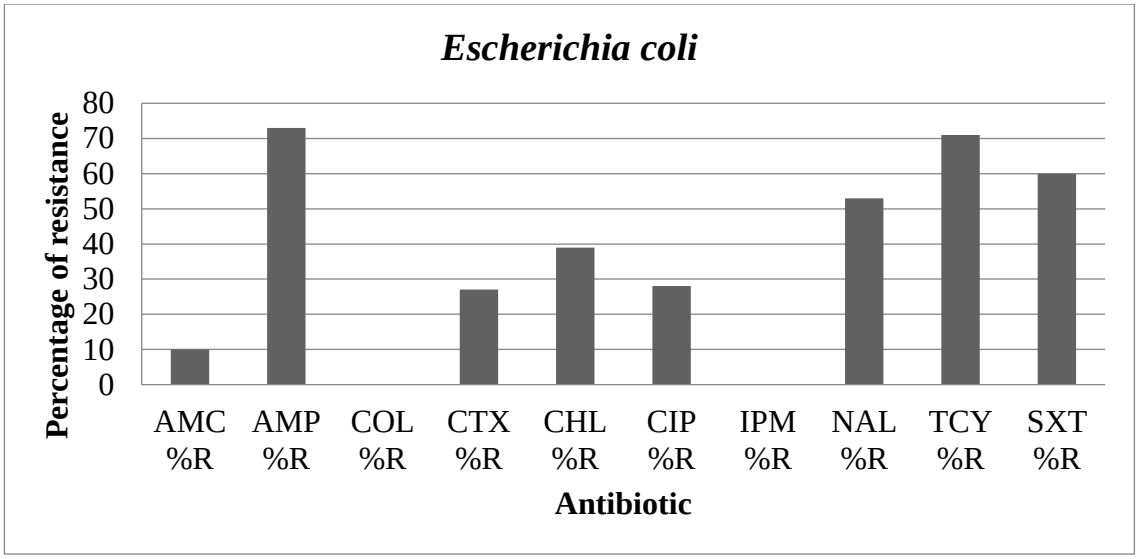

Figure 7: Graphical representation of the antibiotic resistance of *E. coli* for both abattoirs A and B to different drugs (n=118).

4.3 Resistance Profiles for *E.coli* isolates from both abattoirs A and B.

Table 6: Pattern of resistance profiles of *E.coli* to antibiotics

Resistance profile	No. isolates (n = 118)	% Isolates	Observation
None	11	9.3	Resistant to no antibiotic
TCY	6	5.1	Resistant to one antibiotic
NAL	5	4.2	Resistant to one antibiotic
CTX	2	1.7	Resistant to one antibiotic
AMP	4	3.4	Resistant to one antibiotic
TCY SXT	3	2.5	Resistant to two antibiotics
AMP TCY	3	2.5	Resistant to two antibiotics
AMC AMP	2	1.7	Resistant to two antibiotics
NAL TCY SXT	2	1.7	Resistant to three antibiotics
AMP TCY SXT	2	1.7	Resistant to three antibiotics
AMP NAL TCY	2	1.7	Resistant to three antibiotics
AMC AMP CTX	1	0.8	Resistant to three antibiotics

