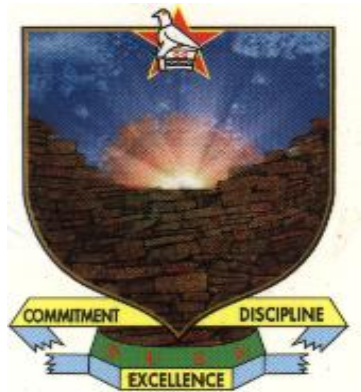


**BINDURA UNIVERSITY OF SCIENCE EDUCATION
FACULTY OF AGRICULTURE AND ENVIRONMENTAL SCIENCE
DEPARTMENT OF ANIMAL SCIENCE**



**COMPARATIVE ANALYSIS OF THE EQUIVALENT OF ANTIBIOTIC-RESISTANT
ESCHERICHIA COLI FROM BROILER AND ROADRUNNERS CHICKEN S
PRODUCED IN MIXED FARMING IN SEKE RURAL DISTRICT.**

A RESEARCH PROJECT

BY

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SUPERVISOR: Dr CHABWANDA

**A RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS OF BACHELOR OF SCIENCE HONOURS DEGREE
IN ANIMAL HEALTH AND PRODUCTION EXTENSION**

JUNE 2026

DECLARATION

I, Tarisai Matika, declare that the project which is hereby submitted for the qualification of Bachelor of Science Honours Degree in Animal Health and Production Extension at Bindura University of Science Education, is my own independent work and has not been handed in before for a qualification at/in another University/Faculty/School. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references. I further cede copyright of the dissertation to Bindura University of Science Education.

Signature TMatika

Date 01/09/26

APPROVAL FORM

The undersigned certify that they have supervised, have read and recommend to the University for acceptance and examination a research project entitled “*Comparative analysis of the prevalence of antibiotic-resistant Escherichia coli from broilers and roadrunner chickens produced in mixed farming in Seke District*” submitted by Tarisai Matika in partial fulfilment of the requirements for the award of the degree of Bachelor of Science Honours Degree in Animal Health and Production Extension.

Supervisor: Dr Chabwanda

Signature

A handwritten signature in black ink, appearing to read 'Chabwanda', with a vertical line extending upwards from the end of the signature.

Date: 02/09/26

DEDICATION

I dedicate this dissertation to my friends and family. I adore each and every one of you.

ACKNOWLEDGEMENTS

I could not have completed this dissertation on my own. First and foremost, I would want to thank my supervisor, Dr. Chabwanda, whose door was always open and whose compassion and understanding helped me overcome the most difficult conditions. I appreciate you believing in both the project and me. I'd also want to thank Dr. Gufe for his support. My family has my heartfelt gratitude. Your efforts, Mom and Dad, have given me this opportunity. And you were my rock, hubby. You encouraged me after every failure and thrilled over every minor win. This thesis is dedicated to you.

ABSTRACT

Antimicrobial resistance (AMR) within food animal production is a major reservoir of antimicrobial-resistant microorganisms posing a serious threat to public health worldwide. The prevalence, antibiotic susceptibility, and multidrug resistance of *Escherichia coli* from live broiler and roadrunner chickens from the same farms in Seke District, Zimbabwe, were investigated. A cross-sectional study comparing both live chicken species was conducted. A total of 150 cloacal swab samples were collected from live chickens (75 broiler and 75 roadrunners). *Escherichia coli* was cultured on MacConkey agar, Blood agar, TBX agar, and Gram staining tests and biochemical tests (motility, citrate, and indole tests). Antimicrobial susceptibility tests using the Kirby-Bauer disc diffusion method were used on selected antibiotics. The most resistant antibiotics in both groups were tetracycline, erythromycin, sulphamethoxazole, and nalidixic acid. The most common antibiotic pattern in all *Escherichia coli* isolates from chickens (100%) was resistance to tetracycline, nalidixic acid, erythromycin, and sulphamethoxazole, which indicates multidrug resistance (MDR) in resistant *Escherichia coli* (resistance to three or more antibiotic classes). The resistance rates of *Escherichia coli* to roadrunners were significantly higher than broilers ($p=0.00663$). Furthermore, roadrunner *E. coli* showed lower sensitivity to gentamicin (32% susceptibility) and meropenem (60% susceptibility). The high levels of antimicrobial resistance in both types of chickens from the same farms challenge the notion that raising chickens in free-range systems reduces the risk of antimicrobial resistance. Furthermore, the presence of antimicrobial resistance in both species of chickens indicates the presence of common reservoirs of antimicrobial-resistant pathogens and genes within the environment. Given the threat that antimicrobial-resistant *Escherichia coli* from chickens poses to public health, interventions must be made within the One Health framework to reduce such risks to the community.

Keywords: *E. coli*, mixed farming, poultry, antimicrobial resistance, Seke District.

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LIST OF ACRONYMS AND ABBREVIATIONS

Acronym/Abbreviation	Meaning
AMR	Antimicrobial Resistance
AMP	Ampicillin
AST	Antimicrobial Susceptibility Testing
CFU	Colony Forming Unit
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
DNA	Deoxyribonucleic Acid
<i>E. coli</i>	<i>Escherichia coli</i>
ERY	Erythromycin
ESBL	Extended-Spectrum Beta-Lactamase
FAO	Food and Agriculture Organization
GEN	Gentamicin
I	Intermediate
KB	Kirby–Bauer
kg	Kilogram
MHA	Mueller–Hinton Agar
MIC	Minimum Inhibitory Concentration

mL	Millilitre
MDR	Multidrug Resistance
MDR-EC	Multidrug-Resistant <i>Escherichia coli</i>
MER	Meropenem
mm	Millimetre
NA	Nalidixic Acid
OH	One Health
PCR	Polymerase Chain Reaction
R	Resistant
S	Susceptible
SPSS	Statistical Package for the Social Sciences
STR	Streptomycin
SUL	Sulphamethoxazole
TBX	Tryptone Bile X-Glucuronide Agar
TET	Tetracycline
WHO	World Health Organization
WOAH	World Organisation for Animal Health
°C	Degrees Celsius
µg	Microgram
χ^2	Chi-Square Statistic

MDR Pattern Abbreviations Used in the Study

Pattern	Meaning
TET-NA-ERY-SUL	Tetracycline–Nalidixic Acid–Erythromycin–Sulphamethoxazole

TET-NA-ERY- SUL-STR	Tetracycline–Nalidixic Streptomycin	Acid–Erythromycin–Sulphamethoxazole–
TET-NA-ERY- SUL-AMP	Tetracycline–Nalidixic Ampicillin	Acid–Erythromycin–Sulphamethoxazole–
TET-NA-ERY- SUL-CRO	Tetracycline–Nalidixic Ceftriaxone	Acid–Erythromycin–Sulphamethoxazole–
TET-NA-ERY- SUL-CIP	Tetracycline–Nalidixic Ciprofloxacin	Acid–Erythromycin–Sulphamethoxazole–

CHAPTER 1

1.0 Introduction

1.1 Background

Antibiotic-resistant *Escherichia coli* (*E. coli*) has become a growing problem globally in the context of agriculture and the extensive use of antibiotics on farm animals (FAO, 2025; Xexaki et al., 2023). In areas that employ mixed agricultural methods, such as the Seke District in Zimbabwe, both broiler and roadrunner chickens are raised together. This investigation aims to determine the prevalence of antibiotic-resistant *E. coli* in these two breeds of chickens. Antibiotics are often used in the management of broiler and roadrunner chickens (BMC Vet Res., 2025; systematic review of poultry, 2024). However, the use of antibiotics in chickens is associated with the development of antibiotic-resistant bacteria, which threaten the health of chickens and humans (systematic review of poultry, 2024). *E. coli* is a normal bacterial inhabitant of the chicken intestinal tract (BMC Vet Res., 2025; PubMed horizontal transfer study, 2020). The antibiotic-resistant forms of this bacteria can cause serious illnesses in chicken and humans that are often difficult to treat (systematic review of poultry, 2024).

The Seke district is well known to employ a mixed form of agriculture that raises both broiler and roadrunner chickens. Broilers are often raised for high meat yields and have intensive farming methods that include the use of antibiotics. Roadrunner chickens are indigenous to the area and are raised in more extensive farming methods that include fewer antibiotics. The difference in these two types of farming techniques may have an impact upon the forms of antibiotic-resistant bacteria that live in these two types of chickens. An understanding of the prevalence of antibiotic-resistant bacteria in both types of chickens is crucial to the assessment of the potential health risks of these birds to the population. The ability to determine the prevalence of antibiotic-resistant *E. coli* in these two types of chickens will allow for the gain of essential knowledge regarding the risks of raising these types of chickens in the agricultural systems in which they live, as well as how to best manage the health of these birds within those systems. Furthermore, such knowledge may allow individuals to develop sustainable methods of farming these types of chickens to ensure the health of these birds within those systems (Spain longitudinal study, 2022). Thus, the performance of such a study will allow for the gaining of essential baseline data regarding

antibiotic-resistant *E. coli* in the Seke District, Zimbabwe, and in its mixed farming systems of broilers and roadrunner chickens. The prevalence of antibiotic-resistant bacteria is currently one of the major public health problems of our time (Nigeria poultry MDR, 2024). Thus, by performing this research into the antibiotic-resistant *E. coli* in broilers and roadrunner chickens in the Seke District, we can contribute to the knowledge of the impact of antibiotic resistance in mixed farming systems, and take steps to protect the health of both farming communities and the public from such antibiotic-resistant bacteria.

1.2 Problem Statement

The resistance of strains of bacteria like *E. coli* to the antibiotics that are used to treat or remove those bacteria from the bodies of chickens is an increasing problem for the health of those farm birds and the humans that consume those birds (Bangladesh One Health, 2022). Within the Seke district, Zimbabwe, the farming of both broiler and roadrunner chickens presents the potential for antibiotic-resistant *E. coli* to be present within those birds due to the way in which those chickens are managed and farmed. Broiler chickens are managed in ways that introduce them to antibiotics, while the indigenous roadrunner chickens are managed in methods that do not include the use of antibiotics.

Therefore, the roadrunner may become a reservoir of antibiotic-resistant bacteria due to these differing farming techniques and potential exposure to environmental toxins. Despite the known threats that antibiotic-resistant bacteria can pose to the health of both poultry and human populations, there exists a lack of data regarding the prevalence of antibiotic-resistant *E. coli* in broilers and roadrunners specifically within the Seke district. Therefore, this knowledge gap presents a challenge to the ability of individuals to understand the risks that the chicken products can pose to the health of consumers of those products, as well as to develop methods of controlling or eliminating those risks. Thus, the aim of this study is to provide insights regarding the antibiotic resistance of *E. coli* from chickens in the Seke district.

1.3 Justification

There are several reasons that this study is to be performed. The emergence of antibiotic-resistant bacteria is one of the major health problems facing the planet today (Greece prevalence study, 2023). Furthermore, *E. coli* is one of the bacteria that is often associated

with foodborne illnesses, making its antibiotic resistance another threat to the health of those who consume chicken products. A understanding of the prevalence and antibiotic-resistant profiles of bacteria like *E. coli* in different types of chickens within the Seke district will allow for the understanding of the potential health threats that those different types of chickens can pose to the health of the local and surrounding communities.

