INVESTIGATING ANTIMICROBIAL USE AND OCCURRENCE OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL)-*ESCHERICHIA COLI* IN COMMERCIAL AND SMALL-SCALE POULTRY FARMS IN SELECTED DISTRICTS OF ZAMBIA

By

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements for the master of science degree in microbiology by research

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DECLARATION

I, **TAONA SINYAWA**, declare that the work in this dissertation is my own. Furthermore, to the best of my knowledge, this work has never wholly or partially been presented at this university or any other institution for the award of a master's degree or any other qualification.

Student's signature:  Date submitted: ……

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CERTIFICATE OF APPROVAL

This dissertation submitted by Taona Sinyawa, has been approved as fulfilling the requirements for the award of Master of Science degree in Microbiology by The University of Zambia.

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ABSTRACT

Antimicrobial resistance (AMR) among *Escherichia coli* (*E. coli*) from food animals is a rising problem with overuse, underuse, or prolonged usage of one type of antimicrobial in poultry as a contributing factor. In Zambia, studies linking poultry-associated AMR and antimicrobial use (AMU) are rare. This study aimed to investigate commercial and small-scale poultry farmers' usage of antimicrobials using a pre-tested structured questionnaire in ten districts of Zambia. A total of 119 questionnaires were administered to 35 commercial farmers and 84 small-scale farmers. In addition, the study detected extended-spectrum *β*-lactamase (ESBL)-producing *E. coli* isolates obtained from poultry in the same districts. Further, three cloacal swabs were collected in each of the 269 poultry houses and pooled into a single sample for analysis of the presence of *E. coli* and resistance profiles**.** A total of 232/269 (86.2%) *E. coli* isolates were isolated and tested for antimicrobial susceptibility using eight antibiotic classes and stored. The overall prevalence of AMR was 98% and Multidrug resistance (MDR) was identified in 70.7% of the *E. coli* isolates. However, 150/232 (64.7%) *E. coli isolates* were recovered and further screened for ESBL production by streaking them on cefotaxime (CTX)-supplemented MacConkey agar, 20% (30/150) of the third-generation cephalosporins (3GC) resistant isolates were ESBLs. Of these isolates, those with CTX Minimum Inhibitory Concentration (MIC) $\geq$ 4 μg/mL were then subjected to sequencing on a NextSeq. The questionnaire information showed that more small-scale than commercial poultry farmers used antimicrobials (OR = 7.70, 95% CI = 2.88–20.6, $p < 0.001$ but less prescriptions (OR = 0.02, 95% CI = 0.00–0.08, $p < 0.001$). Altogether, the questionnaire survey results showed a higher proportion of AMU and lower prescription usage among small-scale farmers**.** Overall, susceptibility testing revealed that resistance was highest to ampicillin (128/148, 86.5%) and tetracycline (101/136, 74.3%) and that the presence of multidrug-resistance (MDR) (28/30, 93.3%) among the third generation cephalosporins (3GC) (28/150) was high. Whole-genome sequencing (WGS) of eight (8/30, 26.7%) revealed (6/8,75%) the presence of ESBL-encoding genes $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-55}}$ and bla_{TEM}. WGS also detected other AMR genes for quinolones, aminoglycosides, phenicols, tetracycline, macrolides, and folate-pathway antagonists. Survey results indicated that there was higher usage of antimicrobials without

prescription. In addition, the results give insights into the occurrence of ESBL-producing *E. coli strains* with associated MDR in both commercial and small-scale poultry farms. It will be important to educate farmers on AMR risks and to encourage responsible usage of antimicrobials. There is a need to strengthen regulations limiting access to antimicrobials and establish a one health system to guide public health response.

DEDICATION

This work is dedicated, in particular to my husband, Dr. Ilunga Mutwale, and my Children (Lazau Mutwale and Tukisanta Mutwale), for their outstanding assistance throughout my time as a University of Zambia student. I am incredibly grateful to them for their unwavering moral support during the entire study period.

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LIST OF ABBREVIATIONS

3GC	Third Generation Cephalosporins
AGP	Antimicrobial Growth Promoters
AMP	Ampicillin
AMR	Antimicrobial resistance
AMU	Antimicrobial use
ARGs	antibiotic resistance genes
AST	Antimicrobial Susceptibility Testing
BPW	Buffered Peptone Water
CHL	Chloramphenicol
CIP	Ciprofloxacin
CRO	Ceftriaxone
CTX	Cefotaxime
CTX-M	Cefotaxime-Munich
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
ESBLs	Extended-spectrum β-lactamases
GEN	Gentamicin
GIT	Gastrointestinal tract
HGT	Horizontal Gene Transfer
IMP	Imipenem
MDR	Multidrug resistance
MGEs	Mobile genetic elements
MIC	Minimum Inhibitory Concentration
NT	Not tested
OXA	Oxacillinase
PCR	Polymerase Chain Reaction
R	Resistant
S	Susceptible
SHV	Sulphydryl Variable

SXT	Sulfamethoxazole/trimethoprim
TEM	Temoniera
TET	Tetracycline
β	beta

CHAPTER ONE

1.0 INTRODUCTION

1.1 Study background

Poultry is one of the most widespread types of meat produced globally with over 90 million tons of chicken meat produced annually. To counteract this demand, an annual 3% increase in worldwide production is anticipated (Van Boeckel *et al.*, 2015; Agunos *et al.*, 2016; Agyare *et al.*, 2019). Poultry production accounts for 45% of the value of livestock produced in Central and Southern Africa, making it a significant sustainable resource for rural populations in Sub-Saharan African nations (Agricultural Outlook 2021‑2030, 2022). Zambia's poultry sector has seen consistent growth due to the rising human population and concomitant increased demand for chicken meat and eggs, with a bird stock close to 4.7 million broilers, 21 million village chickens, and 2.2 million (Zamstats, 2022). The poultry product consumption trends generally show greater demand and wider acceptance across socio-economic, cultural, religious, and other barriers than any other meat of animal origin (CSO, 2017). Its approval is also anchored on its relative affordability, with prices ranging from $ 2.0 to $ 3.5 per kg. Furthermore, poultry production is attractive as it requires less financial capital and takes less time to reach the point of sale. This industry contributes 5% of the nation's Gross Domestic Product (AgriProFocus, 2015).

Swift urbanization and rising disposable income are other elements driving up demand for poultry and poultry products (AgriProFocus, 2015). There is pressure on farmers to increase production to meet this demand. Livestock intensification approaches are necessary to increase production and address food shortages, but they are also linked to the improper use of antibiotics (Lazarus *et al.*, 2015). In some cases, this has led to irrational use of antimicrobials, such as during the treatment of infectious diseases, prophylaxis, meta-phylaxis, and growth promotion (Selaledi *et al.*, 2020). Furthermore, less than 20% of households engaged in poultry production have access to veterinary

services, a situation likely to promote the abuse of antimicrobials through self-prescriptions and treatment (CSO, 2017). Unlike antimicrobials for treatment, the use for growth promotion is usually at subtherapeutic amounts, which may drive the development of Antimicrobial resistance (AMR) (Aidara-Kane *et al.*, 2018). Abuse or overuse of antimicrobials results in continuous exposure of an animal's (the avian) intestinal flora to antimicrobials, creating a selection pressure that leads to the emergence of AMR, which may be transmitted to humans AMR (Poirel *et al.*, 2018; Wang *et al.*, 2019).

The more prevalent sale of live poultry entails that people are more likely to be exposed to these resistant organisms via poultry than other livestock (Nhung, Chansiripornchai and Carrique-Mas, 2017). This implies that poultry can contribute to the spread of antibiotic-resistant genes to bacteria in humans via the food chain or through direct and indirect mechanisms (Department of Health and Human Services, 2019). Antimicrobials play an essential role in human and food- animal health and represent one of the main therapeutic tools for human and veterinary medicine (Prestinaci, Pezzotti and Pantosti, 2015; Wallace, Fishbein and Dantas, 2020). As, a result AMR is a global public health threat that has a bearing on humans, animals, and the environment (Wallace, Fishbein and Dantas, 2020). AMR is driven by multifaceted and cross-sectoral factors but the biggest culprits are overuse and abuse of antibiotics in humans and animals. AMR is also caused by problems with global sanitation and water pollution, such as manure runoff, waste from hospitals and pharmaceutical manufacturing facilities, and open sewage systems that impede access to clean water (Wall *et al.*, 2016).

Furthermore, AMR is fuelled by differences in healthcare infection control practices, the use of fake medications, a lack of antimicrobials and diagnostics, travel, marked changes in dietary intake as a result of high population densities, and the spread of infectious diseases in humans and animals (Holmes *et al.*, 2016). AMR is significantly impacted by farming systems' subpar antimicrobial regulation, inadequate vaccination schedules, poor temperature controls, poor biosecurity, inadequate ventilation, improper nutrition, and housing, as well as poor quality-assurance programs (Wall *et al.*, 2016).

The emergence and spread of resistant bacteria and their resistance genes in soil and adjacent ecosystems have been linked to several mechanisms, including wastewater treatment plants, agricultural waste, and hospital effluent (Irfan, Almotiri, and AlZeyadi, 2022). Consequently, AMR has emerged as a global public health concern. The Centres for Disease Control and Prevention (CDC), in a 2019 report, summarised that humanity would face increasingly resistant infections, potentially extending to all treatments available, leading to what is coined the "post-antibiotic era" (Department of Health and Human Services, 2019).

Novel resistance mechanisms are thus evolving and spreading rapidly through astronomical increases in human travel and trade, consequently threatening our ability to manage common infectious diseases in humans and animals (Holmes *et al.*, 2016). This inadvertently leads to increased costs of healthcare (Department of Health and Human Services, 2019) and the emergence of AMR in food animal production. There are reports that by 2050, worldwide mortality stemming from AMR is likely to reach 10 million annually, in the absence of intervention, exceeding cancer (Marshall and Levy, 2011; Shankar, 2016).

While AMR can take many forms, ESBLs have great clinical significance in medical bacteriology as they threaten both therapeutics from antimicrobials and infection control in humans and animals (Drieux *et al.*, 2008). ESBLs are a rapidly evolving group of *β*-lactamases that confer resistance to most *β*-lactam antimicrobials, including penicillin, third-generation cephalosporins, and the monobactam aztreonam by hydrolysing their *β*-lactam ring yet can be inhibited by clavulanic acid (Ghafourian *et al.*, 2014). This development of ESBL genes is linked to enteric bacteria that produce ESBLs, like *E. coli*, which are evolving into multidrug-resistant (MDR) bacteria due to their possession of genes encoding enzymes like extended-spectrum *β*-lactamase, together with other genes or mutations in genes that confer resistance to a broad spectrum of antibiotics (Tamma *et al.*, 2022). Genes coding for carbapenemase, aminoglycoside-modifying enzymes (phosphorylating, adenylylating, and acetylating enzymes), porin mutations,

and enhanced transmembrane efflux pump expression may be extra genes associated with ESBL-producing enteric bacteria (Assefa, 2022).

Since the gastrointestinal tract (GIT) serves as a reservoir for enteric bacteria that produce ESBLs, it is crucial to implement appropriate infection-control practices and barriers to stop the spread of these bacteria and prevent outbreaks. Human healthcare workers should wash their hands often, refrain from using intrusive equipment like intravenous lines or urinary catheters, and isolate patients who are colonized or infected with ESBL producers (Rawat and Nair, 2010). To prevent the spread of ESBL-producing bacteria in animals, new approaches must be developed. These include bettering animal husbandry techniques, using alternative therapies, and raising public awareness (Sun *et al.*, 2021).

Typically, ESBLs are derived from the narrow spectrum *β*-lactamases, TEM-1, TEM-2, and SHV-1, which usually give rise to ESBLs through point mutations. However, a relatively recent group, the CTX-M type, has become more dominant (Chileshe *et al.*, 2024). ESBLs are often plasmid-encoded, and these plasmids frequently carry genes encoding resistance to other drug classes. Therefore, antibiotic options in treating ESBL-producing organisms are extremely limited (Shakil *et al.*, 2012). The zoonotic potential of most ESBL-producing organisms is a significant public health concern.

While the selection of AMR is often associated with hospital antibiotic use (Chiyangi *et al.*, 2017), many animal reservoirs now exist. There are several reports of drug-resistant *Enterobacteriaceae* in various livestock, including poultry, sheep, cattle, and pigs (Munk *et al.*, 2018; Subbiah *et al.*, 2020; Atlaw *et al.*, 2021; Peng *et al.*, 2022;). Additionally, AMR has been documented in green sea turtles, black rhinoceroses, and monkeys, despite the absence of prior antibiotic exposure in wildlife (Ahasan *et al.*, 2017; Kipkorir *et al.*, 2020; Abdallah *et al.*, 2022), probably due to exposure to antibiotic-resistant organisms at the human-animal-environment interphase. AMR is a threat to humans, livestock, and the environment because of the inappropriate use of antimicrobials and the use of antimicrobials as growth promoters in food animals.

In Zambia, poultry is one of the most studied sectors in terms of ESBLs. Since the first report by (Chishimba *et al.*, 2016) many studies have been published with ESBL prevalence ranging from 3.4% to 20.1 % (Mwansa *et al.*, 2023; Mwasinga *et al.*, 2023; Chileshe *et al.*, 2024). The commonly isolated ESBL-producing organism is *E. coli* with sequence types (STs) 55 and 69 reported so far (Shawa *et al.*, 2021). Furthermore, Zambian ESBL strains of poultry origin show that the $bla_{\text{CTX-M}}$ gene is the commonest ESBL gene, but bla_{TEM} and bla_{SHV} have also been reported (Chishimba *et al.*, 2016; Mwansa *et al.*, 2023; Chileshe *et al.*, 2024). Notably, a 2021 study in Zambia found a link between MDR *E. coli* from humans and poultry (Shawa *et al*., 2021), suggesting that poultry could be a reservoir of resistant *E. coli* strains in humans. Still, despite these reports, the extent of the problem in poultry remains unclear. Additionally, the relationship between AMR and antibiotic usage in poultry has not been fully explored. This study reports ESBL patterns in commercial and small-scale poultry farms of selected districts in Zambia and relates this to antimicrobial use.