AMP NAL TCY SXT	5	4.2	Resistant to four antibiotics
AMP CIP NAL TCY	3	2.5	Resistant to four antibiotics
AMP CTX NAL TCY	3	2.5	Resistant to four antibiotics
AMC AMP TCY SXT	1	0.8	Resistant to four antibiotics
AMC AMP CTX TCY	1	0.8	Resistant to four antibiotics
CHL CIP NAL TCY SXT	2	1.7	Resistant to five antibiotics
AMP CIP NAL TCY SXT	1	0.8	Resistant to five antibiotics
AMP CHL NAL TCY SXT	6	5.1	Resistant to five antibiotics
AMP CHL CIP TCY SXT	1	0.8	Resistant to five antibiotics
AMP CHL CIP NAL TCY	1	0.8	Resistant to five antibiotics
AMP CTX CIP NAL TCY	1	0.8	Resistant to five antibiotics
AMP CTX CHL TCY SXT	2	1.7	Resistant to five antibiotics
AMP CTX CHL NAL SXT	4	3.4	Resistant to five antibiotics
AMC AMP CHL TCY SXT	1	0.8	Resistant to five antibiotics
AMC AMP CTX NAL TCY	1	0.8	Resistant to five antibiotics
AMP CHL CIP NAL TCY SXT	**17**	**14.4**	**Resistant to six antibiotics**
AMP CTX CIP NAL TCY SXT	1	0.8	Resistant to six antibiotics
AMP CTX CHL NAL TCY SXT	4	3.4	Resistant to six antibiotics
AMC AMP CIP NAL TCY SXT	1	0.8	Resistant to six antibiotics
AMC AMP CHL NAL TCY SXT	2	1.7	Resistant to six antibiotics
AMC AMP CTX NAL TCY SXT	1	0.8	Resistant to six antibiotics
AMC AMP CTX CHL NAL SXT	1	0.8	Resistant to six antibiotics
AMC AMP CTX CHL NAL TCY	1	0.8	Resistant to six antibiotics
AMP CTX CHL CIP NAL TCY SXT	6	5.1	Resistant to seven antibiotics
AMC AMP CHL CIP NAL TCY SXT	3	2.5	Resistant to seven antibiotics
AMC AMP CTX CIP NAL TCY SXT	2	1.7	Resistant to seven antibiotics

AMC AMP CTX CHL NAL TCY SXT	1	0.8	Resistant to seven antibiotics
AMC AMP CTX CHL CIP NAL SXT	1	0.8	Resistant to seven antibiotics
AMC AMP CTX CHL CIP NAL TCY SXT	1	0.8	Resistant to eight antibiotics

One hundred and seven (107/118) 90.7% *E.coli* isolates were resistant to at least one antibiotic (Table 6). Sixty two (53.2%) *E.coli* isolates were found to be resistant to at least three different classes of antibiotics indicating multi-drug resistance (MDR). Ampicillin (AMP), Chloramphenicol (CHL), Ciprofloxacin (CIP), Tetracycline (TCY) and Trimethoprim/sulfamethoxazole (SXT) were six antibiotics with the highest number of isolates exhibiting resistance.

4.4 Effects of the hypothesised variables on the likelihood of chicken batch AMR status to *E.coli.*

The results shown in Table 7 indicate the results of tests of association between AMR-chicken-batch outcome and hypothesized risk factors in Stata. The dependent variable here was the chicken batch AMR status (defined as resistance to at least three different classes of antibiotics) and the independent variables being; antibiotic use, withdraw period, source of antibiotics, prescription, who administered the antibiotic, type of production, presence of other animals, isolation of sick birds, age at which birds get sick, and use of growth promoters/boosters. Cross-tabulations of Fishers exact test in Stata® statistical software (Table 7) did not reveal any statistical association as all the variables had p-value > 0.05.

Table 7: Fisher's exact test of association between the dependent variable (chicken batch *E. coli* AMR status) and predictor variables.

Variables (independent)	Level	Proportion	P-value
Use antibiotics	Yes	8 (47.1%)	1.000
	No	9 (52.9%)	
Follow withdraw period	Yes	7 (41.2%)	0.406
	No	1 (5.9%)	
	Do not know	9 (52.9%)	
Where do you buy drugs	Drug store	8 (47.1%)	0.335
	Non-response	9 (52.9%)	
Are you asked for a Prescription	Yes	6 (35.3%)	0.208
	No	2 (11.8%)	
	Non- response	9 (52.9%)	
Access of drugs	Very Easy	4 (23.5%)	0.511
	Easy	4 (23.5%)	
	Do not know	9 (52.9%)	
Who administers	Veterinary officer	4 (23.5%	1.000
	Trained farm personnel	4 (23.5%)	
	Farm owner	5 (29.4%)	
	No response	4 (23.5%)	
Farm category	Small scale (1 – 1000)	-	0.353
	Medium scale (1000-5000)	1 (5.9%)	
	Large scale	16 (94.1%)	
Production type	All in all out	12 (70.6%)	0.102
	Continuous	5 (29.4%)	
Other animals	Yes	12 (70.6 %)	1.000
	No	5 (29.4%)	