The Seke district farms both broiler and roadrunner chickens. Broiler chickens are raised with intensive farming methods that include the use of antibiotics to manage their health. In contrast, roadrunner chickens are raised in extensive systems that do not use antibiotics as often. Thus, the ability of broilers and roadrunners to contain antibiotic-resistant bacteria can help to reveal the impact that farming techniques have upon the development of antibiotic resistance. Furthermore, the results of this study will allow for the improvement of various farming methods for the chickens in question. Despite the recognized threats of antibiotic resistance in farm animals, there is a dearth of information regarding the problem in the Seke district. Thus, the performance of this study will help to provide information regarding antibiotic-resistant *E. coli* in the area, and contribute to the knowledge of the risks of antibiotic resistance to those farming communities.

Additionally, roadrunners and broilers are among the types of chickens that are most commonly farmed in Zimbabwe. Thus, information gained from this research will provide essential knowledge to the stakeholders of the chicken farming industry in Zimbabwe. Finally, the knowledge of the presence of antibiotic-resistant bacteria within farm animals will allow for the development of methods of reducing their prevalence or impact upon health. Thus, the information gained from this research will allow for the development of methods to reduce the excessive use of antibiotics in farming chickens (Spain longitudinal study, 2022). Furthermore, increasing public awareness regarding the threats to health posed by antibiotic-resistant bacteria can allow individuals to make more informed decisions about the consumption of chicken products. Finally, the knowledge of antibiotic resistance within these types of farming systems can lead to the development of methods that are both sustainable and helpful to the farming communities of the Seke district. Thus, this study will allow for the development of actions that can benefit those communities and the ecosystem in which they exist.

1.4 Aim

The aim of the study is to determine the prevalence of antibiotic-resistant *E. coli* from live broiler and roadrunner chickens in Seke district.

1.5 Specific Objectives

The specific objectives of the study are to:

1. Determine the prevalence of antibiotic-resistant *E. coli* from live broiler and roadrunner chickens in Seke district.
2. Identify the antibiotic resistance profiles of *E. coli* bacteria strains from live broiler and roadrunner chickens in Seke district.
3. Assess the risk factors for antibiotic resistance of *E. coli* strains from live broiler and roadrunner chickens in Seke district.

1.6 Research Questions

The study was guided by the following research questions:

1. What is the prevalence of antibiotic-resistant *Escherichia coli* among live broiler and roadrunner chickens in Seke District?
2. What are the antibiotic resistance profiles of *Escherichia coli* strains isolated from live broiler and roadrunner chickens in Seke District?
3. What risk factors are associated with the occurrence of antibiotic-resistant *Escherichia coli* strains in live broiler and roadrunner chickens in Seke District?

1.7 Research Hypotheses

Hypothesis 1: Prevalence of Antibiotic-Resistant *E. coli*

Null Hypothesis (H_0):

There is no significant difference in the prevalence of antibiotic-resistant *Escherichia coli* between live broiler and roadrunner chickens in Seke District.

Alternative Hypothesis (H_1):

There is a significant difference in the prevalence of antibiotic-resistant *Escherichia coli* between live broiler and roadrunner chickens in Seke District.

Hypothesis 2: Antibiotic Resistance Profiles

Null Hypothesis (H_0 2):

There is no significant difference in the antibiotic resistance profiles of *Escherichia coli* isolates obtained from live broiler and roadrunner chickens in Seke District.

Alternative Hypothesis (H_1 2):

There is a significant difference in the antibiotic resistance profiles of *Escherichia coli* isolates obtained from live broiler and roadrunner chickens in Seke District.

Hypothesis 3: Risk Factors Associated with Antibiotic Resistance

Null Hypothesis (H_0 3):

There is no significant association between identified risk factors and the occurrence of antibiotic-resistant *Escherichia coli* strains in live broiler and roadrunner chickens in Seke District.

Alternative Hypothesis (H_1 3):

There is a significant association between identified risk factors and the occurrence of antibiotic-resistant *Escherichia coli* strains in live broiler and roadrunner chickens in Seke District.

1.8 Chapter summary

This chapter introduced the topic of antibiotic-resistant *E. coli* among live broiler and roadrunner chickens in the Seke district of Zimbabwe. Antibiotic-resistant bacteria poses a threat to public health worldwide today (FAO, 2025; Xexaki et al., 2023). Broilers are managed in intensive farms with antibiotic use, while roadrunners are raised in extensive farms without the use antibiotics (BMC Vet Res., 2025; systematic review of poultry, 2024). The goal of this study is to determine the antibiotic resistance of *E. coli* strains from both types of chickens from the Seke district, as well as justify the performance of such a study. Thus, the aim and specific objectives of the study were determined. Additionally, the research question and hypotheses of the study were also defined in this chapter. Overall, the knowledge of the risks and antibiotic resistance among the types of chickens in Zimbabwe will help to establish the rationale for this research, as well as provide an understanding of the framework that will be utilized in performing the research to answer the research question and test the hypotheses.

CHAPTER 2

LITERATURE REVIEW

2.1. Overview of *Escherichia coli*.

E. coli, a rod-shaped Gram-negative bacteria found in the Enterobacteria *Escherichiaceae* family, is among the most commonly found microorganisms in human and animal intestinal tracts. As such it is classified as a facultative anaerobe, because it can live and multiply in both presences of (or without) oxygen. Normal gut function is maintained by the majority of *E. coli* strains in healthy hosts, which helps with digestion and prevents colonization by harmful microorganisms. As a result, the bacterium is an essential element of the natural intestinal microbiota in several animal species and humans (Khong et al, 2022; Li dc.

Despite the fact that many *E. coli* strains are harmless, some have virulence factors that enable them to cause disease. Genetic traits of these pathogenic strains are unique and allow for invasion of host tissue, generation of toxins (endemic species), avoid immune system responses and creation of infections. *E. coli* can cause numerous illnesses in both humans and animals. This is the case. The pathogenicity of the organism is primarily determined by environmental factors, virulence genes, and host susceptibility. To prevent and control disease, it is important to have knowledge of the diverse *E. coli* strains (Manges et al, 2022; Khong & al." in 2021).

The classification of pathogenic *E. coli* strains into intestinal or extra-intestinal groups is based on the site of infection. The gastrointestinal tract is the primary site of colonization for intestinal pathogenic *E. coli*, which are associated with diarrheal diseases. Certain extra-intestinal strains have specialized virulence determinants that enable them to survive outside the intestinal environment, leading to diseases like urinary tract infections and respiratory infections. These strain. These differences in the pathogenic mechanisms point to a clear complexity of *E. coli* infections among different host species (Dogan et al, 2023; Li: 2026).

E. coli intestinal pathotypes have been identified through their virulence features and mechanisms of disease production. These strains can attach to and colonize the intestinal lining of their hosts by means of adhesins, fimbriae, toxins, and other virulence factors. This is possible. These adaptations enable bacteria to create infections and overcome host defense mechanisms.. Both domesticated and wild animals contain high levels of pathogenic strains of *E. coli*, which can be spread through transmission from the environment to other organisms such as animals or humans. Thus, certain strains of *E. coli* are classified as

significant environmental pollutants with important public health consequences (Manges et al, 2022; Dogan & Co-2019, 2003).

Commercial and backyard flocks of poultry are frequently affected by Avian Pathogenic *Escherichia coli* (APEC), which is one of the most significant bacterial pathogens in poultry production. The global poultry industry suffers significant losses due to *colibacillosis*, which is contracted by APEC. It can manifest in different ways, such as airsacculitis, pericarditis, and hepatitis diseases like polyoserositis or septicaemia, as well the presence of peritonitis. Bird health, bird productivity, feed conversion efficiency, and farm profitability are all negatively impacted by these conditions.

Colibacillosis has other effects, including economic and production benefits, besides mortality losses. Reduced growth rates, higher veterinary expenses, lower egg yields, poor hatchability, and reduced carcass quality are common symptoms of infection. Additionally, APEC infections are frequently observed in conjunction with other diseases, intensifying health concerns associated with poultry production. Through the use of recent molecular biology developments, scientists have been able to sequence and analyse the genomes of significant APEC strains, providing valuable information on their susceptibility and antimicrobial resistance. This information is important for the development of effective prevention, surveillance and control measures aimed at lessening the burden of *E. coli* infections in poultry production systems (Mellata, 2022; Kabir et al, 2004) as well as for research purposes.

2.2. Antibiotic Resistance: Definitions and Implications.

One of the major global health challenges that humans and animals face is antibiotic resistance. This occurs when the bacteria are able to survive exposure to antibiotics which would normally kill or inhibit their growth. Increasingly, bacteria become less susceptible to treatment when exposed to repeated antimicrobials and are therefore harder to control. The presence of antimicrobial resistance is a significant public health concern that the World Health Organization views due to its impact on essential medicines for treating bacterial diseases. Hence, resistant infections lead to extended illness, higher healthcare expenses, and elevated mortality rates worldwide (WHO 2023; Khong et al, 2022). However,

The development of antibiotic resistance is a result of natural evolutionary processes, but it is made more severe by the inappropriate use of antimicrobial agents. The frequent use of antibiotics results in selective pressure that permits resistant bacteria to persist and prevents

the destruction of susceptible organisms. These resistant bacteria then multiply and create populations that are difficult to treat with common treatments. Additionally, resistance genes can be transferred horizontally between bacteria through horizontal gene transfer mechanisms that allow resistance traits to be rapidly transferred between species and environments. This process has been a key factor in the global increase of antimicrobial resistance observed over the past few decades (Szmolka et al, 2021; Murray & al.").

Neither pathogenic nor non-pathogenous bacteria are immune to antibiotic resistance.? Resistance genes can be stored in non-pathogenic bacteria, which may not necessarily lead to disease, but could still transfer to pathogenic organisms. The likelihood of failure in both human and veterinary treatment is raised by this transfer. The occurrence of resistant bacteria is particularly problematic in livestock production systems, where animals may act as reservoirs for resistant pathogens that can be transmitted to humans through contact with animals, food items, or environmental contamination. This is an issue. Public health authorities worldwide have prioritized monitoring resistance patterns in bacterial populations (Khong et al, 2022; Murray & al: 2021).

Antibiotic resistance is influenced by a variety of factors, including antibiotic type, dosage, treatment duration, frequency of administration, and management strategies. However, there are several important exceptions to this rule. The primary roles of antibiotics in animal agriculture are to treat disease, prevent disease and promote growth.. Excessive or inappropriate use of these drugs is a significant factor in the development resistant bacteria.? Hence, antimicrobial stewardship programmes have been introduced in several countries to encourage the effective use of antibiotics and decrease the proliferation of resistant microorganisms (Szmolka et al, 2021; WHO, 223).