1.2 Problem statement

According to Phiri *et al.,* (2020), the demand for poultry in Zambia is high. Driven by the quest for maximal profit and rapid modernization of the developing world, poultry is often raised under intensive conditions moving disease build-up from host to host is very easy (Van Boeckel *et al.*, 2015). Consequently, most poultry farmers resort to using large amounts of antimicrobials to prevent and treat disease and for growth promotion (Nhung, Chansiripornchai and Carrique-Mas, 2017). Most treatment in poultry is delivered orally, continuous exposure to the enteric microbes some of which are predominant facultative flora of the GIT is likely to result in the development of AMR (Abreu *et al.*, 2023). Prolonged usage of antimicrobials may result in the development of AMR including ESBL in pathogens that can cause diseases in poultry. This can result in economic losses due to treatment failure. Furthermore, increased usage of antimicrobials results in the emergence of antibiotic-resistance genes that could be transmitted to the human biome. Poultry could be a reservoir for ESBL *E. coli* in humans.

1.3 Justification of the study

Because of the rampant use of antimicrobials in poultry production, antibiotic-resistance genes are likely to emerge (Mencía-Ares *et al.*, 2021). *E. coli* is an enteric bacterium and represents the most urgent global concern (Prestinaci, Pezzotti and Pantosti, 2015). Studies suggest that the commensal bacteria have a vital role in spreading AMR through acquiring and disseminating AMR genes by horizontal transfer (Cordero *et al*., 2019; Leekitcharoenphon *et al*., 2021).

Since poultry is a potential AMR reservoir, restricting antibiotic access by farmers may be an essential public health entry point. However, the AMR contribution from farmers' knowledge, attitudes, and practices on antimicrobial usage remains unknown, and the relationship between such usage and AMR is unclear. Therefore, there was a need to determine the relationship between AMR and farmer's knowledge, attitudes, and practices regarding antimicrobial usage in poultry. *E. coli* is particularly notable as it is intrinsically susceptible to almost all clinically relevant antimicrobial agents but also represents a major reservoir of AMR genes as it can accumulate resistance genes (Peng *et al.*, 2022).

1.4 Research questions

1. Are there differences in terms of antimicrobial use among commercial and small-scale poultry farmers?
2. What are the resistant profiles of *E. coli* circulating in commercial and small-scale poultry farms in Lusaka, Southern, Central, Western and Eastern provinces?
3. Are there ESBL *E coli* strains circulating in commercial and small-scale poultry farms of Lusaka, Southern, Central, Western and Eastern provinces?

1.5 Study objectives

1.5.1 General objective

To understand antimicrobial use, determine the antimicrobial resistance profiles in *E. coli* isolates and investigate the presence of ESBL *E. coli* in commercial and small-scale poultry farms in selected districts of Zambia.

1.5.2 Specific objectives

1.5.2.1. To compare antimicrobial use among commercial and small-scale poultry farmers.

1.5.2.2. To determine the antimicrobial susceptibility and resistance profiles of *E. coli* isolated from poultry.

1.5.2.3. To investigate the possible presence of ESBL-encoding genes in *E. coli* isolated from poultry.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Knowledge, Attitudes, and Practices (KAP) among poultry commercial and small-scale farmers

Antimicrobial resistance in food-producing animals is a major public health threat on a global scale (Almansour *et al.*, 2023). One of the key causes of AMR is the overuse and abuse of antimicrobials (Tufa *et al.*, 2023). It is, therefore, important to assess the Knowledge, Attitudes, and Practices (KAP) on antimicrobial use (AMU) and AMR.

In Asia, a study in Nepal among poultry farmers revealed an overall 87% AMU, and less than half of them 49.8% (n = 301/605) aware of AMR. Comparatively layers farmers (OR: 4.63; 95% CI: 1.75-12.3; p = 0.003) were more likely to practice prudent usage of antimicrobials (Subedi *et al.*, 2023). In Bangladesh, the KAP study on commercial poultry farmers (broiler and layer farmers) demonstrated that most of the respondents had insufficient KAP regarding AMU and AMR. The respondents used a variety of antimicrobials primarily in the treatment of various diseases in poultry and one-third of the farmers did not seek antimicrobials from registered vets. Layer farmers were more likely to seek out antimicrobials from a registered vet compared to broiler farmers (73% and 60%, respectively) (Hassan *et al.*, 2021).

In Africa, antibiotics are among the commonest prescribed medicines (Okedo-Alex *et al.*, 2023). According to a Nigerian study of small-scale poultry farmers, 83% of the farmers utilized antimicrobials inappropriately while using them for disease prevention, growth promotion, and treatment. Surprisingly, farmers who were knowledgeable about AMU and AMR (OR = 4.5; 95% CI = 1.26-15.8) were more likely to abuse antibiotics than those who were not (OR = 4.2; 95% CI = 1.03-17.2) (Chah *et al.*, 2022). Research conducted on commercial poultry in Southern Togo revealed that 21% of farmers did not know about antibiotics, 39% did not know about AMR, and 63% obtained antibiotics based on prescriptions from veterinarians (Bedekelabou *et al.*, 2022). A rural

chicken farm study conducted in Cameroon revealed low mean scores for knowledge of AMU (0.30 ± 0.24) and AMR (0.26 ± 0.22), a desirable attitude (0.29 ± 0.29), acceptable practice (0.58 ± 0.18) toward AMU, and a positive risk assessment of AMR (0.37 ± 0.21) (Moffo *et al.*, 2020). The majority of commercial farms (97%, n = 32) and 43% (n = 56) of domestic farms used antimicrobials, according to a Ghanaian study on the use of antibiotics in poultry farming. Nonetheless, compared to domestic poultry keepers, commercial farmers were 6.1 (CI: 3.2–11.8) times more likely to read and abide by antimicrobial prescription guidelines. A further 11% of domestic farmers and 34% of commercial farmers reported having received education on the use of antibiotics (Paintsil *et al.*, 2021). Research conducted in Tanzania, however, found that just 41% of poultry farms there followed the withdrawal period and that the majority of farms 87.7% used antimicrobials for therapeutic purposes (Azabo *et al.*, 2022).

The Zambian poultry sector has seen rapid growth triggered by not only an increase in the human population but also increasing investments and competition (Samboko, Zulu-Mbata and Chapoto, 2018). Zambia's total poultry population is estimated at 30.5 million. Of these, 21 million are village chicken farmers accounting for 1% (209,163/21,232,477) commercial farmers and 99%, (21,023,285/21,232,447) small-scale farmers. On the other hand, there are 4.7 million broiler farmers of them, 14% (743317/5486509) are commercial farmers and 86% (4743192/5486509) are small-scale farmers. Meanwhile, there are 2.2 million layers of farmers of which 0.02% (364/2244070) are commercial establishments and 99.98% (2,244,070/2244070) are small-scale farmers (Zamstats, 2022). A study towards a bottom-up understanding of antimicrobial use and resistance reported that Zambian poultry farmers were among the highest users of antimicrobials (3–5 times per month) ahead of Zimbabwe, Kenya, and Ghana. In this study, the majority of households acquired antimicrobials from agrovet shops (83%) and 80% (158/198) of them acquired them without a prescription. Poultry farmers with 10.2% higher knowledge levels were linked to occasionally obtaining guidance from government veterinarians, while those with 14.7% higher scores were linked to practically always receiving advice (Caudell *et al.*, 2020). In a different study, the likelihood of having AMR concerns was lower for farmers who knew about it

compared to those who didn't (OR = 0.26, 95% CI: 0.08%–0.86%) (Mudenda *et al.*, 2023). Altogether, all these studies only focused on a single production type, and very few related AMU to AMR.

2.2 Poultry production and drivers of antimicrobial resistance

Poultry products are among the most consumed products worldwide, which warrants increased use of antimicrobials to improve productivity (some livestock farmers resort to giving low doses of antimicrobials to animals to promote their growth or increase feed efficiency) (Hassan *et al.*, 2021). The use of antimicrobials for growth promotion in animal production helps to control bacterial disease. While the animals grow bigger faster, the bacteria they carry are exposed to low doses of antimicrobials over a long period. This practice sets up a perfect breeding ground for the development of resistant bacteria as the susceptible ones are eliminated, leaving resistant variants to multiply (Subbiah *et al.*, 2020). Resistant bacteria transmit genetic traits among enteric bacteria, a key component of the AMR challenge (Holmes *et al.*, 2016).

2.3 ESBL-producing bacteria

Extended-spectrum *β*-lactamases are gram-negative bacteria belonging to the Enterobacteriaceae family. The World Health Organization (WHO) lists *E. coli, Klebsiella pneumoniae,* and *Klebsiella oxytoca* as among the main types of ESBLs (WHO_OMS, 2024). However, other types include *Acinetobacter baumannii, Pseudomonas aeruginosa, Citrobacter spp, Salmonella spp, Enterobacter spp, Providentia spp, Morganella morganii* and Proteus *spp* (Chileshe *et al.*, 2024). Bacteria that produce β-lactam hydrolysing enzymes carry ESBL genes in their chromosomes or plasmids. TEM-1, TEM-2, or SHV-1 are the usual sources of ESBL genes, which are produced through mutations that change the arrangement of amino acids surrounding the active site of β-lactamases. This broadens the range of *β*-lactam antimicrobials that these enzymes can hydrolyze. It has recently been reported that the CTX-M, which is neither of TEM or SHV lineage, is rising (Isaiah *et al.*, 2011). In *E. coli* and *Klebsiella spp*., the SHV-1 type ESBLs are frequently found. *S. enterica serovar Typhimurium, K. pneumoniae, E. coli*, and *Shigella species* are known to harbor CTX-M-type ESBL. The isolates of *P. aeruginosa* and *K. pneumoniae* were found to harbor the plasmid-mediated OXA and AmpC-type ESBL while *Salmonella enteritidis, S. newport*, and *S. paratyphi* are among the serovars that have been identified as producing ESBL and have been

connected to severe foodborne gastroenteritis in people. ESBLs are therefore legitimately considered one of the most challenging causes of diseases (Ozsolak and Milos, 2010).

2.4 ESBL-producing bacteria antibiotic resistance

Extended-spectrum beta-lactamases-encoding plasmids are often multidrug-resistant due to the presence of resistance genes for other antibiotic classes, including sulfonamides, aminoglycosides, and fluoroquinolones (Chileshe *et al.*, 2024). Treatment options for infections due to these multidrug-resistant organisms are therefore limited, and initial empirical therapy is often ineffective and associated with increased mortality. When treating ESBL infections, carbapenems have long been the drug of choice (Kettani Halabi *et al.*, 2021). However, the situation is evolving due to the rise of bacteria that produce carbapenemase, among other factors. Therefore, alternate strategies must be developed (Nordmann and Poirel, 2019).

2.5 Antibiotics affected by ESBL-producing bacteria

Extended-spectrum beta-lactamases (ESBLs) are enzymes that provide resistance to penicillin, aztreonam, and first, second, and third-generation cephalosporins, and the monobactam aztreonam. However, they are not capable of hydrolysing cephamycin or carbapenems (Song *et al.*, 2020). Co-resistance on the same mobile genetic elements allows ESBL-producing bacteria to show resistance to several different drugs. In addition to the ESBL enzymes, the same mobile genetic elements might harbor modifying enzymes that impart resistance to aminoglycosides, alternative routes for folate (such as trimethoprim/sulfamethoxazole resistance), and target mutations for DNA gyrase and topoisomerase (fluoroquinolone resistance) (Parmanik *et al.*, 2022;Walker *et al.*, 2022).

2.6 ESBL-producing *E. coli* in Zambia

In Zambia, ESBL-producing *E. coli* has been the topic of several research on food, humans, animals, and the environment. In animals, the first study on ESBLs-producing *E. coli* in healthy chickens revealed an overall 20.1%, of ESBL-producing isolates

carrying *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV,}}$ and *bla*$_{\text{TEM}}$ ESBLs genes (Chishimba *et al.*, 2016). A study on *E. coli* isolated from diseased broiler chickens in Lusaka detected 33.3% (2/6) *bla*$_{\text{CTX-M}}$ and 66.7% (4/6) *bla*$_{\text{TEM}}$ positive ESBL - encoding genes (Chileshe *et al.*, 2024). Determination of antimicrobial resistance patterns of *E. coli* isolates from farmworkers in broiler poultry production revealed a prevalence of two (3.4%) ESBL isolates that harboured the CTX-M gene (Mwansa *et al.*, 2023). A comparative study on multidrug-resistant (MDR) *E. coli* collected from poultry in Lusaka against *E. coli* found in patients at the University Teaching Hospital found a related ESBL gene *bla*$_{\text{CTX-M-14}}$ (Shawa *et al.*, 2021) Another study on ESBL-producing *E. coli* found *E. coli* harbouring *bla*$_{\text{TEM–1}}$ (13%) and *bla*$_{\text{TEM–1}}$ (56%) ESBL genes in dairy animals and human isolates from a Lusaka hospital respectively (Mainda *et al.*, 2019). Meanwhile, an assessment of AMR profiles and risk factors associated with *E. coli* in human and environmental settings found 31.8% ESBL-producing *E. coli*, of which 70.1% were clinical isolates (Kasanga *et al.*, 2023). A study investigating the AMR profiles of *E. coli* isolated from clinical and environmental samples in Lusaka found 42.8% ESBL-producers (Kasanga *et al.*, 2024). Another study in humans revealed higher phenotypic ESBL-producing *E. coli* prevalence in inpatients at 52% compared to 12% in outpatients (Nagelkerke *et al.*, 2017).

On the other hand, some food safety studies isolated *E. coli* from flies on fresh fish revealing an overall, 17.2% of total samples analysed containing ESBL-producing *E. coli* alongside *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ genes in *bla*$_{\text{CTX-M}}$ ESBL genes (Songe *et al.*, 2017). Another study in raw cow's milk found *E. coli* harbouring *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{TEM}}$ ESBL genes indicating a serious public health risk for consumers (Mwasinga *et al.*, 2023).

2.7 Sources of ESBL bacteria

Outside of the clinical setting, ESBL-producing bacteria have been found in the community and environment, including in food products, healthy people, wastewater, environmental water, wild animals, livestock, and companion animals (Cho, Jackson and Frye, 2023). ESBL-producing bacteria have been isolated from many regions of the world. In sample types such as human blood (Sangare *et al.*, 2015), human stool (Ding

et al., 2021;Amare *et al.*, 2022), salad vegetables (Parker *et al.*, 2021), boot swabs in broilers (Daehre *et al.*, 2018), farm wastewater, feed, human and animal rectal faecal swabs, and faecal samples (Zhang *et al.*, 2016;Yasmeen *et al.*, 2023). Faecal carriage of ESBL-producing bacteria is one of the commonest and has a potential risk for transmission of infection with resistant strains (Daehre *et al.*, 2018).