Do you isolate sick birds	Yes	3 (17.6%)	0.568
	No	12 (70.6%)	
	Do not know	2 (11.7%)	
Age birds get sick	Week 2	2 (11.7%	0.349
	Week 4	1 (5.9%)	
	Week 5	2 (11.7%)	
	Don’t know	12 (70.6%)	
Use of boosters/growth promoters	Yes	2 (11.7 %)	1.000
	No	5 (29.4%)	
	Don’t know	10 (58.9%)	

CHAPTER FIVE

DISCUSSION

5.1 Summary of the study

This study aimed at determining the occurrence of antimicrobial resistance *Salmonella sp*. and *E. coli* in broiler chickens slaughtered in commercial abattoirs in Lusaka Province, Zambia. As an emerging problem worldwide, gram-negative bacterial organisms are increasingly becoming resistant to antimicrobials, in particular, resistance to common causes of diarrhoea *Salmonella sp*. and *E. coli*. In this study both *Salmonella sp*. and *E. coli* were isolated though *E.coli* isolation frequency was higher compared to *Salmonella sp.* as would be expected.

5.2 Bacteria isolated from cloaca and carcass swabs

The presence of *Salmonella sp.* in any food meant for human consumption specifically chickens is a major public health concern. The recovery rate of *Salmonella sp*. from chickens in this study from the two abattoirs (1.3%) was lower than previously reported by Mpundu et al., (2019), found 2.6, Shamaila et al. (2018) reporting about 2%, Hang'ombe, (1999) who reported a prevalence of 28% and 16.2% in a study done by William et al., (2012). However, the finding in this study was in agreement with previous studies of *Salmonella sp.* in poultry, were low *Salmonella sp.* detection rates of 0 to 17.0% (Ata and Aydın, 2008) and 9.9 to 17.9% by Van Hoorebeke et al., (2010) were reported. In a comparison of isolation of *Salmonella sp.* at farm and retail level, Goncagul et al., (2005) isolated *Salmonella sp.* and other non-tippable *Salmonella sp.* from 7 out of 8 broiler carcass producers at prevalence levels of 8.6% and 9.5%, respectively. In a study carried out in China, 1152 chicken samples were analysed for the presence of *Salmonella sp.* from three types of retail markets and the overall prevalence was 52.2% (Yang et al., 2010). Studies done by Carraminana et al., (2004) and Goksoy et al., (2004) on poultry carcass contamination also found a high prevalence of *Salmonella sp*. at the rate of 43.6% and 50%, respectively.

The low *Salmonella sp*. isolation in this study could mainly be attributed to intermittent low shedding patterns of *Salmonella sp.,* level of pathogen exposure and whether the strains carried genetic factors that facilitate evasion of host defenses. *Salmonella sp*. unlike other enterobacteria such as *E.coli,* is transmitted through the faecal-oral route and is associated with poor hygiene practices (Tessari et al., 2012). Salmonellosis is an important disease worldwide and it remains important that surveillance of poultry species at farms and processing plants is conducted regularly. These zoonotic organisms (*Salmonella sp.* and *E. coli*), may not necessarily cause any health problems in animals where they may exist as commensals but may pose a health challenge in humans, especially the young, elderly and the immuno-compromised, through enteric infections.

High use of antibiotics is another factor that could have contributed to the low isolation of *Salmonella sp*. Most antibiotics fail to eliminate *Salmonella sp*. from the animals although shedding is (temporarily) decreased (Arora et al., 2013). The use of sub-therapeutic doses of antibiotics as growth promoters is a public health problem, because many resistant microorganisms may transfer resistance to microorganisms found in bird feces. This kind of use may be responsible for the selective pressure that generates resistant bacteria, due to the risk of dissemination of pathogens and transfer of resistance genes, through the food chain, to pathogenic and commensal microorganisms of humans, decreasing the treatment options for infections (Medeiros, 2011). It is clear that antibiotics are not the primary choice to control *Salmonella sp.* in poultry flocks. This is mainly due to the fact that drugs, when applied at therapeutic level, are able to clear some and most of the bacteria depending on the spectrum of the antibiotic used. However, some of these bacteria acquire mechanism of resistance which makes them survive in the presence of these antibiotics and hence can be isolated from the cloacal and carcass swabs (Arora et al., 2013, Goksoy et al., 2004).