2.3. The significance of Mixed Farming in Seke District.

A significant proportion of agricultural productivity and livelihoods in Seke District, Zimbabwe are attributed to mixed farming systems. These systems enable farmers to increase their income opportunities by combining crop cultivation with livestock and poultry rearing, which contributes to improving the food security of households. These farming systems rely heavily on the production of poultry, such as roadrunner and broiler chickens. Poultry is a vital source of protein, income generation, and cultural value for many rural households. Additionally, smallholder farmers can produce poultry as it requires comparatively low capital investment in contrast to other livestock industries (FAO 2022; Mapiye et al, 2023).

In Seke District, both roadrunner and broiler chickens are raised under a mixture of farming methods. Roadrunner chickens are typically reared in semi-scavenging or free-range systems, where they forage for food and are augmented with household leftovers and grain. The ability to adapt to local environmental conditions is remarkable, and these birds are coveted for their resistance to diseases and limited management. Broiler chickens are mainly raised for commercial meat production purposes and necessitate more stringent management methods, such as specially fed diets, housing facilities, and veterinary interventions (Mapiye et al, 2023; Chimonyo & al: 2022).

Women and children are crucial participants in poultry management within mixed farming systems. Poultry sales are a means of financing household expenses, educational expenses and health care costs. How does this relate to the industry? Moreover, rural families benefit from the inclusion of chickens in their diets as they provide both meat and eggs, which helps to improve their food security. Incorporating poultry into mixed farming systems can enhance soil fertility and crop productivity, which helps to optimize farm resources through the efficient use of poultry manure. Thus, the cultivation of poultry in Zimbabwe remains a vital component of sustainable rural livelihoods (FAO, 2022; Chimonyo et al, 2020).

Poultry being farmed and extensively using antimicrobial agents has led to concerns about the emergence of antibiotic-resistant bacteria in mixed farming systems. *E. coli*, which is present in the digestive tract of poultry, has become a popular indicator organism for assessing antimicrobial resistance due to its natural presence. The examination of resistance patterns in *E. coli* from different poultry production systems can provide valuable information about the dissemination of resistant genes within farming settings. These facts are important in developing effective interventions that can reduce antimicrobial resistance and protect public and animal health (Braykov et al., 2016; Khong & al., 2018).

2.4. *E. coli* that responds to antibiotics is present in poultry.

Due to the rising global demand for poultry meat, worldwide poultry production systems have experienced significant growth. The adoption of intensive and extensive production systems by farmers to meet consumer demand often involves raising different breeds of poultry in the same environment. Both methods are highly successful across industries. The use of mixed farming systems in Zimbabwe has led to the rearing of roadrunner and broiler chickens as key ingredients for ensuring food security and household income. Concerns have

been raised about the potential emergence of antibiotic-resistant bacteria due to increased poultry production and increased use of antimicrobial agents (Varga et al, 2019; FAO, 2022).

Many cases of antibiotic-resistant *E. coli* in poultry populations have been reported globally. *E. coli* colonizes the intestinal tract of birds, making it a crucial source of antimicrobial-resistant bacteria. This is particularly important in poultry production. Antibiotics are commonly used to prevent disease transmission, treat infections, and promote growth, but they have a selective effect on resistant strains. As a result, resistant *E. coli* isolates have been observed in both commercial and free-range poultry systems, but the prevalence rates may vary depending on antimicrobial exposure levels and management practices (Samun Sarker et al, 2019; Thomrongsuwannakij & Al., 2021).

The exposure to antibiotics and environmental conditions in Roadrunner chickens is a significant difference from that in broiler chicken. The use of intensive production systems is more common among broiler chickens, which have a higher incidence of antimicrobial use, while roadrunner chicken breeds typically have lower levels of antibiotic exposure due to their management systems. Despite these differences, both types of poultry are resistant to *E. coli* strains. According to Wang, the evidence suggests that antimicrobial resistance can arise through multiple mechanisms, including environmental contamination, exposure to other animals, and transfer of resistance genes between bacterial populations (Khong et al, 2022; Thomrongsuwannakij & Al., 2021).

Poultry is known to harbor antibiotic-resistant *E. coli*, which has far reaching effects on animals and on humans. The transmission of resistant bacteria to humans can occur through the handling and consumption of contaminated poultry products, direct contact with infected birds, or exposure to tainted environmental sources. Moreover, the presence of resistance genes in *E. coli* can be transferred to other pathogenic bacteria, making it more challenging to control disease. Keeping track of antimicrobial resistance in poultry production systems is crucial for food safety and public health (Murray, 2022; WHO research, 223).

2.5. Factors Influencing Antibiotic Resistance.

Multiple interacting biological, environmental, and management factors contribute to the development of antibiotic resistance.' Antibiotic usage in livestock production is a significant factor. Through selective pressure, bacterial populations are encouraged to survive and multiply by antimicrobial agents, which can result in the development of resistant strains. Gradual replacement of susceptible populations by resistant bacteria leads to a decrease in the

effectiveness of antimicrobial treatments. Both human and veterinary medicine have reported this phenomenon extensively (Braykov et al, 2016; Murray , 2022).

A poor stewardship of antimicrobials is also an important factor in the emergence of resistance. Resistance mechanisms in bacteria can be established through the use of incorrect dosages, inappropriate treatment duration and administration of antibiotics without proper diagnosis. Antibiotics are often misused and over-used, especially in developing countries where veterinary prescription is not required. The process of selecting resistant strains is accelerated, which undermines efforts to control antimicrobial resistance within animal production systems (WHO, 2023; Szmolka et al, 2020).

Environmental factors are equally crucial in the propagation of resistant bacteria.. The accumulation of antimicrobial-resistant microorganisms and resistance genes can be facilitated by the presence of animal manure, wastewater, or contaminated feed, as well as farm runoff. Soil, water bodies, wildlife populations and local communities are susceptible to the spread of resistant bacteria. Studies indicate that farming surroundings are significant conduits for the transmission of resistance between animals, people and ecosystems. Hence, the One Health approach, which acknowledges the interdependence between human, animal, and environmental health (Davis et al, 2018; WHO, 2023), is increasingly being utilized to combat antimicrobial resistance.

In addition to farm management practices, such as inadequate sanitation and biosecurity, overcrowding, and improper waste disposal contribute to resistance development. These conditions often lead to increased disease incidence and a greater need for antimicrobial treatments. Given the surge in animal protein demand caused by poultry production, effective biosecurity and antimicrobial stewardship strategies are essential to prevent the transmission of resistant bacteria populations.

2.6. *E. coli* Resistance and the Impact of Farming Practices.

Poultry production and transmission of antibiotic-resistant *E. coli* are affected by the agricultural practices involved. Antimicrobial use, environmental contamination (fungal growth factors), stocking densities, and disease challenges are all factors that can affect birds in different management approaches. Collectively these factors are responsible for selecting and maintaining resistant strains of bacteria. Therefore, variations in antimicrobial resistance patterns tend to arise among poultry produced with different production systems (Musa et al, 2020; Khong et al., 2021).

Most Roadrunner chickens are raised in free-range systems that allow them to roam outdoors and receive minimal veterinary attention. The selective pressure for resistance development is reduced in them due to their lower exposure to antibiotics compared to broiler chickens. The extensive contact with soil, water sources, wildlife and other domestic animals in which these birds are found can result in exposure to environmental reservoirs of resistant bacteria..... Accordingly, free-landing systems can still assist in the transmission and preservation of antimicrobial resistance among farmers. (Musa et al, 2020; WHO, 2023).

Unlike other poultry, broiler chickens are typically raised using intensive production systems that incorporate high stocking levels, well-managed feeding regimens (controlled intake), and regular health care. Antibiotics are frequently prescribed as treatment and for disease prevention, which creates conditions that favor the development and persistence of resistant strains of *E. coli*. Additionally, keeping a large number of birds closely at rest results in the rapid transference of resistant bacteria. Antimicrobial resistance is often higher in intensive poultry production systems than in extensive production (Khong et al, 2022; Thomrongsuwannakij et al., 2021), hence more resistant to antimbiotic agents.

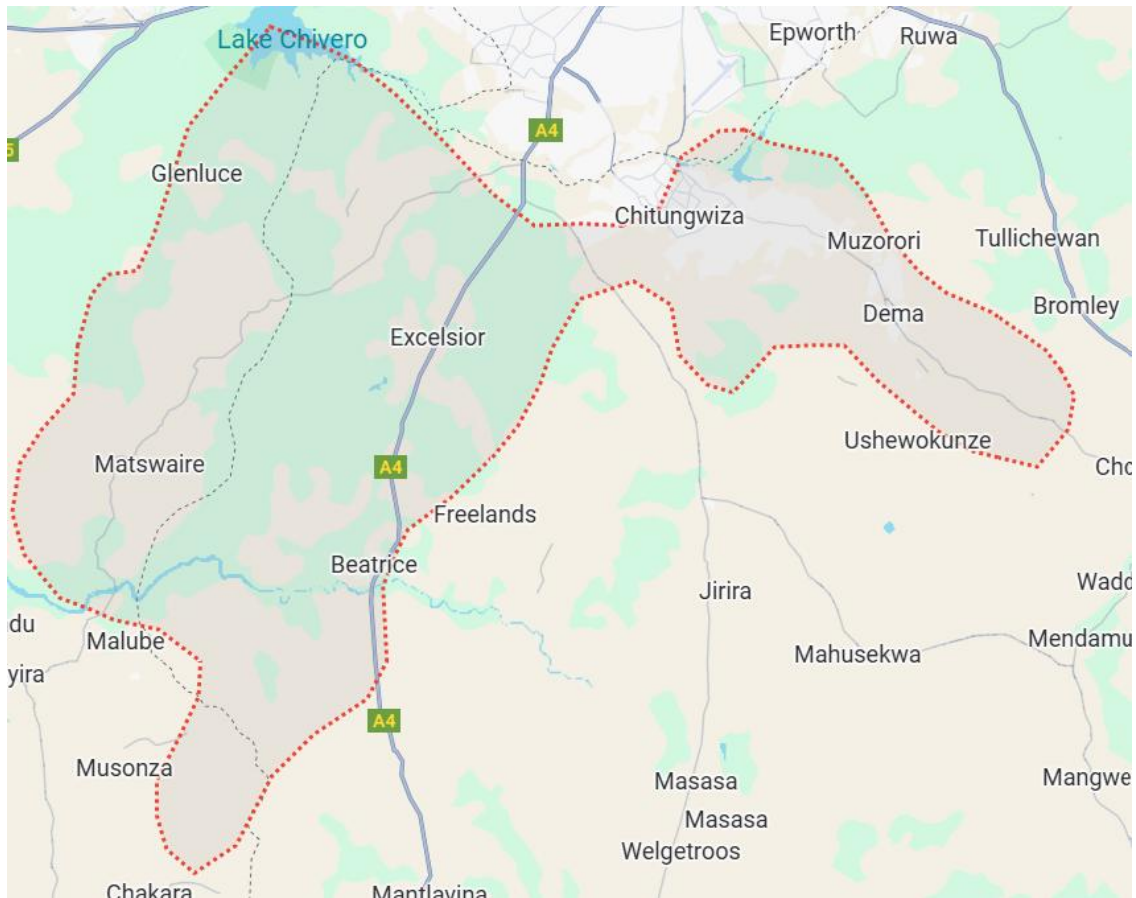
To effectively control antimicrobial resistance, it is important to understand the interdependence between farming practices and this process. A comparison between broiler and roadrunner chickens can provide insight into how management systems affect resistance development. The exchange of resistance genes and bacteria through interactions between different poultry breeds is a common occurrence in mixed farming systems, particularly in Seke District. Improved antimicrobial stewardship, better poultry health management, and reduced public health risks associated with antibiotic-resistant bacteria can be achieved through ongoing monitoring of *E. coli* resistance patterns (Musa und al, 2020; Murray et al., 2023)

CHAPTER 3

METHODOLOGY

3.1 Study site

The study was carried out around the Seke district. Figure 3.1 is a map of the study site of



Seke District.