2.8 Movement of resistant bacteria and antibiotic-resistant genes

Over time, there has been a steady rise in both global trade and human mobility. This has had significant effects on the spread and evolution of genes that confer antibiotic resistance (Ellabaan *et al.*, 2021). AMR can occur naturally by bacterial chromosomal DNA mutation or through the acquisition of AMR genes from other bacteria or the environment via horizontal gene transfer (HGT) (Aminov, 2009; van Schaik, 2015; Schrader, Vaubourgeix and Nathan, 2020). However, plasmid-mediated transmission of resistance genes is the most common route for the acquisition of outside genetic material (Reygaert 2018). Genotypically, ESBL bacteria emerge and spread ESBL-encoding genetic elements like $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV}, as well as AmpC-encoding genes like *DHA*, *CMY*, and *CIT*. Furthermore, ESBL-producing bacteria carry additional genetic elements like bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{\text{OXA-48}}$, and bla_{NDM}, which encode carbapenemases i.e., VIM (veronica integrin Metallo-beta-lactamases), IMP (imipenemase), KPC (*Klebsiella pneumoniae* carbapenemase), OXA-48 (oxacillinase-48), and NDM-1 (New Delhi Metallo-beta-lactamase-1), respectively (Mayanja *et al.*, 2023). The CTX-M group's ESBLs have emerged and expanded over the last 20 years, which is undoubtedly the most significant breakthrough in the field of antibiotic resistance (Ellabaan *et al.*, 2021).

2.9 Clonal transmission of ESBL-producing bacteria at the human-animal interfaces

Understanding the clonal diversity of ESBL-producing bacteria is important for determining the potential risk of transmission of these resistant bacteria (Schaufler *et al.*, 2016). Most of the resistance is shared by environmental bacteria, human pathogens, and animal pathogens. Studies in humans and animals found clonal transmission of

ST131 as the commonest (Adefioye *et al.*, 2021; Pérez-Etayo, González and Vitas, 2022). In sub-Saharan Africa, a study in healthy chickens in Tunisia found sequence types ST542, ST155, ST349, ST405, ST1056, ST117, ST2197, and ST155 that showed that healthy chickens constitute an important reservoir of ESBL-producing *E. coli* isolates that potentially could be transmitted to humans via the food chain or by direct contact (Maamar *et al.*, 2016). In Ghana, a study on ESBL producing-*E. coli* isolates found Sequence (ST) ST10 (5/46, 11%) as the most dominant (Eibach *et al.*, 2018).

However, a Zambian study on phylogenetic analysis and hierarchical clustering showed a high degree of genetic relatedness between *E. coli* O17:H18-ST69 from poultry and humans. Meanwhile, another study in Tunisia found minor clones belonging to ST410 and ST471 shared by chickens from both households (Benlabidi *et al.*, 2023). A One Health study evidenced interspecies transmission of a new successful clone of ST410 *E. coli* between wildlife, humans, companion animals, and the environment. The results underline the zoonotic potential of clinically relevant multi-resistant bacteria found in the environment as well as the mandatory nature of the 'One Health' approach (Schaufler *et al.*, 2016).

2.10 Detection and confirmation of ESBL-producing *E. coli*

2.10.1 Phenotypic methods

Phenotypic methods determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to specific antibiotic concentrations, aiding physicians in selecting appropriate treatment options (Hudzicki, 2012). Bacteria are grown in the presence of specific antibiotic concentrations to be tested to evaluate the phenotypic AMR. Phenotypic methods can be divided into manual and (semi) automated. Some manual methods include the disk diffusion method, which involves inoculating agar with bacterial suspension beforehand and then placing several disks impregnated with antimicrobials on top of it. An antibiotic concentration gradient is created as the antimicrobial agent diffuses into the agar and inhibits the growth of the bacteria, producing a "zone of inhibition" around the disk, which is measured after overnight incubation. Using Clinical Standard Laboratory Institute (CSLI) guidelines, a qualitative

report of susceptible, intermediate, or resistant can be obtained (Clinical Laboratory Standard Institute antimicrobial susceptibility testing, 2021; Gajic *et al*., 2022).

Dilution methods- (broth/agar dilution method) used to determine the lowest concentration of an antibiotic that inhibits bacterial growth. To determine the broth dilution, which is commonly done using 96-well microtiter plates, bacteria are inoculated into a liquid growth medium in various antimicrobial agent concentrations. The process of agar dilution entails adding varying concentrations of the antimicrobial agent to a nutrient agar medium and then applying a standard number of cells to the plate's surface. The MIC values are read following growth assessment after 16 – 20 hours of incubation(Wiegand, Hilpert and Hancock, 2008). Gradient methods like the Epsilometer test (E-test) are used to determine if bacteria are susceptible, intermediate, or resistant to an antibiotic. They are quantitative techniques based on a combination of the concept of dilution and diffusion principle for susceptibility testing. The E-test strip, a 5mm wide, 60mm long plastic carrier strip, provides a stable antimicrobial gradient upon application to an inoculated agar plate. After overnight incubation, a symmetrical inhibition ellipse is produced, indicating the MIC value is read from the scale in terms of µg/ml where the ellipse edge intersects the strip (Nachnani *et al.*, 1992). On the other hand, automated methods include the VITEK®2 an automated accurate and reliable identification and antimicrobial susceptibility system that utilises growth-based technology systems that accommodate self-contained, disposable test colorimetric reagent cards to identify Gram-positive and Gram-negative bacteria plus yeast that are incubated and interpreted automatically (Pincus 2010; Kaprou *et al.*, 2021).

2.10.2 Molecular methods

Molecular methods used for the detection of AMR genes include amplification tests such as Polymerase Chain Reaction (PCR), which is a laboratory technique for the amplification of DNA or RNA sequences using DNA polymerase I enzyme, specifically Taq DNA from Thermus aquaticus known as Taq DNA (Khehra, Padda and Swift, 2024). Furthermore, molecular methods also include sequencing (determination of the sequence of units of a linear polymer) using methods and technologies that identify and

order the four nucleotide bases (adenine, guanine, cytosine, and thymine) in a segment of DNA tests such as whole genome sequencing to distinguish organisms down to species and individual levels, sequencing is a powerful tool in forensics and may be used in the future to characterize various diseases, identify therapeutic targets, and personalize treatments (‘The principles of DNA Sequencing’, 1977). Mass spectrometry method -Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) detects microbial samples, absorbs energy, and forms crystals. Primarily, exposure to laser irradiation vaporizes the sample and matrix, accelerating ionized proteins under an electrostatic field (Chen *et al.*, 2021). Molecular methods are slowly replacing phenotypic methods as they provide faster and more accurate detection of the underlying mechanisms of AMR (Anjum, Zankari and Hasman, 2017).

2.11 Control and prevention of ESBL-producing *E. coli*

ESBL-producing *E. coli* are a major public health concern as they are associated with severe healthcare-associated infections worldwide (Teerawattanapong *et al.*, 2017). Maintaining high standards of cleanliness or adherence to strict hand washing, glove use, isolating sick animals with ESBL-producing *E. coli*, regular cleaning, and disinfection of animal housing, equipment, and surfaces, sterilising medical devices as well as the use of personal protective equipment when interacting with infected animals are the best approaches to controlling the spread of ESBL-producing *E. coli* (Lowe *et al.*, 2012; Economou *et al.*, 2023).

ESBL-producing *E. coli* can be prevented by practicing improved hygiene when managing sick animals. Reduce AMU in livestock production by encouraging good animal husbandry practices. Additionally, improved wastewater management by proper wastewater treatment and disposal can prevent ESBL-producing *E. coli* transmission(Department of Health and Human Services, 2019) (Davis, Mayer, and Pacilli, 2024). Above all, education and training of animal health-care professionals and farmers can go a long way in preventing the spread of ESBL-producing *E. coli* (Magiorakos *et al.*, 2012).

2.12 Antimicrobial resistance in enteric bacteria

Enteric bacteria are generally organisms of the family *Enterobacteriaceae*. They typically exist in the intestines of animals and humans. Enteric bacteria can have a symbiotic existence in healthy individuals and, therefore, be harmless, such as gut flora or microbiota. They may also be pathogenic in the very young, those with weakened immune systems, or in a naive host without a prior encounter with the microbe (van Schaik, 2015). The normal enteric flora plays a vital role in maintaining organismal health. It has beneficial effects in the competitive exclusion of potentially pathogenic organisms, facilitating the production and uptake of nutrients such as short-chain fatty acids and vitamins, detoxification, and interactions with the immune system (Milani *et al.*, 2017).

The gut microbiota of healthy individuals contains a densely populated microbial ecosystem dominated by non-pathogenic gut commensals (van Schaik, 2015) that are considered good indicators to monitor AMR as they are subjected to the continuous selection pressure of antimicrobials via the targeted use of antimicrobials against pathogenic gut bacteria. This situation may contribute to the development of AMR in gut commensals as they develop ways to protect themselves against antimicrobials (Aasmäe *et al.*, 2019). There are four primary mechanisms of resistance, and these are drug inactivation, drug target alteration, drug uptake limitation, and drug active efflux. Gut microbes have ample opportunity to interact closely with each other; therefore, genetic AMR transfer can occur.

2.13 Factors associated with AMR in enteric bacteria

Antimicrobial resistance develops naturally in bacteria (Allen and Stanton, 2014). The emergence, spread, and persistence of AMR in intestinal bacteria are, however, generally associated with prolonged usage, misuse, underuse, and inappropriate use of antimicrobials, as well as bacterial mobility. (Wallace, Fishbein, and Dantas, 2020). The prevalence of AMR is commonly attributed to questionable livestock production practices that began in the 1950s, like the use of subtherapeutic amounts of

antimicrobials in feed or water to improve livestock production performance (Franklin *et al.*, 2016).

A study in commensal *E. coli* reviewed the highest prevalence of resistance in broiler and turkey isolates, whereas it was rare in cattle (Wasyl *et al.*, 2013). The resistance patterns specific to the isolation source were suggestive of the antimicrobial consumption, usage preferences, or management practices in particular animals. To that effect, over 70% of global antimicrobial consumption is in livestock production (Tiseo *et al.*, 2020). Besides antimicrobials, there are other factors associated with AMR in enteric bacteria that would contribute to the spread and persistence of antimicrobial-resistant enteric bacteria. Implementation of standards of infection control practices such as good management practices, good biosecurity measures, good hygiene (hand washing), routine isolation and quarantine of new animals, routine surveillance, vaccination, and clean buildings and equipment can prevent the spread of AMR enteric bacteria (Ackers *et al*., 2020; Ugandan Ministry of Agriculture Animal industry and fisheries 2020). Good agricultural practices reduce infections, which in turn reduces the need for antimicrobials and the emergence of AMR. Poor sanitation and lack of access to clean water can promote the spread of AMR enteric bacteria through contaminated drinking water and the environment via improper waste disposal of human excreta and sewage (Subbiah *et al.*, 2020). Access to quality antimicrobials and diagnostics can limit AMR enteric bacteria spread threatening survival rates and health benefits. However, this requires new healthcare financing and a one-health perspective in expanding appropriate access while restricting inappropriate access (Laxminarayan *et al.*, 2016). Traveling and migration can spread AMR enteric bacteria from humans, animals, contaminated food and water, and medical services. Emerging data indicates that traveling internationally is contributing to the worldwide spread of antibiotic resistance over 65% of which are enteric bacteria and 35% of them are β-lactam drug-resistant (Bokhary *et al.*, 2021).

2.14 Genetic drivers associated with antimicrobial resistance in enteric bacteria

Antimicrobial resistance in enteric bacteria can be intrinsic, acquired, or adaptive (Langendonk, Neill, and Fothergill, 2021). Enteric bacteria can naturally express intrinsic resistance without prior exposure to antibiotics or HGT. This can be achieved through genetically encoded genes in their chromosomes that code for the expression of efflux pump systems. Other bacteria can confer intrinsic resistance by having resistance genes or enzymes (e.g., gram-negative bacteria's inducible AmpC cephalosporinases and β-lactamases, which hydrolyze β-lactam antimicrobials); still others can confer resistance by having an impermeable outer membrane (Hasan, Dutta and Nguyen, 2022). While acquired resistance results from the acquisition of resistance genes from other species or the selection of favorable mutations, adaptive resistance mechanisms are those that are generated by environmental stimuli (Langendonk, Neill, and Fothergill, 2021).

It is important to understand the genetic drivers of AMR in enteric bacteria that are responsible for bacterial evolution (Pfeifer *et al.*, 2021). Enteric bacteria can acquire resistance genes externally through mobile genetic elements (MGEs) that transfer DNA between different bacterial cells. MGEs can be extra-chromosomal-like plasmids, or they can be embedded within bacterial chromosomes as insertion sequence elements or transposons (Ghaly and Gillings, 2022). HGT occurs by three mechanisms which are transformation (resistance genes from the dead bacterial DNA can be taken up and integrated into the recipient’s chromosomes by homologous recombination), conjugation (bacteria directly transfer genes to other bacterial species), and transduction (resistance gene transfer is mediated by bacteriophage which infects and transfers resistance genes to new bacterial species). These allow genetic information, including AMR genes, to be horizontally transferred among enteric organisms in the gut, driving pathogen evolution (Burmeister, 2015). Enteric bacteria can also acquire resistance genes as a result of continuous exposure to antimicrobials. This pressure from antimicrobial selection can lead to the evolution of resistance through point mutations. A random alteration in the sequence of a single base pair of DNA can arise from the substitution, insertion, or deletion of nucleotides during replication and repair processes.

Enteric bacteria with resistance mutations are selected and other enteric bacteria are eliminated when antimicrobials are present. Resistant strains continue to proliferate and eventually spread throughout the population because they have the advantage of survival. Since resistant strains have the advantage of survival, they continue growing and take over the population. The AMR genes continue to evolve and spread to bacteria among humans, animals, and the environment (Samreen *et al.*, 2021).