This study found a high prevalence of *E .coli* at both abattoir A and abattoir. *E.coli* compared to *Salmonella sp*. is a normal commensal bacteria hence high isolation is expected. The high prevalence of *E.coli* in this study is in line with those previously done in Zambia by Chishimba, et al. (2016) and Hang'ombe, (1999), who similarly

found a high prevalence of *E. coli* including extended-spectrum beta-lactamases (ESBLs).

Broilers chickens arriving at poultry slaughterhouses are generally highly contaminated with bacteria, especially with potential human pathogenic bacteria, such as coliforms and *Salmonella sp.* (Goksoy et al., 2004). A high contamination level of *E. coli* on chicken carcasses was associated with carcass contamination with gut products during the process of evisceration. *E. coli* is the most commonly used indicator of faecal meat contamination, using culture-based methods (Delcenserie et al., 2008). A study by Delcenserie et al., (2008) found high contamination level of *E. coli* on beef carcasses which were attributed to carcass contamination with gut products during the process of evisceration (Delcenserie et al., 2008).

5.3 Antibiotic Resistance Patterns of *Salmonella sp*. and *E. coli* isolates

This study isolated *Salmonella sp* that was resistant to Amoxicillin-clavulanic acid, Ampicillin and Cefotaxime. The resistance of *Salmonella sp* to Ampicillin and Amoxy-clav, both at 18%, respectively has been also reported in other studies (Arora et al., 2013) as well as resistance to cefotaxime by a study done by (Shah and Korejo, 2012). Other drugs aswell which belong to third generation cephalosporins Ceftiofur 61% and Ceftriaxone (4.9%) were isolated from the abattoir (Hanson et al., 2003, Logue et al., 2003). The frequency and extent of *Salmonella sp*. resistance to antimicrobials vary based on the use of antibiotics in humans and animals and on ecological differences in the epidemiology of Salmonella infections (Zhao et al., 2008). Resistance to *Salmonella sp*. transmitted by contaminated foods of animal origin is undesirable, but it is an inevitable consequence of the use of antimicrobials in animals used in food production (Diarrassouba et al., 2007).

The antibiotic resistance pattern of the 118 *E.coli* isolates revealed that all the isolates were sensitive to Colistin sulfate (100%) and Imipenem (100%). This could be attributed to the fact that colistin and Imipenem are the last line of treatment drugs hence non-resistance to the antibiotics is expected because of non exposure of bacteria to these particular antibiotics. However, most of the isolates were sensitive to

Amoxicillin/clavulanic acid (82.2%), followed by cefotaxime (71.2%), ciprofloxacin (65.3%), and chloramphenicol 54.2%). Above all, maximum resistance was obtained against ampicillin (72.9), followed by tetracycline (71.2%), trimethoprim/sulfamethoxazole (60.2%) and nalidixic acid (53.4%). The observation in this study are consistent with those observed by Chishimba et al., (2016) who found high resistance of *E.coli* isolated from abattoir carcass swabs to antibiotics which included Tetracycline, Cefotaxime, Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Nalidixic acid and Sulfamethoxazole/trimethoprim and furthermore observed that multidrug resistance (MDR) of *E. coli* isolates to six or more drugs was most frequent (45.5%, 35/77). The findings are similar to studies conducted by Mainda et al., (2015) who also found the resistance of *E.coli* isolated from cattle Cefpodoxime, Ciprofloxacin, Ampicillin, Sulfamethoxazole/trimethoprim and Tetracycline.