Figure 1: Google map showing the Seke district

3.2 Study design and sample collection

A comparative laboratory-based cross-sectional study design was employed, and 150 cloacal swabs were collected from broiler ($n = 75$) and roadrunner ($n = 75$) chickens, raised on the same compound, month and year in Seke. Sample size was calculated using the sample size

calculator (<https://www.calculator.net>), at a 95% confidence level, and a prevalence of 0.97 was employed. The samples were labelled and placed in a cool box. The samples were transported to Harare Central Veterinary Laboratory for laboratory analysis and placed in a -20°C freezer at the veterinary lab, awaiting testing.

3.3 Laboratory Analysis

The laboratory methodologies were performed using the methods adopted from Markey *et al.* (2013) and Quinn *et al.* (1994). Samples were collected from clinically healthy live broiler and roadrunner chickens using sterile cloacal swabs.

3.3.1 *E. coli* Isolation

The swabs were then streaked on Blood Agar (BA) and MacConkey Agar (Mac) and incubated at 44°C for 24 hours. BA promotes the growth of a wide variety of bacteria, differentiating haemo-lytic from non-haemolytic bacteria. Mac isolate gram-negative bacteria and differentiates between lactose vs non-lactose fermenters. Microscopic morphology of the colonies was noted and compared with control pure cultures available in the laboratory.

3.3.2 Gram Stain Test

Blood agar plates with growth were selected, and different colonies on each plate were selected for Gram staining. Glass slides were labelled with the sample number and colony type. Using a flamed wire loop, sterile saline was inoculated onto the slide; this slide was used to make a suspension of the colony of interest on the side of the slide. The slide was air dried then heat fixed by passing through the flame and placed on a rack. It was then flooded with Crystal Violet dye for 60 seconds and washed with water, then flooded with Iodine solution for 60 seconds which binds the dye to the bacterial cell walls, then washed with water. Acetone was added to each slide to decolourise the bacteria with thin cell walls for 15 seconds, then washed off with water. The slides were then flooded with a counter stain Carbon Fuchsin for 60 seconds, washed with water and allowed to dry. A drop of immersion oil shall be added onto each slide and observed under light microscope's high power lens (X100). Results were recorded.

3.3.3 Biochemical Tests

Biochemical tests shall be done depending on Gram stain test results of each suspected bacterium to confirm suspected bacteria. Each suspected bacterium shall have different biochemical tests done to it, however, overall each sample shall undergo the following tests:

3.3.3.1 Citrate Test

To confirm usage of citrate as their only carbon source Bijoux bottles with Simmons' Citrate shall be labelled with the sample number and colony type. Single colonies of interest shall be picked up using a flame straight wire and stabbed into the base of Bijoux bottle media, which shall be streaked onto the slant of the media. These shall be incubated at 37°C for 24 hours. Results shall be checked against positive and negative controls available in the laboratory.

3.3.3.2 Motility Test

To confirm that organism is motile, Bijoux bottles with Motility agar shall be labelled with the sample number and colony type. Single colonies of interest shall be picked up using a flame straight wire and stabbed into the centre of the media. These shall be incubated at 37°C for 24 hours. Results shall be checked against positive and negative controls available in the laboratory.

3.3.3.3 Indole Test

To confirm that organism can split tryptophan in the media to indole, Bijoux bottles with Peptone water shall be labelled with the sample number and colony type. These shall be picked using a flame straight wire and a suspension shall be made in Peptone water. These shall be incubated at 37°C for 24 hours, then two drops of Kovac's reagent shall be added to each bottle after which results shall be checked against positive and negative controls available in the laboratory.

3.3.4 Antimicrobial Susceptibility Test of *E. coli*

The Clinical & Laboratory Standards Institute (CLSI) breakpoints were used to interpret the findings from testing antibiotic susceptibility using a Kirby-Bauer agar disc diffusion method (Quinn et al., 2002). Sterile cotton wool bud was used to swab four to five well-isolated

colonies of similar morphological type from pure culture (Quinn et al., 2002). The cotton wool swab was dipped into a bijoux bottle of peptone water (PW) to transfer his inoculum into the PW and the bacterium emulsified (Quinn et al., 2002). The inoculated PW was placed in an incubator at 37 °C for two hours until slightly visible turbidity appeared before removal (Quinn et al., 2002). Another sterile cotton bud was taken and dipped into the PW suspension, where excess fluid was expressed by pressing and rotating the swab against the inside of the bottle above fluid level, but then released it by planting it back in the upper section of the bottle after dipping it into PD. The cotton swab was removed from the bottle straight after it dipped into PW; it was then streaked in three different directions on the surface of some MHA plates so that uniformity was achieved under PPE conditions as described earlier, but only MHA plates were needed for this procedure; experimental scientist was to use sterile petri dish sealer instead of tape to cover MHA plates while being mindful not to press or damage their surface; they are not held at an angle but held parallel to worker throughout handling; only sterile forceps are used; etc.). The inoculated plates shall be allowed to stand for an unspecified time until ooze dries; then take out an antibiotic disc dispenser from the refrigerator after which it can ooze i.e., wait 15min at room temperature before application; use disc dispenser kit instructions (in this kit there's also a ruler/disc dispenser pre-packaged); after application, push down on it within 15 min, rate inhibition zone = clearance zone around antibiotic disc when testing is finished (yet placed +/- right before picking can thoroughly cleanse nostrils); keep record re: any place where anything touches or open bugs/insects around); results should either show that bacterium is susceptible or resistant to all drugs tested (ciprofloxacin, erythromycin, gentamycin, streptomycin, sulphamethoxazole, chloramphenicol, ertapenem ampicillin & penicillin)

3.4 Data analysis

All data recorded in Excel & Chi-squared testing performed to see if there is significant difference between *E.coli* prevalence & antibiotic resistance patterns among broilers & roadrunners in Seke.

CHAPTER 4

RESULTS

4.0 Introduction

Chapter Four presents the findings that resulted from the laboratory analysis of the *E. coli* isolates collected from broiler and roadrunner chickens reared in mixed farming systems in Seke District. The findings are presented according to the objectives of the study to determine the presence of *E. coli*, antibiotic-resistant isolates, the susceptibility of the antibiotic-resistant isolates, the resistance of the isolates to antibiotics, and the multidrug-resistant profiles of the isolates. The findings are presented in tables, bar graphs, and pie charts.

4.1 Isolation and Identification of *Escherichia coli* from Broiler and Roadrunner Chickens

A total of 150 cloacal swab samples were collected from live chickens that were reared in mixed farming systems in Seke District. Out of the total number of samples collected, 75 samples were collected from broiler chickens, while the remaining 75 samples were collected from roadrunner chickens.

Results from the samples revealed that the bacterium *E. coli* was successfully isolated from all the chicken samples collected from both broiler and roadrunner chickens. Thus, the isolation rate of the bacterium *E. coli* from the two types of chickens was 100%. The isolation of the bacterium was performed by culturing the samples on MacConkey agar, TBX agar, and blood agar, and identifying the cultured bacteria based on the characteristics of the colonies that grew on these culture media.

The colonies that grew on MacConkey agar were large and pink in color, indicating that the *E. coli* bacteria were lactose fermenters. The colonies that grew on TBX agar were blue-green in color. The colonies that grew on blood agar were large in size and grey in color and did not exhibit haemolysis. The bacteria that grew on these media were Gram-negative and rod-shaped. Furthermore, the *E. coli* isolates were confirmed through the positive reactions that they exhibited for the tests for motility and indole, and the negative reactions that they exhibited for the tests for citrate and urease.

Table 4.1 presents the isolation rate of *E. coli* from broiler and roadrunner chickens that were sampled for the study.

Table 4.1 Isolation rate of *Escherichia coli* from broiler and roadrunner chickens

Chicken Type	Number Sampled	Number Positive	Isolation Rate (%)
Broilers	75	75	100
Roadrunners	75	75	100

4.2 Prevalence of Antibiotic-Resistant *Escherichia coli* Isolates

Antimicrobial susceptibility testing was carried out on all isolates of *E. coli* recovered from broiler and roadrunner chickens using the Kirby-Bauer disc diffusion method. All isolates of *E. coli* from both groups of chickens exhibited resistance to at least one antibiotic. The resistant isolates contained resistance to three or more classes of antibiotics, making them classified as multidrug-resistant isolates.

The prevalence of antibiotic-resistant *E. coli* isolates was 100% from both broiler and roadrunner chickens. The prevalence of multidrug-resistant *E. coli* isolates was also 100% from both groups of chickens. There was no variation in the prevalence of antibiotic-resistant and multidrug-resistant *E. coli* between broiler and roadrunner chickens. All isolates of *E. coli* from the chickens exhibited resistance to multiple antibiotics.

Table 4.2 presents the prevalence of antibiotic-resistant and multidrug-resistant *E. coli* isolates recovered from broiler and roadrunner chickens in Seke District.

Table 4.2 Prevalence of antibiotic-resistant and multidrug-resistant *Escherichia coli* isolates

Chicken Type	Antibiotic-Resistant Isolates (%)	Multidrug-Resistant Isolates (%)
Broilers	100	100
Roadrunners	100	100

4.3 Antimicrobial Susceptibility Profiles of *Escherichia coli* Isolates from Broiler Chickens

Antimicrobial susceptibility testing of *E. coli* isolates recovered from broiler chickens revealed the presence of antibiotic resistance, intermediate susceptibility, and susceptibility to the antibiotics tested. The isolates exhibited higher levels of susceptibility to meropenem and

gentamicin as compared to the other antibiotics tested. Lower levels of susceptibility were exhibited to tetracycline, sulphamethoxazole, nalidixic acid, and erythromycin.

The levels of resistance to the antibiotics tested by the isolates of *E. coli* from broiler chickens exhibited resistance to various antibiotics. The highest levels of resistance were exhibited to erythromycin and tetracycline, followed by sulphamethoxazole and nalidixic acid. Intermediate levels of resistance were exhibited to ampicillin and streptomycin. The lowest levels of resistance were exhibited to meropenem and gentamicin.