Antimicrobial resistance in enteric bacteria may be promoted on a farm setup where many antimicrobials are poorly absorbed in animal guts, resulting in as much as 30% – 90% of the parent compound being excreted into manure and urine (An *et al.*, 2015). Due to the high rate of antimicrobials on animal farms, a high concentration of antibiotic residues in farm waste contaminates the environment creating a genetic selection pressure for the emergence of MDR bacterial infections (Samreen *et al.*, 2021). The use of an antibiotic targeting one invading organism places a challenge in the therapeutic management of enteric diseases as it invariably elicits resistance in commensals which become the reservoirs for resistance genes (Juricova *et al.*, 2021). The non-selective nature of antimicrobials may place adaptive pressure on normal flora, further facilitating the transfer of antibiotic-resistance genes (ARGs) within the microbial community. This can further facilitate the interspecies and intraspecies transfer of ARGs, which can, in turn, efficiently contribute to the acquisition, maintenance, and spreading of ARGs within bacterial communities by the acquisition of resistance (Berendsen *et al.*, 2015). Metal and antibiotic resistance mechanisms share some structural and functional similarities. Pal *et al*., (2017) reported that among 20 metal-resistance genes, copper (Cu) resistance is the most commonly distributed following the decreasing abundance of non-essential metals in the BacMet database. Bacteria harbouring metal-resistance genes have been reported to carry AMR genes more frequently than those without metal-resistance genes, often located on plasmids (Di Cesare *et al.*, 2016).

2.15 Extended-spectrum *β*-lactamases producing-*E. coli* and multidrug resistance in poultry

A few studies in Zambia have focused on ESBL-producing *E. coli* in poultry. The first study focused on ESBLs-producing *E. coli* in healthy broilers and the results showed an overall 20.1%, ESBL-producing isolates carrying $bla_{\text{CTX-M}}$, $bla_{\text{SHV,}}$ and bla_{TEM} ESBLs genes (Chishimba *et al.*, 2016). Meanwhile, another study on *E. coli* focusing on diseased broilers detected 33.3% (2/6) $bla_{\text{CTX-M}}$ and 66.7% (4/6) bla_{TEM} positive ESBL - encoding genes (Chileshe *et al.*, 2024). A comparative study on multidrug-resistant (MDR) *E. coli* collected from poultry against *E. coli* found in patients at the University Teaching Hospital found a related ESBL gene $bla_{\text{CTX-M-14}}$ (Shawa *et al.*, 2021).

In sub-Saharan Africa, a study in healthy chickens in Tunisia detected $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14,}}$ and $bla_{\text{CMY-2}}$ (Maamar *et al.*, 2016). Another study in Tunisia detected genes 35 $bla_{\text{CTX-M-1,}}$ 5 $bla_{\text{CTX-M-55}}$, 5 $bla_{\text{CTX-M-15}}$, 1 $bla_{\text{SHV-2,}}$ and 1 $bla_{\text{SHV-12}}$ in free-range chickens (Benlabidi *et al.*, 2023). A study in chicken liver in Algeria found 86.3% MDR *E. coli* isolates and ESBL genes detected among *E. coli* were as follows (number of isolates): $bla_{\text{CTX-M-15}}$ (3), $bla_{\text{CTX-M-1}}$ (3), $bla_{\text{CTX-M-55}}$ (1), and $bla_{\text{SHV-12}}$ (1) (Chenouf *et al.*, 2021). Additionally, another study in Algeria found MDR ESBL-producing *E. coli* detected in the chicken ovaries of four different broiler breeder flocks. Molecular characterization revealed that three isolates harboured the $bla_{\text{CTX-M-1}}$ gene and one isolate expressed the $bla_{\text{SHV-12}}$ gene. In addition, one $bla_{\text{CTX-M-1}}$ -producing *E. coli* co-harboured the $bla_{\text{TEM-1}}$ gene (Benameur *et al.*, 2019). In an Egyptian poultry study, 65.7% of the isolates were confirmed to produce ESBL. Out of the 92 ESBL phenotypes, 55 (59.7%), 32 (34.7%), 18 (19.6%), and 37 (40.2%) isolates harbour $bla_{\text{TEM-3}}$, $bla_{\text{SHV-4}}$, $bla_{\text{CTX-M-1}}$, and $bla_{\text{CTX-M-14}}$ genes, respectively (Ali *et al.*, 2024).

Other African countries like Ghana found the most ESBL producing-*E. coli* isolates harboured the $bla_{\text{CTX-M-15}}$ gene (61/81, 75%) (Eibach *et al.*, 2018). Another Ghanian study in free-range poultry found 56.2% (n/N = 81/144) positive for ESBL-producing *E. coli* with the majority of them carrying the $bla_{\text{CTX-M}}$ genes, with $bla_{\text{CTX-M-15}}$ (95.1%, n/N = 77/81) (Akenten *et al.*, 2023). In a study in Nigeria, ESBL producing-

E. coli harboured the ESBL gene variants $bla_{\text{CTX-M-15}}$ (n = 49), $bla_{\text{CTX-M-14}}$ (n = 2), $bla_{\text{CTX-M-27}}$ (n = 1) or $bla_{\text{CTX-M-55}}$ (n = 1) and all isolates (49/49, 100%) demonstrated multidrug resistance to antimicrobial agents belonging to at least three different classes of antimicrobials (Maamar *et al.*, 2016; Okpara *et al.*, 2018). Signifying that poultry is a reservoir for ESBL-producing *E. coli* and transmission to humans can occur via the food chain or by direct contact.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1Study area

This study was conducted as part of the National Integrated Antimicrobial Resistance Surveillance Strategy (NIAMRSS) (WHO, 2020). The NIAMRSS is a nationwide human and livestock sector-based cross-sectional study that provides a coherent framework for combating AMR using the "One Health" approach. From this framework, the Protocol on AMR Surveillance in Poultry Populations in Zambia 2020 – 2027 was developed. The nationwide surveillance collects samples from all administrative regions (provinces) through a convenient sampling technique down to districts and farms within the province. Briefly, five of 10 provinces of Zambia were selected based on proximity to AMR-testing laboratories. Next, one to four districts were conveniently selected from each province, namely Lusaka province (Chongwe, Chilanga, Lusaka and Rufunsa), Central province (Chibombo and Chisamba), Southern province (Choma), Western province (Kaoma and Mongu) and Eastern province (Petauke). Farms were identified using a list of poultry farmers provided by the local veterinary assistants/livestock officers by simple random sampling.

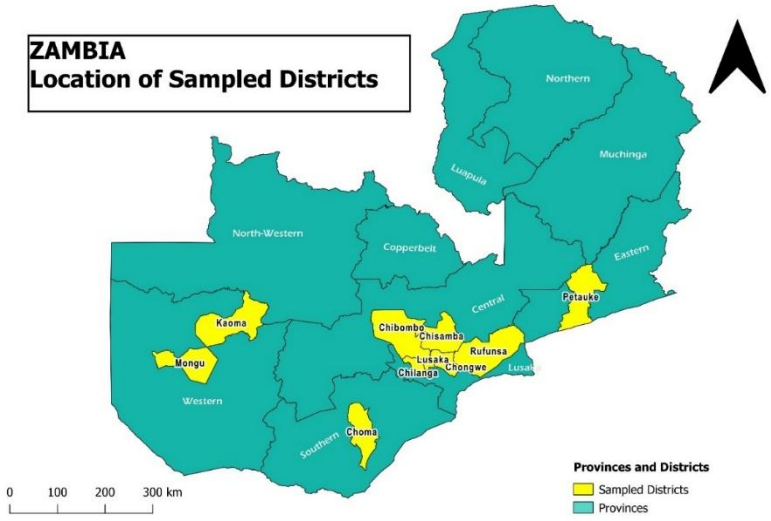

Figure 3.1: Map of Zambia showing the location of the ten sampled districts

3.2 Study design

The study approach taken for this research was a cross-sectional study on laboratory-based *E. coli* archived isolates collected from poultry cloacal swab samples from commercial and small-scale poultry farms. The study was conducted in 10 selected districts of Westen, Southern, Eastern, Central, and Lusaka provinces. Specifically, the following districts were sampled: Chongwe, Chilanga, Chibombo, Chisamba, Choma, Kaoma, Mongu, Lusaka, Petauke and Rufunsa.

A questionnaire with three sections: Sample collection and submission, epidemiological, and antimicrobial use sections was developed and pre-tested on 22 poultry farms of Lusaka (n=6), Chilanga (n=8), and Chongwe (n=8) districts by epidemiology and public health specialists from the University of Zambia and the Ministry of Fisheries and Livestock. However, pilot results were not incorporated into the study.

The primary sampling unit on a farm was an independent poultry house. The poultry house was divided into three sections and from each section a bird was randomly caught and sampled. The study collected samples from poultry houses with market-ready birds

(four weeks and above for broilers and at the point of lay for layers) that were about to enter the food chain since these have a major impact on public health.

Bacterial isolation and phenotypic characterization were done at the Central Veterinary Research Institute (CVRI), while molecular analysis was conducted at the University of Zambia, School of Veterinary Medicine. Whole-genome sequencing of ESBL-positive isolates was done at Noguchi Memorial Institute for Medical Research, University of Ghana.

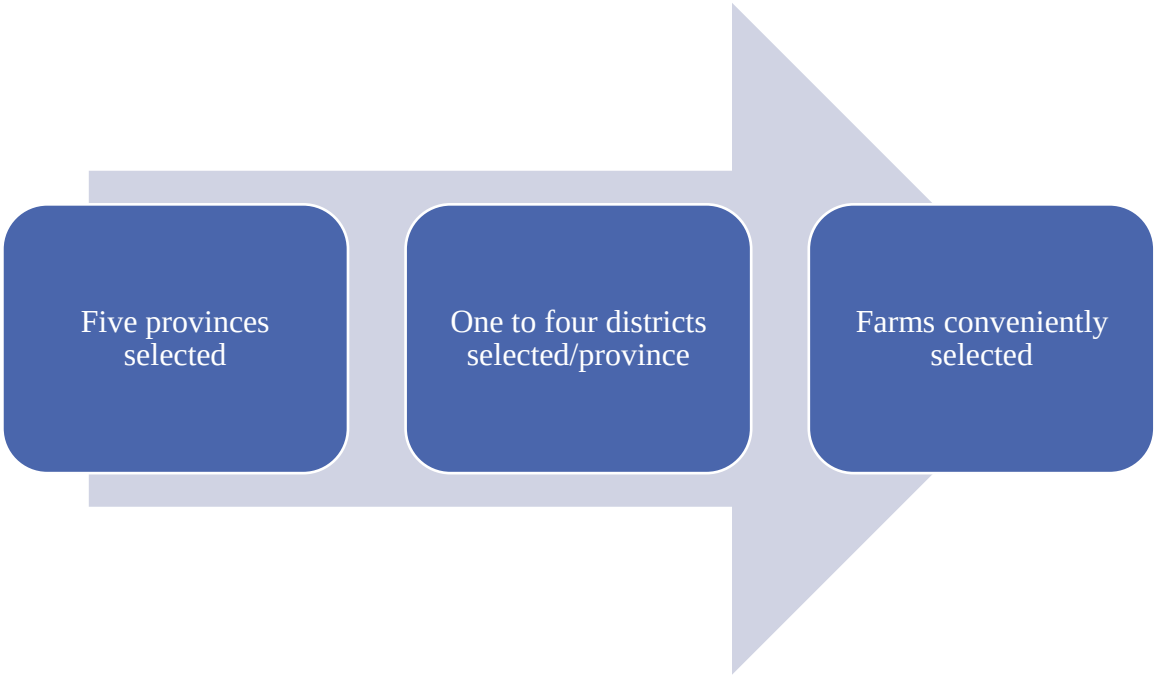

Figure 3.2: Flow chart of the study design

3.3 Sample size

The sample size was determined using the formula for an unknown population. The formula used to determine the target sample size was as follows: $N= (Z)^2 * S * (1 - S)/(E)^2$. Where: N = required minimum sample size; The Z-Standard value (1.96) corresponds to a confidence level at 95%; S= sample standard deviation at 0.5; E = accepted magnitude of error at 0.05. The minimum target sample size was estimated at 384 farms and rounded off to 400 as shown in Table 3.1.

Table 3.1: Estimated sample size distribution by strata for chickens

Sector	**Production Capacity per cycle***	**Proportion (%)**	**Estimated Sample Size (farms)**
Sector 1 (Commercial-scale level 1)	> 50,000	20	80
Sector 2 (Commercial-scale level 2)	10,000 to 50,000	10	40
Sector 3 (Medium-scale level 3)	1,000 – 10,000	35	140
Sector 4 (Small-scale level 4)	< 1,000	35	140
	TOTAL	100	400

3.4 Sampling techniques

The poultry farms within the district were stratified according to the production categories adapted from the Food and Agriculture Organization of the United Nations (Food and Agriculture Organization, 2006), which divides sectors into classes based on the number of birds per sector. In this study, sector class 1 (>50,000 birds) and sector class 2 (10,000–50,000 birds) were classified as commercial, while sector class 3 (1,000 – 10,000 birds) and sector class 4 (below 1,000 birds) were categorized as small-scale.

In a third of a poultry house, one cloacal swab was taken from each bird, and three cloacal swabs were obtained per poultry house. A total of 807 cloacal swabs were collected, labelled with a unique identification code, and pooled in threes (3s) according to each poultry house to make 269 samples. The samples were triple packaged and

placed in ice cooler boxes for transportation to the laboratory for processing within 24 hours.

3.5 Laboratory procedures

3.5.1 Bacterial isolation and identification of *E. coli* isolates

All media was prepared according to the manufacturer's instructions. On arrival in the laboratory, the well-labelled sterile swab sticks in a Cary-Blair transport medium (Oxoid, Basingstoke, UK) were pre-enriched in Buffered Peptone Water (BPW) broth (Oxoid Ltd., Basingstoke, Hampshire, UK) by aseptically pooling three of the pooled swabs from each poultry house into 9ml of sterile BPW and incubated at 37℃ for 24hours. Next, a loop full from an overnight culture of samples was cultured onto MacConkey agar plates and incubated at 37°C for 24 hours. Further, reference strains *E. coli* (American Type Culture Collection (ATCC 25922) and *Staphylococcus aureus* ATCC 25923 were inoculated as well on MacConkey agar and incubated at 37°C for 24 hours as the positive and negative control, respectively. Colonies on MacConkey agar were identified as either lactose fermenters or non-lactose fermenters. The suspected *E. coli* colonies with typical phenotypic characteristics on MacConkey agar (small dry, pink colonies) were sub-cultured on a selective media Eosin Methylene Blue agar (EMB agar) and incubated at 37 °C for 24 hours. The shiny metallic green colonies were selected as presumptive *E. coli*. Further, the presumptive colonies were sub-cultured on nutrient agar (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated at 37 °C for 18 hours for confirmation by biochemical tests (Mwansa *et al.*, 2023).