Tetracycline had been used as a growth enhancer in food-producing animals until 1998 to 2000, when its use was banned in most developed countries (Cardoso et al., 2006). Tetracycline has also been used as a therapeutic agent, thus the high level of resistance to tetracycline is not surprising(Cardoso et al., 2006). In Zambia, use of tetracycline in food animals, particularly, has been used extensively to treat tick-borne diseases and has given rise to the resistance of bacteria in the process (Mainda et al., 2015a). In *E. coli*, tetracycline resistance is frequently regulated by efflux genes that are normally associated with large plasmids, which often carry other antibiotics resistance genes, heavy metal resistance genes, and/or other pathogenic factors such as toxins. Hence, selection for any of these factors selects for the plasmid (Diarrassouba et al., 2007).

In this study, none of the farm level factors investigated was associated with AMR status. This is probably due to a limited sample size. However, according to (Van den Bogaard et al., 2001) and (Diarrassouba et al., 2007), some of the major factors leading to AMR of *E.coli* include factors such as antibiotic use, overcrowding and poor sanitation. These factors are typical of intensive poultry farming and explain the high prevalence and degree of resistance in *E. coli* of poultry in this and other studies (Van den Bogaard, et al. 2001).

In other studies, multi-drug resistance enterobacteria including both *Salmonella sp.* and *E.coli* have been isolated and have been attributed to the use of growth promoters (Ramchandani et al., 2005, Talebiyan et al., 2014). In this study, the high rates of resistance to bacteria, specifically *E.coli,* can be attributed to the widespread use of antibiotics agents given to poultry as prophylaxis, growth and treatment which is line with a study done by (Byarugaba, 2004). In his study, Byarugaba, (2004), found that the use of antimicrobials in veterinary practice as therapeutic and prophylactic agents, in addition to using as antimicrobial growth promoters, greatly influences the prevalence of resistance in animal bacteria and posed some risk for the emergence of antibiotic resistance in human pathogens. Byarugaba (2004) further observed that isolates which are resistant to two or more antibiotics may have originated from high–risk sources of contamination like commercial poultry farms, where antibiotics are commonly used. In this study, we observed that a significant number of isolates were resistant to more than one antibiotic. This is consistent with the study by Byarugaba, (2004), which provided direct evidence that antimicrobial use in animals selects for antimicrobial-resistant bacteria that may be transferred to humans through food or direct contact with animals. This has been observed in a study by Chiyangi et al., (2017), who found multi-resistance enterobacteria including *Salmonella sp.* and *E. coli* in children under the age of 5 years.

The increasing population coupled with hard economic situation has resulted in many Zambian residents to start rearing broiler chickens as an income generating venture. This has resulted in humans coming into close contact with chickens and these chickens could be possible sources of shedding *Salmonella sp*. and *E.coli* bacteria into the environment. Lately, there has been an increase in the number of drugstores which stock human and livestock antimicrobial without proper registration, guidance on the dispensation of drugs (Kalungia et al., 2016). The inadequate monitoring of the drugstores in the nation, especially in Lusaka district, has led people to easily access the livestock antibiotics without following the recommended laid down clinical prescription procedures thereby increasing the risk of resistant *Salmonella sp*. and *E.coli* bacteria in food animals (Mainda et al., 2015a, Mubita et al., 2008). Detection of *Salmonella sp*. and *E.coli*

bacteria isolates in broiler chickens from processing plant (abattoirs) has not been fully conducted in Zambia.

In this study, both resistant *Salmonella sp*. and *E.coli* were detected in broiler chickens. The high prevalence of *E.coli* observed in this study could be due to frequent administration of antimicrobials to poultry which in turn increases the risk of higher antimicrobial-resistant *E. coli* strains in the normal intestinal flora as observed in a study conducted by (Talebiyan et al., 2014, Van den Bogaard et al., 2001). In this study, it was established that broiler chickens were possible reservoirs for resistant faecal flora *Salmonella sp*. and *E. coli*. Furthermore, the high colonization rate could be attributed to the cross-contamination of poultry in abattoirs, particularly during slaughtering and dressing. The processes of slaughtering and dressing are potential risk factors that may exacerbate the transmission rate of *Salmonella sp*. and *E. coli*. resistant genes as described by (Chiu and Ou, 1996).