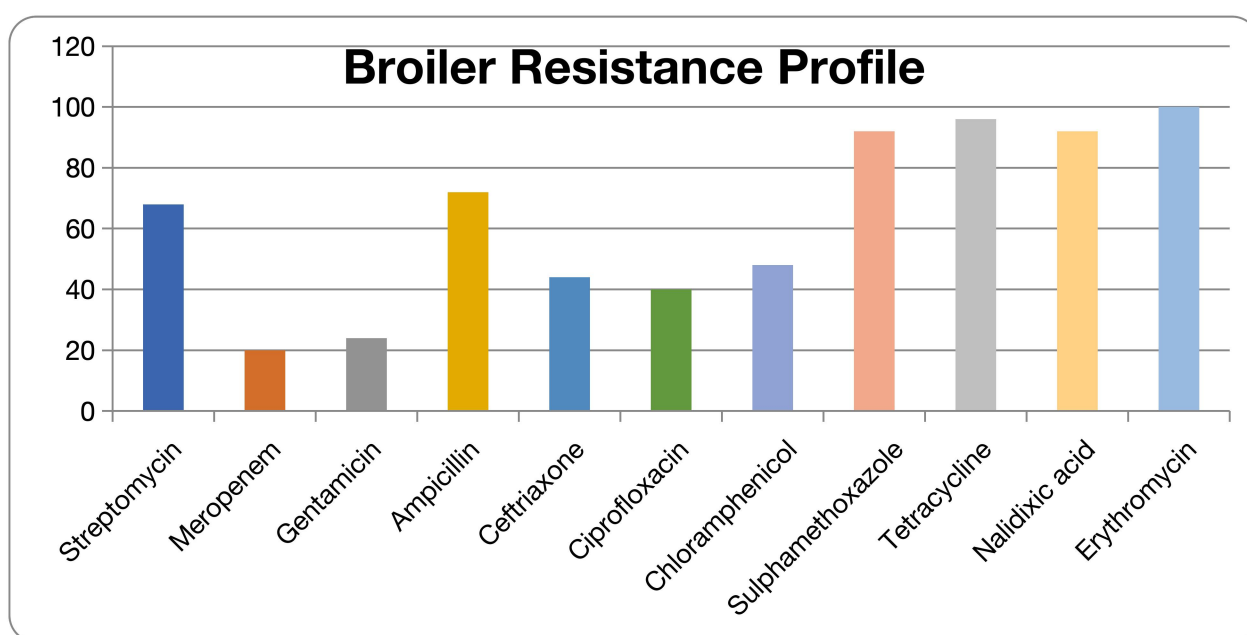


Figure 4.2 shows the antimicrobial susceptibility profiles of *Escherichia coli* isolates recovered from broiler chickens.

4.4 Antimicrobial Susceptibility Profiles of *Escherichia coli* Isolates from Roadrunner Chickens

Antimicrobial susceptibility testing of *Escherichia coli* isolates recovered from roadrunner chickens revealed varying levels of resistance and susceptibility to the tested antibiotics. Antibiotics to which roadrunner chicken isolates exhibited the highest levels of resistance included erythromycin, sulphamethoxazole, tetracycline, nalidixic acid, streptomycin, and ampicillin. The isolates exhibited lower levels of susceptibility to gentamicin, chloramphenicol, ceftriaxone, and ciprofloxacin.

The highest levels of resistance to antibiotics were exhibited by erythromycin, followed by sulphamethoxazole, tetracycline, and nalidixic acid. Resistance levels to streptomycin and ampicillin were also high. The lowest resistance levels were exhibited against meropenem. Antibiotic susceptibility levels varied amongst the tested antibiotics, with meropenem exhibiting the highest level of susceptibility amongst the roadrunner road runner isolates.

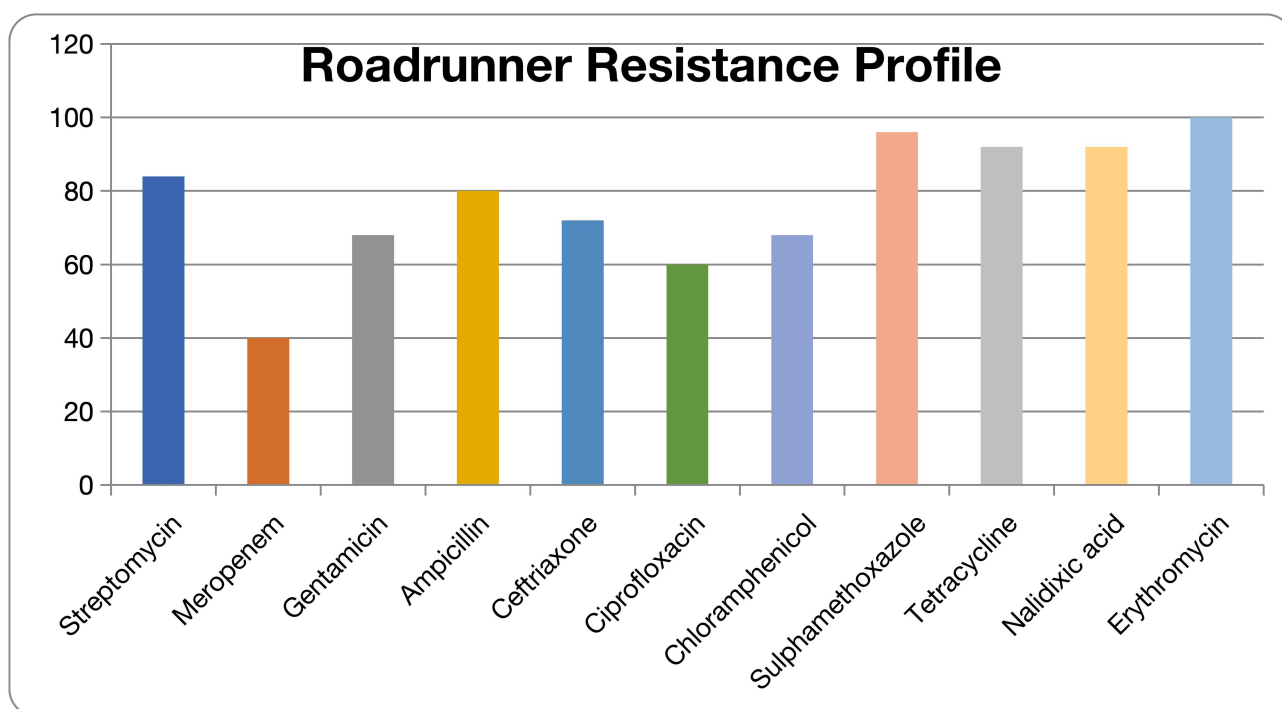


Figure 4.3 shows the antimicrobial susceptibility profiles of *Escherichia coli* isolates recovered from roadrunner chickens.

4.5 Comparative Analysis of Antimicrobial Resistance Profiles between Broiler and Roadrunner Isolates

A comparative analysis of the antimicrobial resistance profiles of the *E. coli* isolates recovered from broiler and roadrunner chickens revealed variations in the proportions of isolates that were resistant to the antibiotics that were tested. Roadrunner isolates contained higher proportions of bacteria that were resistant to the antibiotics as compared to the isolates obtained from broiler chickens. Differences in resistance rates were apparent for gentamicin, ceftriaxone, ciprofloxacin, chloramphenicol, streptomycin, and ampicillin.

High rates of resistance to the antibiotics erythromycin, tetracycline, sulphamethoxazole, and nalidixic acid were present among the isolates from both groups of chickens. Resistance to erythromycin was identified at 100% in both groups of isolates. Resistance rates for sulphamethoxazole and nalidixic acid were above 90% in each of the sampled groups. Additionally, high rates of resistance to tetracycline were present among the isolates from both groups.

A chi-square analysis of the antimicrobial resistance profiles of the two groups of isolates revealed a statistically significant difference between the groups at the 95% confidence level ($p < 0.05$). Thus, the conclusions from this statistical analysis indicate that there are significant differences in the resistance rates between roadrunner and broiler isolates, with the roadrunner isolates having higher rates of antimicrobial resistance.

Figure 4.4 presents a comparative analysis of the antimicrobial resistance profiles of the *E. coli* isolates that were obtained from broiler and roadrunner chickens.

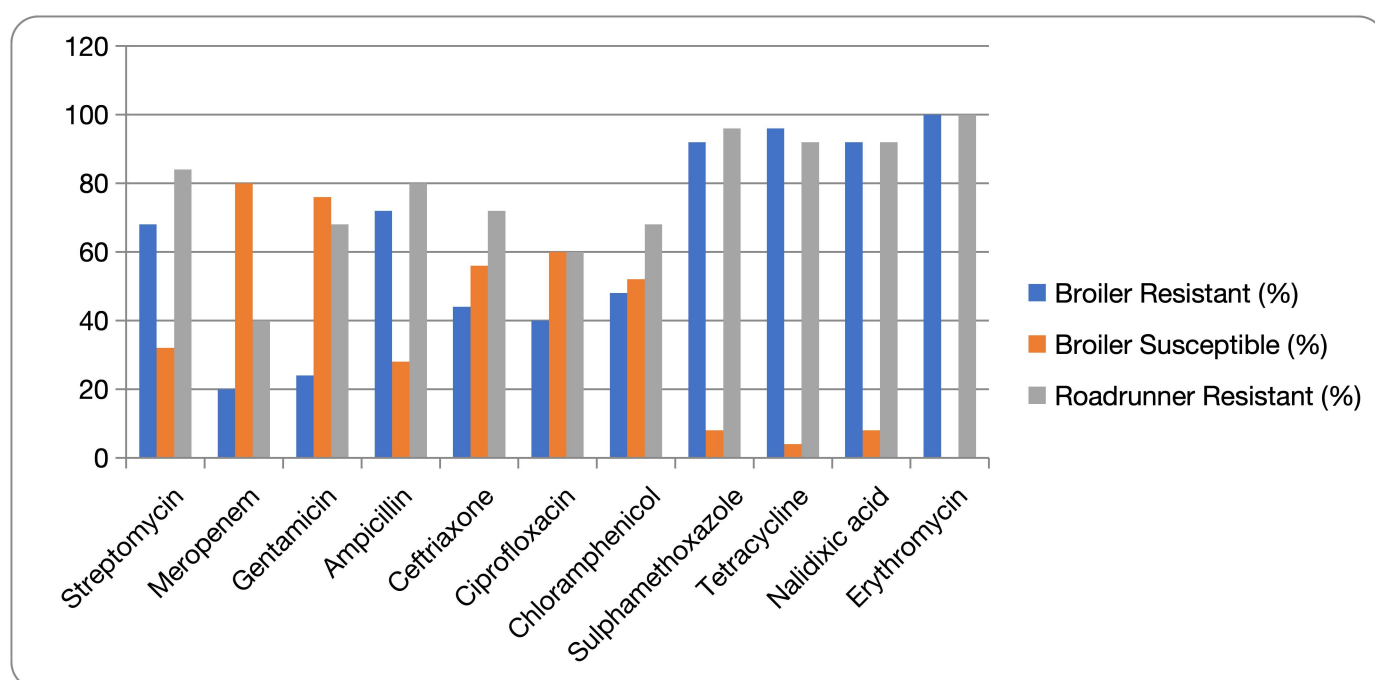


Figure 4.4 shows the comparative antimicrobial resistance profiles between broiler and roadrunner *Escherichia coli* isolates.