3.5.2 Biochemical identification of *E. coli* isolates

The presumptive pure *E. coli* isolates on nutrient agar were subjected to five biochemical tests, including Triple Sugar Iron (TSI), Urease, Citrate, Lysine Iron Agar (LIA), and Sulfide, Indole, Motility (SIM).

3.5.3 Triple Sugar Iron (TSI) test

A single pure colony of the bacterium was picked using a sterile inoculating needle, stubbed into the butt and streaked on the slant of the TSI agar, and then incubated at

37◦C for 24 hours with loosely placed caps. After that, results were examined and interpreted by comparing with *E. coli* positive control results on TSI: Acid slant / Acid Butt / H_2S-Negative / Copious hydrogen sulfide present.

3.5.4 Urease test

A single pure colony of the bacterium was picked using a sterile inoculating needle and stubbed into the butt of urease agar and then incubated at 37◦C for 24 hours with loosely placed caps. After that, results were examined and interpreted by comparing with *E. coli* positive control: media maintains yellow colour.

3.5.5 Simmons citrate test

A single pure colony of the bacterium was picked using a sterile inoculating loop and streaked on the slant of Simmons citrate agar and then incubated at 37◦C for 24 hours with loosely placed caps. After that, results were examined and interpreted by comparing with *E. coli* positive control results on Simmons citrate: Negative media maintains the green colour.

3.5.6 Lysine Iron Agar (LIA) test

A single pure colony of the bacterium was picked using a sterile inoculating needle and stubbed into the butt and streaked on the slant of the TSI agar, and then incubated at 37◦C for 24 hours with loosely placed caps. Next, results were examined and interpreted by comparing with *E. coli* positive control results on LIA: Lysine Iron Agar: Alkaline slant / Alkaline Butt / H_2S – (+)/ Gas variable.

3.5.7 Sulfide, Indole, Motility (SIM) test

A single pure colony of the bacterium was picked using a sterile inoculating needle stubbed into SIM agar and then incubated at 37◦C for 24 hours with loosely placed caps. Thereafter, results were examined and a test for indole production was done by adding 2-3 drops of Kovacs reagent. Interpretation of *E. coli* positive control results on SIM: No H_2S / Indole positive / Motile was done.

3.6 Detection of resistance profiles in *E. coli* isolates

3.6.1 Phenotypic methods

3.6.1.1 Disk diffusion

The antimicrobial susceptibility of *E. coli* isolates was determined by the Kirby-Bauer diffusion method (Hudzicki, 2012) based on the Clinical and Laboratory Standards Institute (CSLI) guidelines. Using a sterile inoculating swab 1-3 well-isolated freshly grown *E. coli* colonies were picked from nutrient agar and suspended in 2 ml of sterile saline in a test tube. An emulsified smooth suspension was used to compare turbidity to 0.5 McFarland standard, containing approximately $1–2 \times 10^8$ colony forming units (CFU)/ml. On Mueller-Hinton agar plates within 15 minutes of preparation. Using a sterile swab, these bacterial suspensions were then spread evenly on a Mueller Hinton agar plate. Antimicrobial disks were gently placed on the Mueller Hinton agar plate using a disk dispenser. The plates were then incubated at 37 °C for 18 hours.

Antimicrobial susceptibility of *E. coli* isolates was tested using Ceftriaxone (30µg) CRO, Ciprofloxacin (5µg) CIP, gentamycin (30µg) CN, Imipenem IMP (10µg), Ampicillin (10µg) AMP, Chloramphenicol (30µg) C, Tetracycline (30µg) TE and trimethoprim/ sulfamethoxazole (25µg) SXT according to the diameters of the inhibited zones measured; including the diameter of the discs to the nearest whole millimeter using rulers and interpreted using the CLSI guidelines (Clinical Laboratory Standard Institute antimicrobial susceptibility testing, 2021). All isolates were stored in 1.5 ml of storage media (Skimmed milk, Tryptone soy broth, Glucose, and Glycerol STGG broth) in cryovials.

3.6.1.2 Detection of ESBL-producing *E. coli* by minimum inhibitory concentration

Preliminary screening of potential ESBL-producing *E. coli* was determined by first ascertaining cefotaxime resistance. The stored *E. coli* isolates were sub-cultured on cefotaxime-infused Luria Bertani (LB) agar (Oxoid Ltd., Basingstoke, Hampshire, UK). To do this, a mixture of 12.5g of LB with 500 ml of distilled water was made and autoclaved at 121°C for 15 minutes to sterilize it. After the mixture cooled down to about 55°C 1 µg/ml of CTX was added to the LB, then poured onto petri dishes and allowed to solidify. Following solidification, the *E. coli* isolates were streaked onto the plates and incubated at 37°C for 18 hours.

Following incubation, test tubes were filled with 3 ml of the LB supplemented with CTX (1 µg/ml). Using sterile loops, individual colonies of the bacteria growing on the solid LB agar plates were selected, emulsified into test tubes, and vortexed. The test tubes were loosely sealed, and placed in a shaker incubator at 175 rpm at 37 °C for 18 hours. The overnight cultures that showed growth were then diluted 10^4-fold and added in triplicates of a serial dilution of cefotaxime ranging from 0.5 to 512 µg/ml in a 96-well plate. The 96-well plate was then incubated at 37°C for 18 hours according to a previously published protocol by Murugappan, Sudarsan and Manoharan, (2006) and Wiegand, Hilpert and Hancock, (2008). Potential ESBL-producing *E. coli* isolates were identified as those with cefotaxime MICs $\geq$ 2 µg/ml. Quality control of the test was done using *E. coli* ATCC 25922.

3.6.2 Molecular Conformation of ESBL producing *E. coli*

3.6.1.1 DNA extraction of phenotypic ESBL isolates

Genomic DNA was extracted from 24-hour cultured isolates using the QIAamp DNA Mini Kit (QIAGEN Inc. GmbH, Holden, Germany) following the manufacturer's instructions. Bacterial overnight cultures of *E. coli* isolates with a CTX MIC $\geq$ 4 µg/ml were centrifuged at 4500 *x g* for 10 minutes at 4°C. The supernatant was pipetted out, and the pellet was subjected to gDNA extraction. A commercial QIAamp DNA mini kit was used to lyse the cells and solubilize the DNA of the ESBL-producing *E. coli* isolates. To break down the cell components, 180µl buffer ATL and 20µl Proteinase K

were added to the pellets in 1.5 ml microcentrifuge tubes and mixed by vortexing then, incubated at 56°C denaturing the proteins until completely lysed for 2 hours. Thereafter, 200µl buffer AL was added and mixed thoroughly by vortexing for 15 seconds and incubated at 70°C for 10 minutes. Then, 200µl of 96% ethanol was added and vortexed for 15 seconds. The mixture was then pipetted out onto the QIAamp Mini spin column in a 2ml collection tube and centrifuged at 6000 *x g* for 1 minute to allow cell constituents such as proteins, carbohydrates and metabolites to flow through while the DNA binds to the QIAamp Mini spin column. To purify the DNA for eluting, DNA bound to the QIAamp Mini spin column was placed in a new 2 ml collection tube for washing with 500µl of buffer AW1 added and centrifuged at 6000 x *g* for 1 minute, the flow-through and collection tube were discarded. This step was repeated with the addition of 500µl buffer AW2 and centrifuged at full speed (20,000 x *g*) for 3 minutes after which, the flow-through and collection tube were discarded. To eliminate carryover buffer AW2, the QIAamp Mini spin column was placed in a new 2 ml collection tube and centrifuged at full speed for 1 minute. Following this step, the QIAamp Mini spin column was placed in a new 1.5 ml microcentrifuge tube, and 200µl buffer AE was added and incubated at room temperature for 1 minute before centrifuging at 6000 x *g* for 1 minute to elute the DNA.

3.6.1.2 Whole genome sequencing of ESBL-positive isolates

For detailed characterization of the strains, WGS of ESBL-producing *E. coli* isolates with a MIC ≥ 4 µg/mL was performed using the Illumina NextSeq platform (Illumina Inc., San Diego, CA, USA). The Qubit 4.0 fluorometer assay kit (Thermo Fisher Scientific, Boston, MA, USA) was used to quantify the concentrations of the extracted DNA. Subsequently, the DNA was diluted to achieve concentrations ranging between 10 and 60 ng/µL in a final volume of 30 µL. Libraries of the DNA were prepared using the Illumina DNA library prep–(M) Tagmentation kit (Illumina Inc. San Diego, CA, USA). Using the Agilent 2100 bioanalyzer system (Santa Clara, CA, USA) and the qPCR kappa library quantification kit (Roche, Porterville, CA, USA), respectively, the quality and concentration of fragmented libraries were assessed. The libraries were pooled together and sequenced using a 2 × 150 paired-end method on an Illumina NextSeq platform (Illumina Inc., San Diego, CA, USA). The raw sequencing reads (fastq files) obtained were quality-filtered to a Phred score ≥ 20 and adaptor-trimmed using Trimmomatic (http://www.usadellab.org/cms/index.php?page= trimmomatic, accessed on 17 August 2023) (Andrew S. 2010; Bolger *et al*., 2014). The FastQC tool was used to assess the quality of reads (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/, accessed on 17 August 2023). Using the Unicycler assembler v0.5.0, the resulting high-quality reads were de novo assembled into contigs. The quality of the assembled genomes was assessed with Quast v5.2.0. Genomes with coverage exceeding 30X and contigs fewer than 300 bases were selected for post-sequencing analysis. The sequences were deposited in the Genbank under the BioProject identifier accession number PRJDB17552.

3.7. Data analysis

The questionnaires were checked for completeness and variables with answers for more than 90% of the questions were retained and included in the analysis. Data collected were entered into Microsoft Excel MS Office 2019 (Microsoft, Redmond, Washington, DC, USA) for antimicrobial use and WHOnet for antimicrobial susceptibility testing, respectively. The epiR statistical package was used to perform Chi-square and Fisher's exact tests on categorical variables and to compute measures of association like the odds ratio. Furthermore, data were manipulated using the dplyr v1.0.7 (Wickham *et al.*, 2023) and reshape2 v1.4.4 (Wickham, 2007) packages and visualized using ggplot2 v3.3.5 (Gentleman, Hornik and Parmigiani, 2008). The tools on the Center for Genomic Epidemiology platform were used for post-WGS sequencing analysis to identify resistance genes using Resfinder (https://cge.cbs.dtu.dk/services/ResFinder/, accessed on 23 September 2023), plasmids using Plasmidfinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/, accessed on 23 September 2023), and sequence types using MLSTFinder (https://cge.cbs.dtu.dk/ services/MLST/, accessed on 23 September 2023).

3.8 Ethical approval and informed consent

The study was approved by the Excellence in Research Ethics and Science, ERES Converge Ethics Committee (Reference number. 2023-Feb-002). Permission to use the archived isolates was sought from the Ministry of Fisheries and Livestock. Further, the study was cleared by the University of Zambia, School of Veterinary Medicine Board of Graduate Studies Committee. The participants gave their consent to participate in the study, and they had the option to withdraw at any moment. The participant's information was kept private even though it was known. Each participant gave both written and verbal consent to participate in the study after being made aware of its objectives. There was no plagiarism in this work, and the outcomes are stated truthfully.

CHAPTER FOUR

4.0 RESULTS

4.1. Sample distribution by production type

A total of 269 farms were sampled comprising commercial farms (*n* = 35) and small-scale farms (*n* = 234). Altogether, the sampled farms included 219 broiler farms, and 50-layer farms (Table 4.1).

Table 4.1: Sample distribution by district, production, scale, and type

S/No	District	Samples	Commercial	Small-scale	Broiler	Layer
1	Chibombo	10	0	10	9	1
2	Chilanga	44	0	44	30	14
3	Chisamba	81	32	49	75	6
4	Choma	13	0	13	9	4
5	Chongwe	43	0	43	37	6
6	Kaoma	12	0	12	12	0
7	Lusaka	30	3	27	16	14
8	Mongu	13	0	13	12	1
9	Petauke	18	0	18	14	4
10	Rufunsa	5	0	5	5	0
	Total	269	35	234	219	50

4.2 Antimicrobial Use (AMU) differed between commercial and small-scale poultry farmers

Of the 269 randomly selected farms, 119 consented to participate in the questionnaire survey. Of these, 35 were commercial and 84 were small-scale farmers; 94 of these were broiler farmers and 25 were layer farmers. Furthermore, 94/119 (79.0%) were broiler farmers and 25/119 (21.0%) were layer farmers. The questionnaire review showed that 99.2% (118/119) had information on antibiotic use on sampled birds. Similarly,

information on prescription use, prophylaxis, antimicrobial growth promoters (AGPs), knowledge of the withdrawal period, and sale of products under treatment was available in 90.8% (108/119), 100% (119/119), 100% (119/119), 99.2% (118/119), and 97.5% (116/119) respectively. The results also revealed that 38.9% (42/108) had acquired a prescription before accessing antibiotics. Further, while most farmers used antibiotics for treatment, 19.3% (23/119) and 12.6% (15/119) used them for prevention and growth promotion, respectively. Meanwhile, 87.3% (103/118) of the farmers expressed knowledge of the antibiotic withdrawal period. Nonetheless, 5.8% (6/103) of the farmers who claimed to be knowledgeable, sold their poultry meat and eggs while birds were under treatment (Figure 4.1).