5.4 Association between the Chicken AMR status and study variables

In this study, the Fishers exact test of association was used to test the association between the chicken batch AMR status and the studied variables which included; use of antibiotics, withdraw periods adherence, source of antibiotics, prescriptions availability, who administers, diseases encountered, type of production, presence of other animals on farm, isolation of sick birds, age birds usually get sick and use of feed additives/promoters during production. None of the variables investigated in this study showed statistical significance as all the variables had $p > 0.05$. This could have mainly been due to the small number of the questionnaires administered in this study which somehow reduced the power of the study in determining the association between the outcome and the predictor variables. However, a study by Van den Bogaard, et al. (2001) identified antibiotic use, overcrowding, use of growth promoters and poor sanitation as some of the major factors associated with antimicrobial resistance in broiler chickens.

Strength and Limitations of the study

The major limitation of this study was the delay in granting permission for the study to be undertaken from the abattoir owners. This was mainly due to the outbreak of cholera during the study period, leading to restriction of access to the abattoirs. One particular abattoir totally refused for a study to be done from their premises because of the same reason.

The other major limitation of this study was that only a selected number of pathogens from the poultry food value chains were investigated and this comes as one of the major limitations. However, it is acknowledged that other organisms outside the scope of selected pathogens could pose a bigger challenge of antibiotic resistance, whereby such agents may serve as reservoirs of resistance genes. Future studies, therefore, should broaden the scope of pathogens and also the food value chains to be investigated. Further, the districts to be investigated were purposively selected based on predetermined criteria. This was necessitated by the limitation of funds which limits the number of such study areas to be included.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The aim of the study was to determine the presence of antimicrobial resistant Salmonella spp. and *E. coli* in broiler chickens at the processing facilities in Lusaka district with a view of determining the public health significance of these resistant bacteria. The study findings revealed that both *Salmonella sp.* and *E. coli*, resistant to a number of antibiotics of both animal and human importance were present and were mainly resistant to Ampicillin and Tetracycline. The *Salmonella sp.* and *E. coli* isolated gave an indication of the levels of resistance of these bacteria in food meant for human consumption and the role they play in resistance in poultry that may find their way into meat products on the market. This study has shown that multidrug resistance of *Salmonella sp*. and *E. coli* in broiler chickens may largely contribute to the wider and broad challenge of antimicrobial resistance and at the same time provide useful information for monitoring and surveillance purposes. The overall implication is that these resistance bacteria may be transmitted to humans and may end up causing treatment failures leading to an increase in mortalities.

6.2 Recommendations

The following recommendations have been made based on results obtained in this study;

I. The Ministry of Health (MOH) and Ministry of Livestock and Fisheries (MLF) should work hand in hand to ensure that essential drugs for human treatment should not be used to treat poultry to avoid development of resistance as exposure tends to lead to the development of resistant bacteria.
II. Policymakers through the Ministry of Livestock and Fisheries should limit the use of antibiotics in poultry farms in order to reduce the antibiotic resistance.
III. The Medicines Regulatory Authority should put strict regulations regarding sale of antibiotics through prescription only.

IV. More studies need to be undertaken to investigate the relationship between AMR and predictor variables using larger sample sizes as the sample size of the questionnaire investigated in this study was small (17).

V. More studies need to be done on the abattoir workers (hands and faecal samples) to ascertain levels of AMR bacteria which could result from cross contamination.

VI. It is also important to continue monitoring pathogens especially those of zoonotic nature such as *Salmonella sp*., *Campylobacter sp.* and *E. coli* maintain the status quo through effective surveillance.

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APPENDICES

Appendix I: The Participant information sheet

University Of Zambia

School of Public Health

P.O. Box 50110

Principle investigator: Nelson Phiri

Study Title: This study focuses on antimicrobial resistance of the common food borne pathogens namely *Salmonella sp.* and *E. coli* in broiler chickens slaughtered from commercial abattoirs in Lusaka province, Zambia.