4.6 Statistical Analysis of Antimicrobial Resistance Profiles

The antimicrobial resistance profiles of *E. coli* isolates from broiler and roadrunner chickens were compared using the Chi-square test to determine whether there was a significant difference in the resistance proportions of the isolates against the antibiotics tested at a 95% confidence level.

The results of the Chi-square test indicated that there was a statistically significant difference in the antimicrobial resistance profiles of the isolates from broiler and roadrunner chickens ($p < 0.05$). The p-value for antimicrobial resistance profiles was calculated to be 0.00663, which shows that there is a significant difference in the proportions of antibiotic-resistant *E. coli* isolates between the two groups of chickens tested.

The results of the antimicrobial susceptibility tests show that roadrunner chickens have significantly higher proportions of antibiotic-resistant *E. coli* isolates than broiler chickens. The differences in the proportions of resistant isolates were noted for gentamicin, ceftriaxone, chloramphenicol, ciprofloxacin, and streptomycin.

Table 4.6 presents the results of the statistical analysis of the antimicrobial resistance profiles of the *E. coli* isolates recovered from broiler and roadrunner chickens.

Table 4.6 Statistical analysis of antimicrobial resistance profiles between broiler and roadrunner isolates

Statistical Test	Confidence Level	p-value	Significance
Chi-square Test	95%	0.00663	Significant

Source: SPSS

The null hypothesis that states that there is no significant difference in the antimicrobial resistance profiles between broiler and roadrunner *E. coli* isolates was rejected.

4.7 Multidrug Resistance Patterns of *E. coli* Isolates

All isolates of *E. coli* recovered from both broiler and roadrunner chickens were multidrug resistant, indicating a 100% prevalence of multidrug-resistant isolates. Multiple resistance combinations were exhibited by these isolates.

The most prevalent resistance pattern among both broiler and roadrunner *E. coli* isolates included resistance to tetracycline, nalidixic acid, erythromycin, and sulphamethoxazole. Additionally, resistance combinations that included antibiotics like streptomycin, ampicillin, ceftriaxone, chloramphenicol, and ciprofloxacin were also observed among these isolates.

While the multidrug resistance patterns were different between the broiler and roadrunner isolates, there were some similarities in the antibiotics to which they were resistant. For instance, resistance to erythromycin, tetracycline, sulphamethoxazole, and nalidixic acid was recognized in isolates from both broiler and roadrunner chickens.

Table 4.7 presents the multidrug resistance patterns of the *E. coli* isolates recovered from broiler and roadrunner chickens.

Table 4.7 Multidrug resistance patterns of *Escherichia coli* isolates

MDR Pattern	Broilers (%)	Roadrunners (%)
TET-NA-ERY-SXT	100	100
TET-NA-ERY-SXT-STR	68	84
TET-NA-ERY-SXT-AMP	72	80
TET-NA-ERY-SXT-CRO	44	72
TET-NA-ERY-SXT-CIP	40	60

4.8 Chapter Summary

High levels of resistance to antibiotics including erythromycin, tetracycline, sulphamethoxazole, and nalidixic acid were present among isolates of *E. coli* from both broiler and roadrunner chickens. However, the resistance proportions between the two types of chickens were different. The proportion of *E. coli* isolates from roadrunner chickens that

were resistant to the antibiotics was higher than the proportion of resistant *E. coli* isolates from broiler chickens. A Chi-square test was used to determine a significant difference in antimicrobial resistance between the two types of chickens at a 95% confidence level.

All isolates of *E. coli* that were tested from both types of chickens were identified as multidrug-resistant bacteria. The most common forms of multidrug resistance included resistance to the antibiotics tetracycline, nalidixic acid, erythromycin, and sulphamethoxazole.

CHAPTER 5: DISCUSSION

5.0 Introduction

The results of antimicrobial susceptibility testing of *E. coli* isolated from both broiler and roadrunner chickens revealed various results regarding the antibiotic resistance of these bacterial isolates. The bacteria was successfully isolated from all chickens of both types. Furthermore, the results of antimicrobial susceptibility testing demonstrated the various levels of resistance and susceptibility that the bacteria exhibited to the antibiotics that were tested.

5.1 Isolation and Identification of *Escherichia coli* from Broiler and Roadrunner Chickens

E. coli was successfully isolated and identified from all chickens of both broiler and roadrunner types. Thus, the rate of isolation of the bacteria was 100% from both types of

chicken samples. The identification of the bacteria from all samples indicates that this bacteria is normally found within the intestinal tracts of chickens. Recent research studies on chickens of various types and locations worldwide have also reported the same results as those obtained from this research study (Ibrahim et al., 2022). Thus, roadrunner and broiler chickens have the potential to normally contain this type of bacteria. Furthermore, the extensive presence of this bacteria among chicken samples from the Seke District indicates that this bacterium is extensively distributed among chickens within this area of study.

The characteristics of the *E. coli* strain that was isolated from the intestinal samples of the chickens were the same as those of normal strains of *E. coli*. The colonies that were produced by the bacteria were pink in color and contained positive results for the presence of lactose fermentation. Additionally, the strains produced blue-green colonies that were positive for TBX agar, were Gram negative, were positive for the tests for indole and motility, but were negative for the tests for citrate and urease. Each of these characteristics for *E. coli* are the same characteristics that have been reported by other researchers who isolated *E. coli* strains from the intestinal systems of chickens (Raza et al., 2023). Thus, these results confirm the successful identification of the bacteria that was isolated from the chicken samples.

The presence of this bacterium from all sampled chickens indicates that the findings of this research are in agreement with other research that has been conducted on chickens from other countries. These other countries have also discovered that *E. coli* is normally present within the chicken samples of both roadrunner and broiler types (Sharma et al., 2023). Furthermore, these other research studies have indicated that the environment in which the chickens are raised has no impact upon the presence of this bacteria from the chickens' intestinal systems. Thus, *E. coli* can be found within the intestinal systems from both types of chicken from the Seke District.

The ability to successfully isolate *E. coli* from the intestinal systems of all sampled chickens from the Seke District is important in recognizing antimicrobial resistance to antibiotics. This bacteria is an indicator species for antimicrobial resistance among bacteria; it is recognized by organizations like the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). Thus, the findings from this research study help to establish a basis for determining the antimicrobial resistance of the broiler and roadrunner chickens from this area. Furthermore, these findings will help to contribute to the available

information regarding antimicrobial-resistant bacteria that are found within the poultry and farming areas from which these chickens are raised.

5.2 Prevalence of Antibiotic-Resistant and Multidrug-Resistant *Escherichia coli* Isolates

The results of the antimicrobial susceptibility testing of the *E. coli* isolates from both types of chickens revealed that 100% of the strains of *E. coli* contained resistance against at least one of the antibiotics that was tested. Furthermore, all of the isolates were multidrug-resistant (MDR) strains of bacteria, as each of the strains exhibited resistance to three or more antibiotics. Thus, these findings indicate that antibiotic resistance is widespread within the chickens that were tested. These findings are in agreement with the findings of other research studies that have conducted similar analyses on *E. coli* isolates from chickens of these two types (Aworh et al., 2023). Furthermore, these findings also help to indicate that antimicrobial resistance is a widespread problem within the chicken farms in the Seke District.

The rates at which multidrug-resistant strains of *E. coli* were found in both types of chickens in this research study were higher than those other studies that have been published on antibiotic-resistant *E. coli* from other parts of Africa and the rest of the world. For instance, Nnadozie et al. conducted a research study on multidrug-resistant strains of *E. coli* from chickens in different areas within Africa, and found that only between 60% and 90% of the *E. coli* isolates from those chickens were multidrug-resistant strains (Nnadozie et al., 2024). Thus, the higher percentage from this study indicates that multidrug-resistant strains of *E. coli* are extensively circulated among the chickens in the Seke District. Furthermore, multidrug-resistant strains of bacteria are generally more difficult to control within a population than antibiotic-resistant strains of that type of bacteria. Thus, antimicrobial resistance among chickens of both of these types is firmly established within the farming systems from which they are raised.

The multidrug-resistant strains of *E. coli* were equally prevalent among both broiler and roadrunner chickens. The findings of multidrug-resistant strains of bacteria among broiler chickens indicates that the problem of antibiotic resistance among this type of chicken is not limited to only certain farming systems within the broiler chicken farms. For instance, various research studies have found that broiler chickens tend to have more exposure to antibiotics than roadrunner chickens, as they are raised for different reasons and for different uses (Moffo et al., 2023). Thus, multidrug-resistant strains of bacteria are able to spread from one group of chickens to another, even though the types of chickens are managed differently.

The presence of antibiotic-resistant and multidrug-resistant strains of *E. coli* among broiler and roadrunner chickens presents a variety of issues for both these types of chickens and for the humans that consume their meat. Antibiotic-resistant bacteria can be transmitted from the chickens to the humans that consume their meat. Furthermore, antimicrobial resistance among food-producing animals like chickens is a growing problem, as indicated by publications of organizations like the World Health Organization (WHO) (World Health Organization, 2023). Thus, these findings from this research study contribute to the knowledge that is required to implement appropriate control measures to reduce the spread of multidrug-resistant bacteria from the farms from which the chickens are raised.

5.3 Antimicrobial Susceptibility Profiles of *Escherichia coli* Isolates from Broiler Chickens

The results of antimicrobial susceptibility testing from isolates of *E. coli* from broiler chickens indicated that the bacteria from these chickens exhibited the highest levels of resistance to the antibiotics of erythromycin, tetracycline, sulphamethoxazole, and nalidixic acid. Furthermore, the levels of resistance to meropenem and gentamicin were lower among these isolates. These findings are in agreement with other published research study findings that analyzed strains of *E. coli* from broiler chickens from various locations (Elbediwi et al., 2023). The high rates of resistance to antibiotics among *E. coli* from broiler chickens is due to the extensive use of antibiotics to manage and treat the chickens from broiler farms.

Among the antibiotics to which the *E. coli* from broiler chickens exhibited the highest level of resistance was erythromycin. All strains of *E. coli* from broiler chickens exhibited resistance against erythromycin. This finding indicates that antimicrobial resistance among broiler chickens is widespread and well-established. As stated by various research studies, macrolide antibiotics like erythromycin are among the antibiotics that are extensively used to manage and care for broiler chickens (Said et al., 2024). Thus, *E. coli* from broiler chickens have become resistant to this antibiotic over time.

The results of antimicrobial susceptibility testing of strains of *E. coli* from broiler chickens revealed that high levels of resistance are exhibited against tetracycline, sulphamethoxazole, and nalidixic acid. These antibiotics are among the most common antibiotics that are prescribed to broiler chickens, as they are among the least expensive antibiotics to purchase. Thus, the high levels of resistance among strains of *E. coli* from broiler chickens against these antibiotics is due to the extensive use of these antibiotics in managing their health (Sharma et al., 2023).