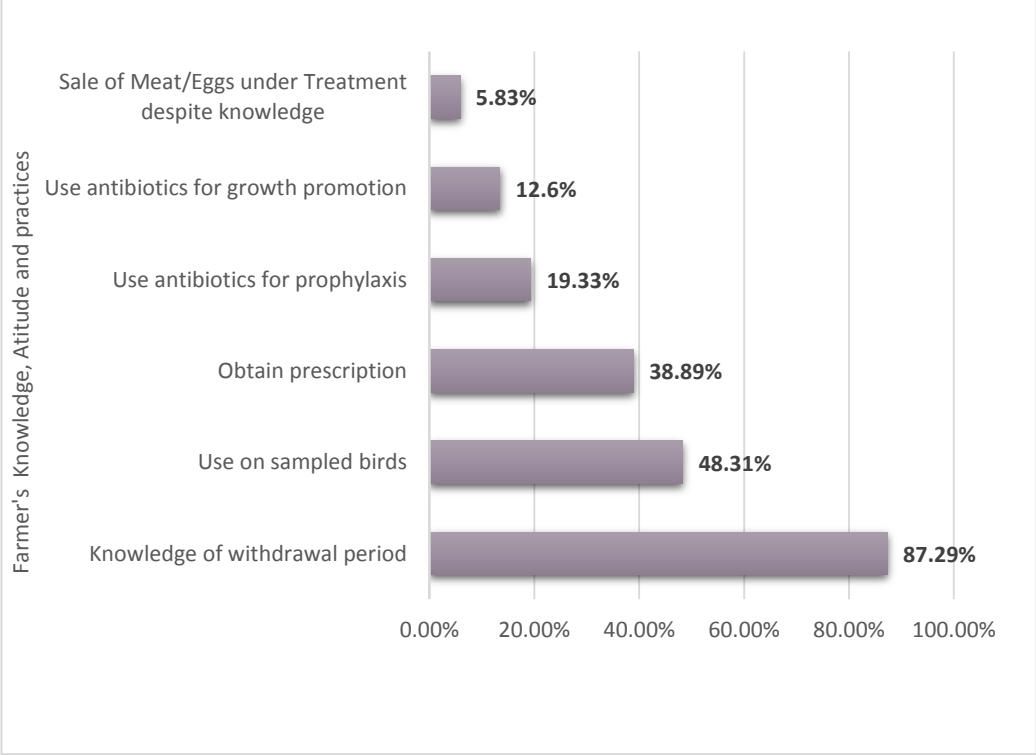

Figure 4.1: Farmer's Knowledge, Attitude and Practices by percentage

Table 4.2: Comparison of antibiotic use among the commercial and small-scale farmers from questionnaire survey results

Production type	Number of responses	Use on sampled bird	Not used on sampled birds	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Commercial (Reference)	35	6	29	1			
Small-scale	83	51	32	7.70	2.88 – 20.61	< **0.001**	Chi-square test
Production type	Number of responses	Prescription used	Prescription not used	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Commercial (Reference)	29	27	2	1			
Small-scale	79	15	64	0.02	0.00 – 0.08	< **0.001**	Fisher's exact test
Production type	Number of responses	Used for prophylaxis	Not used for prophylaxis	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Commercial (Reference)	35	1	34	1			
Small-scale	84	22	62	12.06	1.56 – 93.45	**0.002**	Fisher's exact test
Production type	Number of responses	AGPs used	AGPs not used	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Commercial (Reference)	35	1	35	1			
Small-scale	84	15	69	7.61	0.97 – 59.98	0.053	Fisher's exact test
Production type	Number of responses	Knowledge of withdrawal period	No Knowledge of withdrawal period	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Commercial	35	32	3	1			

(Reference)							
Small-scale	83	71	12	0.55	0.15 – 2.10	0.548	Fisher's exact test
Production type	Number of responses	Sell under treatment	Don't sell under treatment	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Commercial (Reference)	35	3	32	1			
Small-scale	81	11	70	1.68	0.44 – 6.42	0.548	Fisher's exact test

Note: Significant results in bold font.

Table 2.3.: Questionnaire survey results on the Use of antibiotics among Layer and broiler farmers in the study population

Production type	Number of responses	Use on sampled bird	Not used on sampled birds	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Broiler (Reference)	94	39	55	1			
Layer	24	18	6	4.23	1.54–11.6	**0.003**	Chi-square test
Production type	Number of responses	Prescription used	Prescription not used	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Broiler (Reference)	87	33	54	1			
Layer	21	9	12	1.23	0.47–3.23	0.678	Chi-square test
Production type	Number of responses	Used for prophylaxis	Not used for prophylaxis	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Broiler (Reference)	95	15	80	1			
Layer	24	8	16	2.67	0.97–7.34	0.098	Chi-square test
Production type	Number of responses	Used for growth promotion	Not used for growth promotion	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Broiler (Reference)	95	7	88	1			
Layer	24	8	16	6.29	2.00–19.8	**0.002**	Chi-square test
Production type	Number of responses	Knowledge of withdrawal period	No Knowledge of withdrawal	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used

			period				
Broiler (Reference)	95	83	12	1			
Layer	23	20	3	0.96	0.25–3.74	1.000	Fisher's exact test
Production type	Number of responses	Sell under treatment	Don't sell under treatment	Odds ratio (OR)	95% CI of OR	P-value	Statistical test used
Broiler (Reference)	93	7	86	1			
Layer	23	7	16	5.38	1.66–17.4	**0.008**	Fisher's exact test

Note: Significant results in bold font.

To determine the factors associated with the practices mentioned above, the questionnaire-based variables were compared among different production categories and farming scales. Compared to commercial farmers, more small-scale farmers used antibiotics on the sampled birds in general (Table 4.2) (OR =7.70; 95% CI= 2.88 – 20.61; P< 0.001) and for prophylaxis (OR = 12.06; 95% CI= 1.56 – 93.45; P = 0.002). However, small-scale farmers were less likely to obtain prescriptions (OR = 0.02; 95% CI = 0.00 – 0.08; P< 0.001). Furthermore, while there was no antibiotic use for production among commercial farmers, 17.9% (15/84) of small-scale farmers used antimicrobial growth promoters. Despite the observed differences in practices towards antibiotic usage, there was no difference in knowledge of the antibiotic withdrawal period between the two groups (OR = 0.55; 95% CI = 0.15 – 2.10; P = 0.548). Likewise, there was no difference in the proportion of farmers selling products from birds under antibiotic treatment (OR = 1.68; 95% CI = 0.44 – 6.42; P = 0.548).

The results revealed that layer farmers were more likely to use antibiotics on the sampled birds (OR = 4.23; 95% CI = 1.54 – 11.63; P = 0.003) for growth promotion (OR = 6.29; 95% CI = 2.00 – 19.77; P = 0.002) than broiler farmers. Additionally, there was no difference between farmers who reared layers or broilers in obtaining prescriptions to access antibiotics (OR = 1.23; 95% CI = 0.47 - 3.23; P = 0.678). Furthermore, there was

no difference in the usage of antibiotics for disease prevention between broiler and layer farmers (OR = 2.67; 95% CI = 0.97 - 7.34; P = 0.098) or those who were acquainted with the period of withdrawal (OR = 0.96; 95% CI = 0.25 - 3.74; P =1.00) (Table 4.3). However, more layer farmers sold their products during treatment compared to broiler farmers (OR = 5.38; 95% CI = 1.66 - 17.42; P = 0.008).

4.3 Isolation and antimicrobial resistance of *E. coli* isolates

This study managed to revive a total of 150/232, 64.7% *E. coli* isolates (Table 4.4).

Table 4.4: Number of revived *E. coli* isolates per district

S/No	District	Samples	Number of *E. coli* isolates revived
1	Chibombo	10	3
2	Chilanga	44	3
3	Chisamba	81	51
4	Choma	13	13
5	Chongwe	43	12
6	Kaoma	12	8
7	Lusaka	30	32
8	Mongu	13	15
9	Petauke	18	10
10	Rufunsa	5	3
	Total	269	150

In Zambia, all antibiotics are imported into the country. Tetracyclines and penicillins are among the most imported antibiotics for animal administration (Ministry of Fisheries and Livestock Zambia report to OIE, 2019). To determine if the AMR profiles of poultry-associated *E. coli* were related to the national veterinary antibiotic import data, the strains were subjected to antimicrobial susceptibility testing (AST). The results showed the highest resistance to ampicillin (128/148, 86.5%) and tetracycline (101/136, 74.3%) (Figure 4.2). On the other hand, imipenem revealed the lowest non-susceptibility (5/88,

5.7%). Third-generation cephalosporin (3GC) resistance was detected in 20% (30/150) of the isolates, most of which (28/30, 93.3%) exhibited resistance to three or more antibiotic classes.

4.4 AMR patterns of *E. coli* isolates

The AMR patterns of the *E. coli* isolates results showed highest resistance to AMP (128/148, 86.5%) and TET (101/136, 74.3%) (Figure 4.2). On the other hand, imipenem revealed the lowest non-susceptibility (5/88, 5.7%). Third-generation cephalosporin (3GC) resistance was detected in 20% (30/150) of the isolates, most of which (28/30, 93.3%) exhibited resistance to three or more antibiotic classes (Figure 4.3).

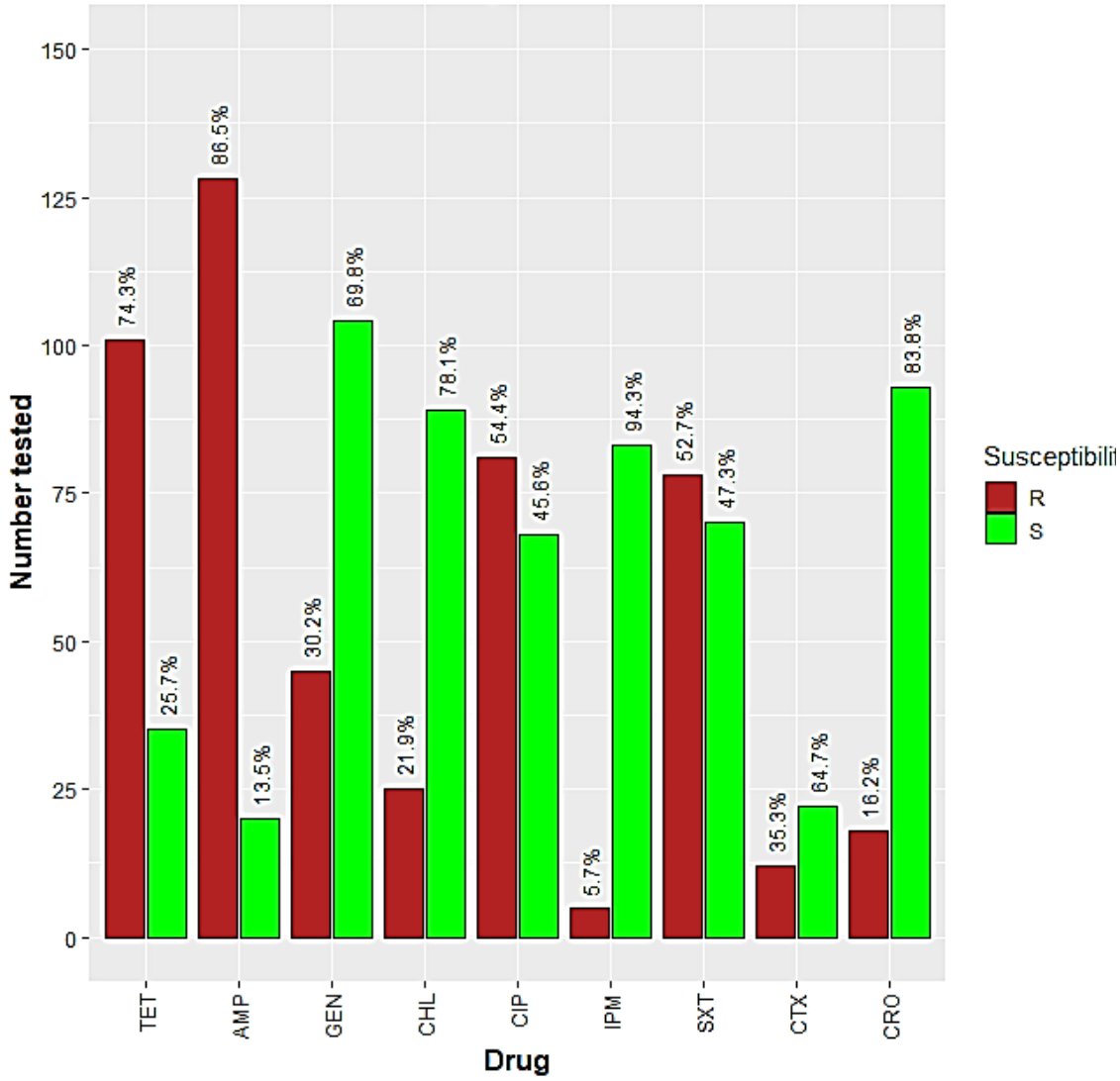

Figure 4.2: AST profiles by isolate

TET-tetracycline, AMP-ampicillin, GEN-gentamicin, CHL-chloramphenicol, IMP-imipenem, SXT-sulfamethoxazole/trimethoprim, CIP-ciprofloxacin, CTX-cefotaxime, CRO-ceftriaxone. R-Resistant, S-Susceptible