I would like to invite you to take part in a research study. Please note this is a voluntary participation therefore before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Ask questions if anything you read is not clear or would like more information.

Purpose of the study: This study is important for the partial fulfillment of Masters of Public Health which I am pursuing under The University of Zambia. Apart from that, the study is important in establishing the current state of antimicrobial resistance *Salmonella sp.* and *E. coli* bacteria pathogens which are a major threat to food safety causing enteric diarrhea in humans. The study will provide valuable insights in identifying resistant strains of pathogens associated with food borne infections in chickens which will ultimately results in improved knowledge for implementation of food safety measures.

Why have I been invited? Your selection to participate in this research has been done purposively. This research is being done in 3 commercial abattoirs currently available in

Lusaka Province. As you happen to work at one of these selected abattoirs, you suit in our study participants hence your being selected.

Do I have to take part? Participation is voluntary, but we would really be grateful if you accept to take part as there are limited study sites. However, it is up to you to decide. We will describe the study and go through the information sheet, which we will give to you. We will then ask you to sign a consent form to show you agreed to take part. You are free to withdraw at any time, without giving a reason if you are not comfortable.

What will happen to me if I take part? When you are recruited to be part of this research you will be required to provide all the necessary information that needed. This will on every visit to the abattoir which be done weekly. Samples will be collected from the premises and these will be taken to the University laboratory for processing. There your assistance to data collection at sample collection centers will be required.

What will I have to do? Apart from providing necessary required information there are no special restrictions as what you are requested to provide.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from the study will help to increase the understanding of antimicrobial resistance *Salmonella sp.* and *E. coli* which are a major causes of diarrhea through consumption of meat which is contaminated with these bacteria's.

Will my taking part in the study be kept confidential?

Yes. All the information, which is collected, about the abattoir and you during the course of the research will be kept strictly confidential, and any information about you which leaves the abattoir will have your name and address removed so that you cannot be recognised.

What will happen if I don't carry on with the study?

You can withdraw from the study but keep in contact with us to let us know your progress. Information collected may still be used. Any samples or information that can still be identified as yours will be destroyed if you wish.

What will happen to the results of the research study?

All the information will be looked at closely at the end of the research. You will be given feedback about the results which may also be published in the medical journal and presented to meetings and national and local authorities.

Further information and contact details:

For further information and clarity don't hesitate to contact the following

Principal Investigator: Nelson Phiri

Email address: nphiri39@yahoo.com Phone Number: 260978167332

Or

The Chairperson

The University of Zambia Biomedical Research Ethics Committee (UNZABREC)

Ridgeway Campus

P.O Box 50110

Lusaka.

Email: unzarec@unza.zm; ***Phone 260-1-256067***

THANK YOU FOR TAKING TIME TO READ!!!

Appendix II: The interviewer consent sheet

I am Nelson Phiri, a student from the University of Zambia, conducting a research on Determination of antimicrobials resistant profiles of *Salmonella sp.* and *E.coli* in chickens slaughtered from commercial abattoirs in Lusaka province, Zambia. You are invited to participate in this Research. The information from this research will be useful in ensuring safety of food for public consumption.

There is an interview schedule which has been designed for you to participate in as an individual. The interview will take a few minutes to complete. The answers to the questions will be treated with high confidentiality and your name will not appear any where.

This research has been reviewed and approved by The University of Zambia Biomedical Research Ethics Committee (**UNZABREC**) conforms to the standards of the National Research Ethics guidelines. If you have any questions about this process or about your rights as a participant in the study, you may contact the Dean for School of Public Health, The University of Zambia. You have the right to withdraw or refuse to participate in the study before questions are asked or during the course of the interview.

Thank you for your willingness to contribute to the success of this research.

Name of Interviewer: .....

Signature: ..... Date: …….

The above information has been explained to me clearly and I fully understand and consent myself to participate in the research.

Name: ……

Signature/thumb print: …….. Date: ……...

Appendix III: Sample collection Form

Data Collection Form

Date……..