Lower levels of resistance against meropenem and gentamicin were exhibited by isolates of *E. coli* from broiler chickens. Various research studies have analyzed the antimicrobial resistance among strains of bacteria from broiler chickens, and have published findings that are in agreement with these results (WHO, 2023). Thus, meropenem and gentamicin are not as extensively used on broiler chickens as are other antibiotics. However, antimicrobial resistance among these antibiotics among broiler chickens is still an issue to address in the care of these chickens.

5.4 Antimicrobial Susceptibility Profiles of *Escherichia coli* Isolates from Roadrunner Chickens

The antimicrobial susceptibility testing that was performed on isolates of *E. coli* from roadrunner chickens indicated that the strains of bacteria from these types of chickens exhibited high levels of resistance against the antibiotics that were tested. The highest rates of resistance against roadrunner chickens was exhibited against erythromycin, sulphamethoxazole, tetracycline, nalidixic acid, streptomycin, and ampicillin. Thus, resistance against antibiotics is widespread among isolates of *E. coli* from roadrunner chickens. These findings are in agreement with other research study findings that determined that the strains of *E. coli* from roadrunner chickens exhibit antimicrobial resistance (Moffo et al., 2023).

Isolates of *E. coli* from the roadrunner chickens exhibited complete resistance against the antibiotic erythromycin. All strains of *E. coli* that were isolated from roadrunner chickens exhibited resistance against erythromycin. Resistance to antibiotics like erythromycin is widespread in the various types of bacteria that are found in the environment, and particularly among strains of Gram-negative bacteria (Elbediwi et al., 2023). Furthermore, resistance against erythromycin has been found to be one of the most common forms of antimicrobial resistance among populations of *E. coli* (Aworh et al., 2023). Thus, findings from this research study support other studies that recognize the antimicrobial resistance of *E. coli* to antibiotics like erythromycin is widespread among roadrunner chickens.

Roadrunner chickens exhibited high rates of resistance against the antibiotics tetracycline, sulphamethoxazole, nalidixic acid, and ampicillin. These antibiotics are among the most common antibiotics that are used upon roadrunner chickens, as they are among the least expensive antibiotics to purchase to treat the various illnesses of the roadrunner chickens in their pens (Said et al., 2024). Thus, the high rates of antimicrobial resistance against these antibiotics among the populations of *E. coli* from roadrunner chickens indicate that

antimicrobial resistance against these antibiotics is well-established within the farms raising roadrunner chickens.

While the levels of antimicrobial resistance from the roadrunner chickens was high against the antibiotics that were tested, the levels of resistance against meropenem were relatively lower to those other antibiotics. Thus, findings from this research study among roadrunner chickens are in agreement with other research studies that determine that carbapenem antibiotics like meropenem are more effective than other antibiotics at clearing infections from *E. coli* strains from roadrunner chickens farms (World Health Organization, 2023). Thus, there is relatively little antimicrobial resistance against meropenem among the *E. coli* populations from roadrunner chickens. Yet, antimicrobial resistance against meropenem is still an issue to manage among roadrunner chickens, as meropenem is an antibiotic that is used to treat the most serious infections in these chickens (World Health Organization, 2023).

5.5 Comparative Analysis of Antimicrobial Resistance Profiles between Broiler and Roadrunner Isolates

The antimicrobial susceptibility profiles for both broiler and roadrunner types of chickens contains differences in the resistance profiles of the bacteria from their samples. More specifically, the *E. coli* isolates from roadrunner chickens contain higher levels of resistance against antibiotics like gentamicin, ceftriaxone, ciprofloxacin, chloramphenicol, streptomycin, and ampicillin. These findings indicate that despite the high instances of resistance against antibiotics among isolates of *E. coli* from these two types of chickens, there are differences in the resistance among the isolates of bacteria from each type of chicken. These findings are in agreement with other published research studies on antimicrobial resistance among isolates of *E. coli* from both types of chickens (Aworh et al., 2023).

Roadrunner chickens exhibit high levels of resistance against gentamicin, ceftriaxone, ciprofloxacin, and chloramphenicol. The reason for such high levels of resistance among roadrunner chickens may be due to the exposure of these chickens to a broader variety of bacteria from their environment. According to other research studies, exposure to other strains of bacteria in the environment of roadrunner chickens is one of the factors that contributes to the high levels of antimicrobial resistance among those bacteria strains (Moffo et al., 2023). Thus, the exposure of roadrunner chickens to other types of bacteria from their environment may be one of the reasons for the high levels of antimicrobial resistance among the strains of *E. coli* from roadrunner chickens.

Despite the differences between the antimicrobial susceptibility profiles of the bacteria strains from these two types of chickens, both types of chickens contain very high levels of resistance against antibiotics like erythromycin, tetracycline, sulphamethoxazole, and nalidixic acid. More specifically, the resistance against erythromycin is 100% among isolates from both types of chickens. Furthermore, the resistance among isolates against the other three antibiotics is above 90%. These findings from both broiler and roadrunner chickens is again in agreement with other research studies that analyzed antimicrobial resistance profiles of *E. coli* strains from each of these types of chickens (Elbediwi et al., 2023). Thus, antimicrobial resistance among these antibiotics is widespread among *E. coli* strains, and it has become well-established within the antimicrobial management of broiler and roadrunner chickens in different parts of the world.

5.6 Comparative Analysis of Antimicrobial Resistance Profiles between Broiler and Roadrunner Isolates

The antimicrobial resistance profiles of the *E. coli* isolates from broiler and roadrunner chickens contain differences between the two types of chickens. The results of antimicrobial susceptibility testing indicate that the isolates of *E. coli* from roadrunner chickens contain higher levels of resistance against antibiotics like gentamicin, ceftriaxone, ciprofloxacin, chloramphenicol, streptomycin, and ampicillin. Thus, these findings indicate that despite the high instances of resistance against antibiotics among isolates of *E. coli* from both types of chickens, each type of chicken contains differences in the resistance of its isolated bacteria strains. Similar findings have been made from other research studies on both broiler and roadrunner chickens from different parts of the world (Aworh et al., 2023).

The high levels of resistance among the isolates of *E. coli* from roadrunner chickens indicates that the roadrunner chickens may have high levels of exposure to other types of bacteria from their environment. Roadrunner chickens are exposed to more of their environment than broiler chickens. Research studies have determined that the exposure of chickens to other bacteria from their environment is one of the factors that explains the high levels of antimicrobial resistance from those bacteria strains (Moffo et al., 2023). Thus, exposure to other antimicrobial-resistant bacteria in the environment of roadrunner chickens is one of the reasons for the differences in the antimicrobial resistance profiles between the *E. coli* strains from roadrunner and broiler chickens.

Despite the differences in the antimicrobial resistance profiles between the two types of chickens, both types of chickens have high levels of resistance against antibiotics like

erythromycin, tetracycline, sulphamethoxazole, and nalidixic acid. More specifically, 100% of the isolates of *E. coli* from both types of chickens are resistant against erythromycin. Furthermore, the resistance of *E. coli* isolates against the other three antibiotics is above 90% of the isolates from both types of chickens. Similar findings have been made from broiler and roadrunner chickens from various locations within the world (Elbediwi et al., 2023). Results from a chi-square test of the antimicrobial resistance profiles of *E. coli* from broiler and roadrunner chickens indicate a statistically significant difference between the two types of chickens ($p = 0.00663$). This p-value of less than 0.05 indicates that the null hypothesis of the research study is to be rejected. Thus, these significant differences in the antimicrobial resistance profiles of *E. coli* from broiler and roadrunner chickens indicates that the type of chicken has an impact upon the antimicrobial resistance of its *E. coli* strains. Similar findings have been made from various studies of antimicrobial resistance profiles of *E. coli* strains from broiler and roadrunner chickens within different parts of the world (Said et al., 2024).

5.6 Multidrug Resistance Patterns of *Escherichia coli* Isolates

The most important finding of this study is the presence of multidrug resistance among all the *E. coli* isolates that were tested from both broiler and roadrunner chickens. All of the isolates displayed multidrug resistance as they were resistant to three or more classes of antibiotics. Multidrug-resistant strains of *E. coli* are widespread within the poultry farms that were investigated in the Seke District. Similar findings have been made from other studies concerning the multidrug resistance of strains of *E. coli* that were isolated from broiler and roadrunner chickens (Nnadozie et al., 2024).

The multidrug resistance of strains of *E. coli* presents a great deal of concern within the management of the broiler and roadrunner chickens in which these strains are present. The multidrug resistance among bacteria strains limits the number of antimicrobial agents that may be used to treat any bacterial infections that may threaten the health of the farm chickens. Among the most common patterns of multidrug resistance of strains of *E. coli* that have been isolated from broiler and roadrunner chickens is resistance to the antibiotics tetracycline, nalidixic acid, erythromycin, and sulphamethoxazole. Similar findings have also been made from different regions of the world, including Africa, Asia, and Europe (Elbediwi et al., 2023). The multidrug resistance among these strains of bacteria presents a challenge to the health of the farm chickens.

In addition to multidrug resistance to tetracycline, nalidixic acid, erythromycin, and sulphamethoxazole, the strains of *E. coli* that were isolated from both types of chickens were

also multidrug resistant to streptomycin, ampicillin, ceftriaxone, chloramphenicol, and ciprofloxacin. The multidrug resistance among these strains presents a challenge in the attempt to treat any infections that may develop within the farm chickens. Recent studies have shown that the multidrug resistance among these strains of *E. coli* is the result of the presence of mobile genetic elements within their genomes that allow for the strains to exchange genetic material that determines their antibiotic resistance (Aworh et al., 2023).

The prevalence of multidrug resistance among strains of *E. coli* that are isolated from broiler and roadrunner chickens in this study surpasses the prevalence that has been noted in most previous studies regarding multidrug resistant strains of *E. coli* from chickens. In various parts of the world, studies have noted that below 100% of strains of *E. coli* from chickens are multidrug resistant (Moffo et al., 2023). Therefore, multidrug resistant strains of *E. coli* are widespread within the poultry farms in the Seke District.

As multidrug resistant strains of *E. coli* are present in farm chickens, these strains can enter the human population through the consumption of chicken meat. Thus, these strains pose a threat to the health of the human population in which they enter, as well. Based on these findings, the recommendations of international organizations should be followed to counteract the problem of antimicrobial resistance (World Health Organization, 2023).

5.7 Chapter Summary

Within this chapter, the findings of this research study regarding the antimicrobial susceptibility of strains of *E. coli* isolated from broiler and roadrunner chickens in the Seke District are presented. All of the strains of bacteria that were isolated from both types of chickens successfully contained the *E. coli* bacteria. Furthermore, the results of testing for multidrug resistance indicate that there are multidrug resistant strains of *E. coli* strains within the farms in which the samples were collected. These findings are similar to those of previous studies conducted with strains of *E. coli* isolated from broiler and roadrunner chickens from various parts of the world (Nnadozie et al., 2024).