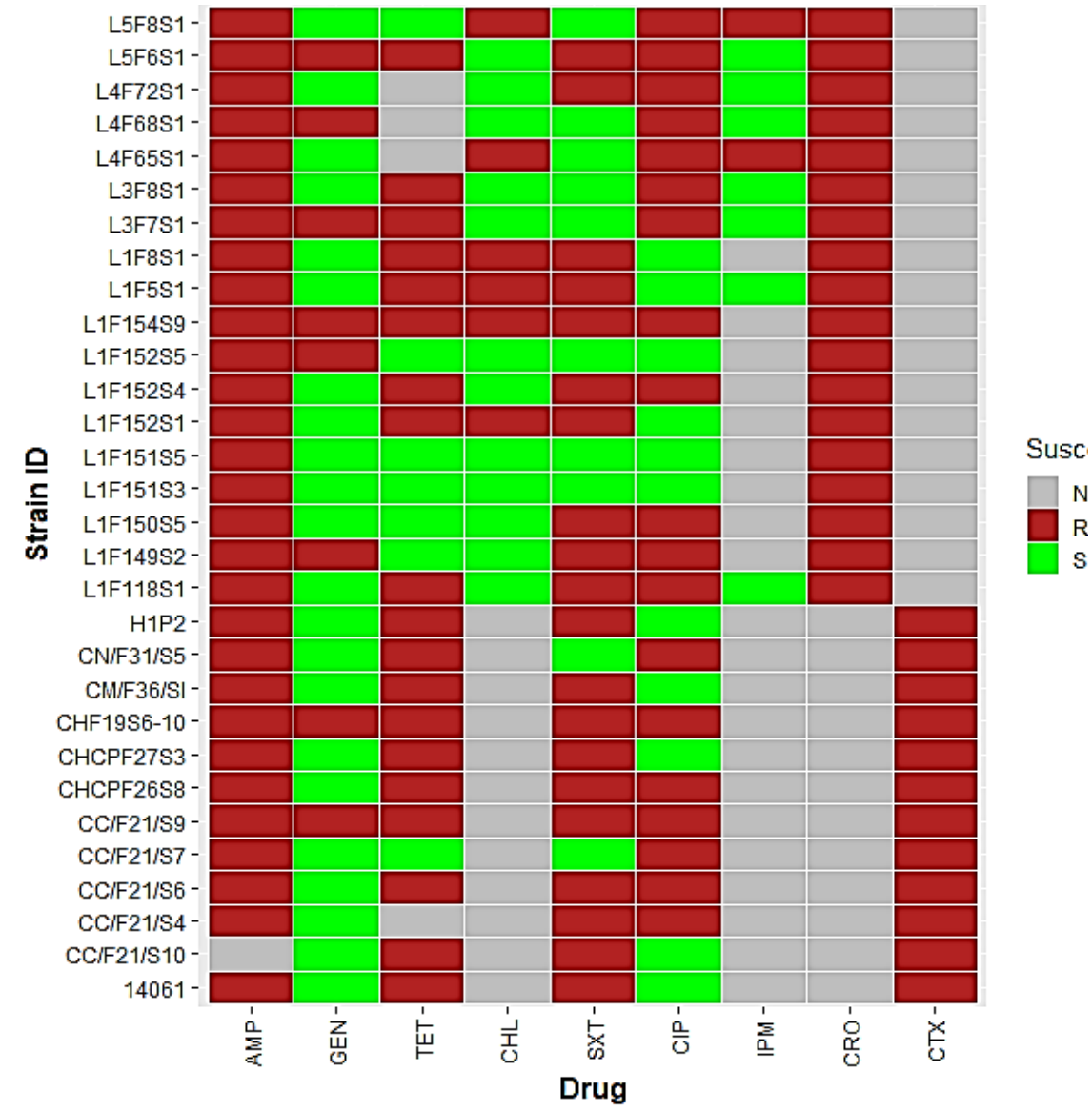

Figure 4.3: AST profiles of 3GC resistance strains

TET-tetracycline, AMP-ampicillin, GEN-gentamicin, CHL-chloramphenicol, IMP-imipenem, SXT-sulfamethoxazole/trimethoprim, CIP-ciprofloxacin, CTX-cefotaxime, CRO-ceftriaxone. R-Resistant, S-Susceptible, NT-Not tested

4.5 ESBL producing *E. coli* isolates

The revived archived150 *E. coli* isolates were screened for cefotaxime resistance by broth microdilution. Thirty isolates met the MIC breakpoint for cefotaxime resistance (≥ 2 µg/ml), which was used as a surrogate for phenotypic ESBL presence. The majority of ESBL positive samples were from small-scale farmers, (22/30, 73%) while the remainder (8/30, 27%) had a commercial-scale origin. Chilanga district had the highest number of isolates with phenotypic ESBL positives at 2/3 (66.7%), followed by Chongwe at 6/12 (50%) and Petauke at 3/10 (30.0%). On the other hand, no ESBLs were detected in Rufunsa and Chibombo districts (Table 4.5).

Table 4.5: Antibiotic Susceptibility Pattern of Suspected ESBL-Producing *E. coli* on cefotaxime screening by District

District	Number of Isolates	Phenotypic ESBL-positive isolates	Number of isolates in each CTX MIC category from 2 to ≥ 64µg/ml					
			2	4	8	16	32	≥64
Chisamba	51	10 (19.6%)	6	0	1	0	1	2
Mongu	15	2 (13.0%)	1	0	0	0	0	1
Lusaka	32	4 (12.5%)	2	1	1	0	0	0
Choma	13	2 (15.3%)	2	0	0	0	0	0
Petauke	10	3 (30.0%)	1	1	0	0	0	1
Chibombo	3	0 (0.0%)	0	0	0	0	0	0
Chilanga	3	2 (66.7%)	2	0	0	0	0	0
Chongwe	12	6 (50.0%)	6	0	0	0	0	0
Rufunsa	3	0 (0.0%)	0	0	0	0	0	0
Kaoma	8	1 (12.5%)	1	0	0	0	0	0
Total	150	30 (20.0%)	21	2	2	0	1	4

4.6 Molecular detection of ESBL-producing *E. coli*

Eight out of nine strains were successfully assembled into nearly complete genomes with the exception of one strain that had poor quality reads. Of the eight assembled strains, WGS data revealed half 4/8,50% of the strains harboured $bla_{\text{CTX-M}}$ gene (i.e., $bla_{\text{CTX-M-14}}$, n = 2; $bla_{\text{CTX-M-55}}$, n = 2). However, the bla_{TEM} gene was more prevalent (n = 5), while the bla_{CMY} existed in three isolates (Table 4.6).

Table 4.6: AMR *bla* genes among sequenced isolates

S/No	Isolate ID	bla_{TEM}	$bla_{\text{CTX-M}}$	$bla_{\text{CMY-2}}$
1	L1F8S1	+	+	-
2	L5F6S1	+	+	-
3	L1F5S1	+	-	-
4	L1F151S5	+	-	+
5	L1F152S5	+	+	-
6	L4F65S1	-	-	+
7	L1F151S3	-	-	+
8	L1F154S9	-	+	-

4.7 Whole-genome sequence characteristics of ESBL-positive isolates

These eight isolates carried a total of 27 different types of AMR genes that encode resistance to eight classes of antimicrobials (Table 4.7). The eight sequenced isolates belonged to six different sequence types (ST), ST770 (3/8, 37.5%) was detected three times of which (2/8, 25%) were in Chisamba and (1/8, 12.5%) in Petauke while the remaining 5 STs were detected only once ST 117, ST 938, ST 155, ST847 and ST 211 (Table 4.8). In addition, a diversity of plasmid replicons was observed across the strains, with incompatibility group F dominating (Table 4.8).

Table 4.7: Detected AMR genes by percentage proportion in *E. coli* isolates

Antibiotic Class	Detected AMR Genes	Gene Percent Proportion
Aminoglycosides	*Aac(3)-Iia, aac(3)-Iid, aac(3)-Via*	(3/8, 37.5%)
	aadA1, aadA5	(2/8, 25%)
	aph(3')-Ia, aph(3")-Ib, aph(6)-Id	(3/8, 37.5%)
β-lactams	$bla_{\text{CTX-M-14}}$	(2/12, 16.7%)
	$bla_{\text{CTX-M-55}}$	(2/12, 16.7%)
	bla_{TEM}	(5/12, 41.7%)
	$bla_{\text{CMY-2}}$	(3/12, 25%)
Folate-pathway antagonists	*dfrA7, dfrA14, dfrA17*	(3/5, 60%)
	sul1, sul2	(2/5, 40%)
Phenicols	*floR*	(1/1, 100%)
Macrolide	*mph(A)*	(1/1, 100%)
Fosfomycin	*fosA3, fosA7*	(2/2, 100%)
(Flouro)quinolones	*qnrB19, qnrS1*	(2/4, 50%)
	OqxA, OqxB	(2/4, 50%)
Tetracycline	*tet(A)*	(1/2, 50%)
	tet(B)	(1/2, 50%)

Table 4.8: Distribution and genetic characteristics of sequenced E. coli isolates

S/ No	Isolate ID	Location	Farm Type	Sequence Type	OH Serotype	AMR Genes	Plasmids
1	L4F65S1	Petauke	Small-scale	ST770	O25H16	*aph(3")-Ib*, *aph(6)-Id*, *bla$_{CMY-2}$*, *floR*, *sul2*, *tet(A)*	IncFIB(AP 001918), IncFII, IncB/O/K/Z, p0111
2	L5F6S1	Mongu	Small-scale	ST117	O45H4	*aadA5*, *aph(3")-Ib*, *aph(6)-Id*, *bla$_{CTX-M-55}$*, *bla$_{TEM}$*, *dfrA14*, *dfrA17*, *tet(A)*	ColpVC, IncFII(pHN 7A8), IncI2
3	L1F154S9	Chisamba	Commercial	ST7938	O32H35	*aac(3)-IIa*, *aadA5*, *aph(3")-Ib*, *aph(6)-Id*, *bla$_{CTX-M-14}$*, *dfrA17*, *floR*, *fosA3*, *mph(A)*, *OqxB*, *OqxA*, *qnrS1*, *sul1*, *sul2*, *tet(A)*, *tet(B)*	IncFIB(AP 001918), IncFIC(FII), IncFII(pHN 7A8)
4	L1F8S1	Lusaka	Small-scale	ST155	O154H51	*aph(3â2)-Ia*, *aph(3")-Ib*, *aph(6)-Id*, *bla$_{CTX-M-14}$*, *bla$_{TEM}$*, *dfrA7*, *floR*, *fosA3*, *fosA7*, *sul1*, *sul2*, *tet(A)*	IncHI2, IncHI2A, p0111
5	L1F5S1	Lusaka	Small-scale	ST847	O108H2	*aph(3")-Ib*, *aph(6)-Id*, *bla$_{TEM}$*, *fosA7*, *qnrS1*, *sul2*, *tet(A)*	IncFII, IncHI2, IncHI2A, IncN, p0111
6	L1F151S5	Chisamba	Commercial	ST211	O22H7	*aac(3)-IId*, *aph(3")-Ib*, *aph(6)-Id*, *bla$_{CMY-2}$*, *bla$_{TEM}$*, *sul2*, *tet(A)*, *tet(B)*	IncFIB(K), p0111
7	L1F151S3	Chisamba	Commercial	ST770	O102H51	*bla$_{CMY-2}$*	IncI1-I(Alpha), IncX4
8	L1F152S5	Chisamba	Commercial	ST770	O102H51	*aac(3)-Via*, *aadA1*, *bla$_{CTX-M-55}$*, *bla$_{TEM}$*, *fosA3*, *qnrB19*, *sul1*, *sul2*	IncFIB(AP 001918), IncFII, IncN

CHAPTER FIVE

5.0 DISCUSSION

5.1 Antimicrobial use among commercial and small-scale poultry farmers

Antimicrobial resistance is fuelled by mostly prolonged usage, misuse, underuse, and inappropriate use of antimicrobial drugs (Mencía-Ares *et al.*, 2021). The findings of the present study suggest that in Zambia both commercial and small-scale poultry farmers use antimicrobials, with nearly 50% of the sampled birds having reported a history of being treated with antibiotics. The results also show that more small-scale than commercial poultry farmers use antimicrobials for prophylaxis. Additionally, the study demonstrated that farmers utilize antimicrobials to promote growth more frequently in layers than in broiler poultry. Furthermore, antimicrobials are used in layers to boost egg production as antibiotics suppress bacterial organisms that may have a negative impact on the birds (Mardones *et al.*, 2023).

While some countries report higher than 90% AMU (Imam *et al*., 2020; Azabo *et al*., 2022), this questionnaire survey highlighted an overall 48.3% (57/118) AMU among poultry farmers, similar to another Zambian survey report by Caudell *et al.*, (2020). However, Caudell *et al.*, (2020) also reported 80% (158/198) lack of obtaining a prescription when purchasing antimicrobials compared to nearly 60% reported (66/108) (OR = 2.51; 95% CI = 1.49 – 4.23; P < 0.001) in this study. This discrepancy could be attributed to the current study covering five provinces (Westen, Southern, Eastern, Central, and Lusaka) and ten districts (Chongwe, Chilanga, Chibombo, Chisamba, Choma, Kaoma, Mongu, Lusaka, Petauke and Rufunsa). While the study by Caudell *et al.*, (2020) covered five districts located in two provinces─Lusaka (Chilanga, Chongwe, and Rufunsa) and Central (Chisamba and Chibombo). Although this study shows lower AMU than other nations, the need to use antimicrobials among these farmers could be exacerbated by poor implementation of biosecurity measures, poor enforcement and adherence to regulations on prescription-only drugs, inadequate veterinary services leading to increased AMU for disease prevention (Mehdi *et al.*, 2018;CSO,

2017;Youssef *et al.*, 2021). Therefore, it is necessary to improve farm-level infection management practices, including the use of vaccines (Ma *et al.*, 2021), phytogenic feed additives, and bioactive phenolic extracts (Salaheen *et al.*, 2017), among other available solutions.

There was no difference in AMU for growth promotion between commercial and small-scale farmers (OR=7.61; CI= 0.97 – 59.98; P = 0.053), suggesting that any difference in the overall usage could be related to infection management. Considering that commercial farmers have more established systems in terms of biosecurity (Mudenda *et al.*, 2022) and thus experience fewer infections, AMU under these conditions is expected to be lower. Consistently, a significant difference in AMU between small-scale and commercial farmers was observed; more small-scale farmers used antibiotics on the sampled birds (OR =7.70; 95% CI= 2.88 – 20.61; P < 0.001). Furthermore, small-scale farmers were comparatively less likely to obtain prescriptions (OR = 0.02; 95% CI = 0.00 – 0.08; P < 0.001). This implies that more small-scale farmers access antibiotics over the counter, promoting abuse and overuse of antimicrobials. This finding indicates a gap in the antimicrobial monitoring of Agro-vet shops.

Nevertheless, there was no difference reported between commercial and small-scale farmers in their being acquainted with the withdrawal period (OR = 0.96; 95% CI = 0.25 – 3.74; P = 0.548). Generally, 87.3% (103/118) of the farmers knew of the withdrawal period. However, despite this knowledge, 5.8% (6/103) of the knowledgeable farmers admitted to having sold their meat/egg of bird products under treatment. In conformity with studies elsewhere (Johnson *et al.*, 2019; Imam *et al.*, 2020), this survey showed that more layer farmers sold their products during treatment (OR = 5.38; 95% CI = 1.66 - 17.42; P = 0.548) than broiler farmers.

According to respondent information, AMU among broiler and layer farmers revealed that more layer farmers compared to broiler farmers used antibiotics on the sampled birds (OR = 4.23; 95% CI = 1.54 – 11.63; P = 0.003), and for growth promotion (OR = 6.29; 95% CI = 2.00 – 19.77; P = 0.002), probably due to the increasing egg demand

(Imam *et al.*, 2020). On the contrary, there was no difference between farmers who reared layers or broilers in obtaining prescriptions to access antibiotics (OR = 1.23; 95% CI = 0.47 - 3.23; P = 0.0678). Additionally, there was no difference in the usage of antibiotics for disease prevention between broiler and layer farmers (OR = 2.67; 95% CI = 0.97 - 7.34; P = 0.098) or those who were acquainted with the period of withdrawal (OR = 0.96; 95% CI = 0.25 - 3.74; P = 1.00) (Table 4.3). However, compared to broiler farmers, more layer farmers sold their products during treatment (OR = 5.38; 95% CI = 1.66 - 17.42; P = 0.008).