Abattoir No.	Sample No.	Sample Id	Salmonella(+ve/ve)	E.coli +ve or -ve	Salmonella sp. Result (AST)			E.coli (AST)		
					S	I	R	S	I	R

Key: S = Susceptible / sensitive, I = intermediate; R = Resistant

AST = Antimicrobial Sensitivity Test

Appendix IV: Questionaire

Section A : Knowledge and General Poultry Farm Management Practices

1. Farm category

   1. Small scale (1-1000 birds) 2. Medium scale (1001- 5000) 3. Commercial (above 5000)

2. What is the farm type?

   (1) All in All out (2) Continous

3. What is the farm's water source drinking and cleaning bird house?

   1. Borehole 2. Well 3. Municipality/ piped 4. Others……

4. Do you mix different species of birds in one house?

   1.Yes 2. No

5. What is the origin of the birds?

   1. Same company 2. Different company

6. What is the source of the feed given to the birds?

   1. Self formulated 2. From feed producers specify…….....

7. Is the housing restricted only to personnel?

1. Yes 2. No

If No to question…. Who else has access to the housing?

……

8. Is the housing cleaned and disinfected before introducing birds?

1. Yes 2. No

9. Are there foot baths and hand sanitizers applied before entering the bird house?

1. Yes 2. No

10. Do you use antibiotics to treat the birds when ill?

(1) Yes (2) No

11. If Yes to question …. Give the types commonly used and for what conditions?

.....

12. If No to question… what other plans are used to treat the birds?

……..

13. Do you know and follow drug withdraw period?

1. Yes 2. No

14. Do you know about antimicrobial resistace?

1. Yes 2. No

15. Do you know if use of antibiotics in food poultry can lead to resistant bacteria in meat that can make people sick?

1. Yes 2. No

16. Do you seek veterinary advice or take samples to the lab before administering antibiotics?

1. Yes 2. No

17. Where do you buy the veterinary antibiotics?

1. Retailor's shop 2.Veterinary clinic 3.Individual Veterinarians (Vet) 4. Other.....

18. Do drug sellers usually ask for a prescription when selling vet antibiotics?

1. Yes 2.No 3. Sometimes 4.Unknown

19. Who administers the antibiotics to the birds?

1.Veterinary officers 2.Trained farm personel 3.Untrained farm personel 4.Farm owner 5. Others

20. Are low doses of antibiotics (such as in feed) given to the birds to promote their growth?

1. Yes 2. No

If Yes to question … indicate the types used

.....

21. Is there a flock health care and monitoring system in place?

1. Yes 2. No

Appendix V: Permission Letter

LETTER REQUESTING PERMISSION TO CONDUCT RESEARCH

University of Zambia
School of Public Health
P.O. BOX 50110
Lusaka.

The Director – Public Health
Lusaka City Council
Lusaka.
Date

Dear Ms/Mr……

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH

I am a registered Masters of Public Health student in the School of Public Health specializing in Environmental Health at the University of Zambia. The proposed topic of my research is: **Determination of antimicrobial resistant *Salmonella sp.* And *E.coli* from chickens slaughtered in commercial poultry abattoirs in Lusaka province, Zambia.**

The objectives of the study are:

1. To isolate and identify AMR *Salmonella sp.* and *E. coli* in broiler chickens slaughtered in commercial abattoirs in Lusaka Province.
2. To determine the AMR patterns of *Salmonella sp.* and *E. coli* in isolates
3. To identify some factors associated with the presence of AMR *Samonella sp.* and

E. coli.

I am hereby seeking your consent to conduct research at the abattoirs located in the district in which you. To assist you in reaching a decision, I have attached to this letter:

(a) A copy of an ethical clearance certificate issued by the University.
(b) A copy of the research instruments to be used in the research.
(c) The information sheet to be used in the study.

Should you require any further information, please do not hesitate to contact the principal investigator or the supervisor.

Your permission to conduct this study will be greatly appreciated.

Yours sincerely,

Nelson Phiri

Contact details: Principal investigator: Nelson Phiri : Cell: +260978167332
Email: nphiri39@yahoo.com

Supervisor: Prof John Bwalya Muma : +260 966744355
Email: jbwalya@lycos.com