While both broiler and layer farmers have an option to observe the withdrawal period and then sell the meat/egg products, in agreement with the speculation, layer farmers are tempted to sell their eggs rather than discard them while treating their chickens (Annan-Prah *et al.*, 2012). This assumption is supported by this data, which showed that most (5/6, 83.3%) of the layer farmers knowingly sold products under treatment.

5.2 Isolation of *E. coli* and observed antimicrobial susceptibility profiles

Ideally, *E. coli* recovery ranges from 80%-90% (Mwansa *et al*., 2023; Mudenda *et al*., 2023). In agreement, the initial National AMR surveillance from August 2019 to September 2021 recovered 232/269, 86.2% *E. coli* isolates from the samples collected across the 10 districts. However, only 150/232, 64.7% of the *E. coli isolates* were successfully revived in this study. The discrepancy might be related to improper storage because some isolates were initially kept at -20°C in 30% glycerol (without skimmed milk, tryptone soy broth, and glucose) which is in addition to power outages.

This study revealed high AMR rates against commonly used antimicrobials such as ampicillin (128/148, 86.5%) and TET (101/136, 74.3%), similar to findings in other Zambian studies (Chishimba *et al*., 2016; Kapena *et al*., 2020). The AMR findings in this study correspond with the Zambian report on AMU by the Ministry of Fisheries and Livestock that cited tetracyclines and penicillins as the most imported antibiotics for administration in animals between 2015 and 2020 (Ministry of Fisheries and Livestock Zambia report to OIE, 2019). The observed high tetracycline and ampicillin resistance

coincides with the genotypic profile of the representative strains subjected to Whole-genome Sequencing (WGS), which possessed *tet* and *bla* genes.

While tetracyclines and penicillins are the most used antibiotics in poultry (Racewicz *et al.*, 2022), 93.3% (28/30) of the 3GC-resistant isolates exhibited MDR (resistance to at least three or more drug classes) which could be due to the existence of multiple AMR genes on the same plasmid, which could be co-selected by a single or few drug classes. The observed MDR could be explained by the several identified genes encoding AMR to eight drug classes (Table 4.7). Importantly, 7.1% (2/28) of MDR strains were resistant to imipenem, a drug of last resort in clinical medicine. However, no carbapenemase-encoding genes were detected, suggesting that the observed resistance could be related to point mutations or novel carbapenemase genes.

Nonetheless, phenotypic resistance to carbapenems has serious clinical implications as it limits the possible treatment alternatives, especially since colistin is not yet available in Zambian hospitals. The two carbapenem-resistant strains in this study were susceptible to Gentamicin, suggesting that aminoglycosides could be potential treatment options. However, the number of isolates was too low for a conclusive inference. In addition to carbapenems, third-generation cephalosporins have an essential role in clinical practice.

5.3 Phenotypic and genotypic AMR of *E. coli*

This study reported a 20% (30/150) 3GC resistance. Considering the close association between 3GC resistance and ESBLs, WGS data was used to screen for various *bla* genes. Previous studies have found the $bla_{\text{CTX-M}}$ gene in nearly all 3GC-resistant isolates (Shawa *et al.*, 2021). However, only half of the strains in this study harboured the $bla_{\text{CTX-M}}$ gene (i.e., $bla_{\text{CTX-M-14}}$, n = 2; $bla_{\text{CTX-M-55}}$, n =2). Meanwhile, the bla_{TEM} gene was more prevalent (n = 5), while the bla_{CMY} existed in three isolates (bla_{CMY}, n =3/8). The presence of $bla_{\text{CMY-2}}$ genes in 3/8 (37.5%) isolates may be a concern for public health since AmpC β-lactamases cause broad-spectrum resistance to β-lactamase inhibitors like clavulanic acid (Heider *et al.*, 2009). *In silico* Multi-locus Sequence Typing (MLST) identified ST155, among other STs, similar to Shawa *et al.*, (2021),

who also found ST155, although their serotypes differed. The presence of AMR genes and plasmids in *E. coli* isolates from poultry may contaminate the environment and food, creating the danger of exposure for humans and animals.

While the study covered five provinces and ten districts, not all samples had epidemiological data. However, not all 119 questionnaires were 100% complete. Furthermore, there was no way to verify the questionnaire data either by antimicrobial residue testing or physical examination of antibiotic sachets or packaging. Also, AMU information could not be verified as there was no access to prescriptions or antibiotic sales statistics. Having sequenced eight strains, sufficient information was gathered upon which future studies should consider incorporating more samples for a more inclusive picture. Finally, determining the AMR genes' location (chromosome or plasmid) was difficult from the short reads data. Therefore, future studies are implored to consider using a hybrid assembly of short and long reads to provide better accuracy in sequence data quality.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. Small-scale farmers use more antibiotics with fewer prescriptions than commercial farmers.
2. Higher ESBL prevalence was reported among small-scale farmers compared to commercial farms.
3. Most *E. coli* isolates obtained from both commercial farms and small-scale exhibited MDR, and WGS revealed β-lactamase (*bla*$_{\text{CMY-2}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-55}}$ and *bla*$_{\text{TEM}}$) and several other AMR genes.

6.2 Recommendations

1. Hold sensitization campaign programs to encourage prudent use of antimicrobials.
2. Raise awareness of the consequences for public health that arise from failing to observe the withdrawal period, focusing on changing layer farmers' behavior.
3. Strengthening knowledge regarding biosecurity, particularly among small-scale farmers.
4. Enhance inspection and enforcement of prescription-only medicines like antibiotics among agrovet shops.
5. Establish a bio-repository for proper storage of bacterial isolates.
6. Development of extension messages for small-scale poultry farmers.
7. Monitoring antimicrobial use in small-scale farms for on-the-spot advice.
8. Develop a digital platform for data collection to avoid missing information.
9. Promote the establishment of initiatives that encourage prudent antimicrobial use like farmer field schools in the poultry industry alongside continued AMR/AMU surveillance.

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APPENDICES

APPENDIX I: Questionnaire

Active Surveillance Data Collection Form

1. SAMPLE COLLECTION AND SUBMISSION FORM		
Sender/Sample collector details		
Name:		
Contact details:	Mobile: …… Email: …… Telephone: ……	
Surveillance Laboratory (e.g., CVRI, UNZA Vet Lab PH; UNZA Vet Lab Microbiology)		
Date of Sample Submission:		
Sample details		
Farm identification number		
Farm Contact details	Contact person: …… Mobile: …… Email: ……	
Number of Samples collected		
Sample Details	Sample ID e.g. L1F6S1	Sample Type e.g., Faecal

Date and time of sampling	Date		Time	
Pooled: Yes [ ] No [ ]	How many individual samples are combined in the pool [ ]			
Type of transport media, if used				
Microorganism to be tested and test required	Microorganism		Test required	
	Campylobacter spp			
	E. coli			
	ESBL *E. Coli*			
	Enterococcus spp			
	Salmonella spp			
	Others			
2. EPIDEMIOLOGICAL DETAILS				
Sample collection point	Farm [ ] Abattoir [ ] Live bird market [ ]			
Name of Sample Collection Point				
	Farm		**Collection Point**	
Geographical location of Farm/Collection point (Coordinates in degree decimals e.g., lat -14.43807 long 28.45251)	Latitude	Longitude	Latitude	Longitude
Province				
District				
Veterinary Camp				

Details of Owner of the farm	Name:		
	Address:		
	Phone number:		
Production category/type	Broiler [ ] Layer [ ] Traditional [ ]		
Sector classification (Broiler)	1[ ] (Above 50,000)	2 [ ] (10,000 to 50,000)	
	3 [ ] (1,000 to 10,000)	4 [ ] (200 to 1,000)	
Sector classification (Layer)	1[ ] (Above 50,000)	2 [ ] (10,000 to 50,000)	
	3 [ ] (1,000 to 10,000)	4 [ ] (200 to 1,000)	
Number of Birds on the farm	**Broiler**	**Layers**	**Traditional**
Number of birds in the pen that was sampled e.g. Pen1: 5 birds	Pen 1	Number:	
	Pen 2	Number:	
	Pen 3	Number:	
	Pen 4	Number:	
Number of pens (e.g. layer houses or broiler sheds)			
Approximate age of the birds (in weeks) that were sampled			
Source of chickens (name of hatchery if known)			
Vaccines administered to the birds during the production cycle			
Diseases commonly encountered on the farm			

Disease encountered during growth period of the birds sampled	**Sampling Unit (Poultry house)**	**Disease**
Average number of birds slaughtered per day if sample collection is being done at;	Sampling Unit	Number
	Farm	
	Abattoir	
	Live bird market	
Average number of farmers supplying birds to the sample collection point per day	Abattoir	
	Live-bird Market	
	Other; specify	
	Species	**Disease**
List other animal species on the same farm (e.g. ducks, geese, guinea fowl, goats, cattle, pigs, fish)	[ ] Poultry	[ ] Guinea fowl [ ] Geese [ ] Ducks [ ] Quails [ ] Doves/Pigeons
	[ ] Pigs	
	[ ] Cattle	
	[ ] Goats	
	[ ] Fish	
	[ ] Others	Specify ……. ……..
3. Antimicrobial Use (AMU) Details		
Use of antibiotics on the birds being sampled	Yes [ ] No [ ]	
If yes, list the antibiotics		

used (in order of quantity usage)		
Common diseases/conditions and corresponding antibiotics used on the birds on the farm	**Disease/Condition**	**Antibiotics**
Common diseases/conditions and corresponding antibiotics used on the birds being sampled		
Do you use any other products to treat your birds?	Yes [ ] No [ ]	
If Yes, List products used (where possible show the container)		
Source of antibiotics used	Agro-Vet shop [ ] Veterinary Officer [ ] General Pharmacy [ ] Fellow farmers [ ] Grocery/Market [ ] Other (specify)……	
Use of a prescription to access antibiotics	Yes [ ] No [ ]	

Knowledge of observation of withdrawal periods	Yes [ ] No [ ] Not sure [ ]
Do you sell eggs and meat of animals while they are under treatment?	Yes [ ] No [ ]
If not, how long do you wait after the end of the treatment to sell eggs? meat?	Number of Days:
Treatment of market-ready birds	Yes [ ] No [ ]
Use of antibiotics to prevent diseases	Yes [ ] No [ ] Not sure [ ]
Use of antibiotics to improve production	Yes [ ] No [ ] Not sure [ ]
Type of feed used	Commercial [ ] Self-formulated [ ] Both [ ]
Use of feed additives	Yes [ ] No [ ]
If yes, provide the name of the feed additives	
Does the feed have antibiotics?	Yes [ ] No [ ] Not sure [ ]
If yes, provide the name of the antibiotics	
At what stage(s) of production is the medicated feed given to the flock	
How long is the medicated feed given to the flock?	

APPENDIX II

A. AMR Surveillance consent form

Purpose of the surveillance

The purpose of this surveillance is to generate data on common infections and the treatment for common diseases in poultry. After the samples have been analyzed, the information will help us determine the best treatment for the common diseases in your poultry. The surveillance will also ensure a regular supply of disease-free poultry.

Your rights as a respondent

a) You are free to withdraw at any time, without giving any reason for your withdrawal.
b) You do not have to answer any question that you are uncomfortable with.
c) You are free to ask any questions related to this surveillance.

The information obtained during this visit will be used for surveillance purposes only. The information you provide is confidential and will be treated as such. Please be reminded that you must provide factual information that will enable the government to make informed policy decisions on the proper management of common diseases in your poultry area.

I do hereby acknowledge that I have fully understood the information provided to me and I voluntarily agree to participate in this surveillance.

Participant	Surveillance Staff
Name……	
Name…….	
Date: …….	Date:
…….	
Signature or Thumbprint:	Signature:
……	

B. Participant Information Sheet- Plain Language Statement

Title: The Fleming Fund Country Grant Antimicrobial Resistance (AMR) and Antimicrobial Use (AMU) surveillance in the animal health sector.

Invitation

You are invited to take part in the Antimicrobial Resistance (AMR) and Antimicrobial Use (AMU) surveillance **that is being implemented by the Government of Zambia with** support from the UK government through the Fleming Fund. The surveillance will collect AMR and AMU data from different parts of the country by use of different methods, including poultry (broiler and layers) faecal samples and cloacal swabs, and a survey for AMR and AMU indicators. The Surveillance has permission to recruit participants from this area.

What is the purpose and significance of the surveillance?

There is a worldwide threat of drug-resistant infections due to bacterial Antimicrobial Resistance (AMR). To understand and improve the AMR/AMU situation in the country, reliable information is required. Through this valuable information including drug resistance of bacteria and antimicrobial use, programs can be designed and implemented to improve the situation as well as raise awareness of the importance of prudent use of drugs among the community and decision-makers.

Why have I been chosen?

You are being asked to participate in this surveillance because you are a poultry producer and the surveillance is partly being conducted in your community the government of Zambia has given us permission to ask you to participate. These activities are earmarked to take place in many districts across the country for a period of up to 3 years.

How do I take part in this study?

Participation is voluntary and there is no monetary benefit. If you agree to take part, you will keep this information sheet. You are free to withdraw at any time.

What does the surveillance involve?

Government officers and/or enumerators will visit you. The sample collector will ask for permission to collect faecal samples from your poultry house(s) and cloacal swabs from the birds and the enumerator will conduct the questionnaire with you on drug use. The questionnaire should take 5-10 minutes to complete.

How will the collected information be managed?

All information, including your identity, will be kept in strict confidence by the surveillance team. In each questionnaire, you will be provided a unique code to ensure that your identity is protected. Any information from the questionnaire will be used anonymously.

What will happen to the results of the surveillance?

The data from the surveillance will be analysed and will be used to better understand and improve AMR/AMU in the country. Reports, user guides, conference presentations, and articles will be published.

What should I do if I have complaints about how the methods are conducted?

If you encounter any issues or have concerns about the procedures, please contact the person in charge of the surveillance (Dr. Geoffrey Mainda, 0977344822, gmainda@hotmail.com, P.O. Box 50600, Mulungushi House, Department of Veterinary Services, Lusaka).