Antimicrobial susceptibility testing revealed that among the two types of chickens in which the strains were isolated, resistance to the antibiotics erythromycin, tetracycline, sulphamethoxazole, and nalidixic acid were high among the strains of *E. coli*. However, multidrug resistance to meropenem and gentamicin among these strains was low. These findings are similar to other studies conducted around the world regarding multidrug resistance among strains of *E. coli* isolated from chickens (Elbediwi et al., 2023).

A comparative analysis of the antimicrobial susceptibility among the strains from both types of chickens revealed significant differences in the resistance profiles of their strains of *E. coli*. The strains of *E. coli* isolated from roadrunner chickens had higher levels of resistance to the antibiotics than those isolated from broiler chickens. Furthermore, statistical analysis of these findings revealed significant differences in their resistance profiles ($p = 0.00663$). Thus, the null hypothesis was rejected.

Additionally, multidrug resistant strains of *E. coli* were widespread among the broiler and roadrunner chickens that were sampled. Furthermore, multidrug resistance to antibiotics tetracycline, nalidixic acid, erythromycin, and sulphamethoxazole were the most prevalent among the strains of *E. coli* isolated from both types of chickens in the Seke District. The presence of multidrug resistance among these strains of bacteria poses a challenge to the health of the farm chickens in which they are found.

In general, then, the findings of this research study indicate that multidrug resistant strains of *E. coli* are a problem among the broiler and roadrunner chickens in the Seke District. Thus, these findings can provide valuable information regarding antimicrobial resistance in the chickens in Zimbabwe.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The objectives of this research study were to isolate and identify the bacteria present in broiler and roadrunner chickens in the Seke district of Zimbabwe, test the antimicrobial susceptibility of those bacteria strains, and draw conclusions regarding multidrug resistance among those strains.

The results of the isolation and identification of *E. coli* strains from the chickens in the Seke district indicate that the bacteria was successfully contained within all samples of both broiler and roadrunner chickens. Thus, each group of chickens contained 100% successful isolation

of the *E. coli* bacteria. Furthermore, the identification of the bacteria confirms that the strains of *E. coli* were successfully isolated from the chickens. Additionally, the results of antimicrobial susceptibility testing reveal that multidrug-resistant strains of *E. coli* were highly prevalent among both types of chickens. All strains of *E. coli* that were isolated contained multidrug resistance to at least one antibiotic. Furthermore, all strains had multidrug resistance as they were resistant to three or more classes of antimicrobial agents. These findings confirm the presence of multidrug resistance among strains of *E. coli* that were isolated from both types of chickens in the Seke district.

Antimicrobial susceptibility testing revealed that the strains of *E. coli* that were isolated from the chickens were highly resistant to the antibiotics erythromycin, tetracycline, sulphamethoxazole, and nalidixic acid. However, they exhibited less resistance to meropenem and gentamicin. These findings are in agreement with other research studies that have been performed around the world regarding the antimicrobial resistance of strains of *E. coli* that were isolated from chickens (Elbediwi et al., 2023).

A comparative analysis of the antimicrobial susceptibility of strains of *E. coli* from broiler and roadrunner chickens revealed significant differences among the two groups of strains. Roadrunner strains exhibited higher levels of resistance among the antibiotics tested compared to those of broiler chickens. These findings are statistically significant as p-values confirm these differences (.00663). Thus, the null hypothesis is rejected.

Another important finding of this research study is that multidrug-resistant strains of *E. coli* were widespread among the chickens of both types that were sampled. Furthermore, multidrug-resistant strains of *E. coli* exhibited the most resistance to tetracycline, nalidixic acid, erythromycin, and sulphamethoxazole. Additionally, multidrug resistance to other antibiotics, such as streptomycin, ampicillin, ceftriaxone, chloramphenicol, and ciprofloxacin were also present among the strains of *E. coli* that were isolated from both types of chickens. Thus, multidrug-resistant strains of bacteria are widespread among the chickens in the farms in which the samples were collected.

Overall, then, the multidrug-resistant strains of *E. coli* were found to be widespread among the broiler and roadrunner chickens in the Seke district. These findings help to demonstrate that the chickens within the area in which the samples were collected are a reservoir of multidrug-resistant and antimicrobial-resistant strains of *E. coli* bacteria.

6.2 Recommendations

In response to the findings of this research study, there are a variety of recommendations that may be made regarding the management of multidrug-resistant strains of *E. coli* among the broiler and roadrunner chickens in the Seke district:

1. Antimicrobial stewardship should be practiced within the poultry farms in the area. Only the amount of antimicrobial agents that are prescribed by the veterinarian for the treatment of the farm chickens should be used within those farms.
2. The use antimicrobial agents on the farms should be supervised by veterinarians in order to ensure that each type of chicken is treated with the appropriate type and dosage of antimicrobial agents.
3. The biosecurity of the poultry farms within the area should be improved to reduce the spread of multidrug-resistant strains of *E. coli* among the different flocks of farm chickens within the area.
4. Programs should be established within the poultry farms to monitor the antimicrobial resistance of strains of *E. coli* among the farm chickens.
5. The farmers within the area should be trained regarding the antimicrobial resistance of *E. coli* and the importance of the responsible use of antimicrobial agents to treat farm chickens.
6. A One Health initiative should be undertaken within the area by the government and other stakeholders to control the antimicrobial resistance among farm chickens (World Health Organization, 2023).
7. Alternative approaches to managing the health of the farm chickens should be encouraged and adopted by the farmers in the area.
8. Future research can be undertaken regarding the determinants of antimicrobial resistance among *E. coli* strains isolated from chickens in the Seke district.

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APPENDICES

Appendix A Appearance of *E. coli* on different media

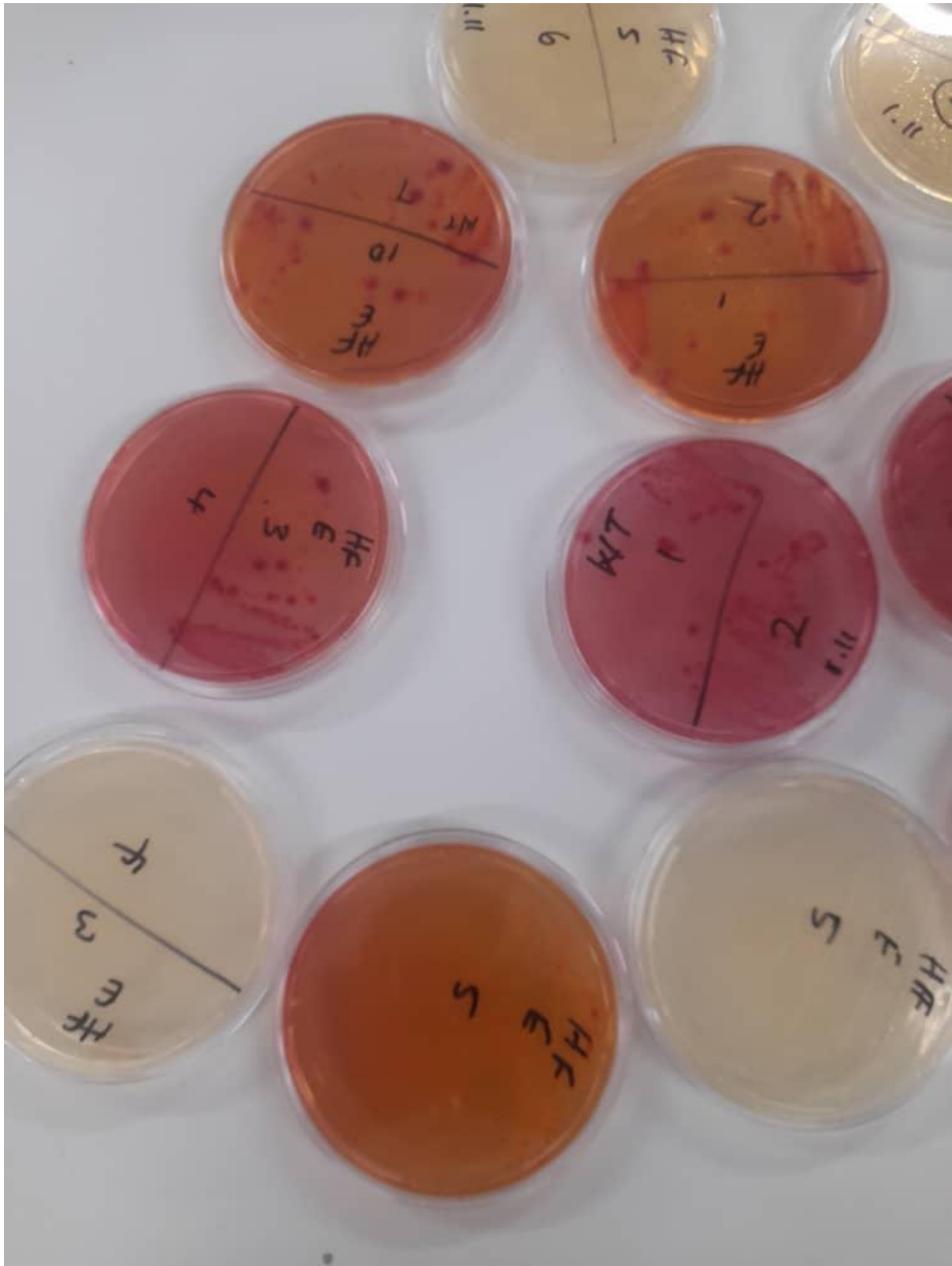


Figure A1 appearance of *E. coli* on MacConkey agar

Figure A2 Appearance of *E. coli* on TBX agar.



APPENDIX B

Antimicrobial Susceptibility Testing Results for *Escherichia coli* Isolates

Table B1. Antimicrobial susceptibility testing results of *Escherichia coli* isolates recovered from broiler and roadrunner chickens

Ani mal Ide ntit y	Cu ltu re Re sul t	Strep tomy cin	Mer open em	Gen tami cin	Am pici llin	Ceft riax one	Cipr oflox acin	Chlora mphen icol	Sulpha methox azole	Tetr acyc line	Nal idi xic Aci d	Eryth romy cin
broi lers 1	<i>E. coli</i> <i>i</i>	R	S	S	I	I	R	S	R	R	R	R
broi lers 10	<i>E. coli</i> <i>i</i>	S	S	I	S	I	S	R	R	R	R	R
broi lers 11	<i>E. coli</i> <i>i</i>	S	S	I	S	I	R	R	R	R	R	R
broi lers 12	<i>E. coli</i> <i>i</i>	S	S	S	S	I	R	R	R	R	R	R
broi lers 13	<i>E. coli</i> <i>i</i>	S	S	S	S	I	I	R	R	R	R	R
broi lers 14	<i>E. coli</i> <i>i</i>	I	R	I	I	R	I	R	S	R	R	S
broi lers 15	<i>E. coli</i> <i>i</i>	R	S	S	R	R	R	S	R	R	R	R
broi	<i>E.</i>	S	I	R	R	R	I	S	R	R	R	R

lers	<i>col</i>											
16	<i>i</i>											
broi	<i>E.</i>	R	I	S	R	R	R	R	R	R	R	R
lers	<i>col</i>											
17	<i>i</i>											
broi	<i>E.</i>	R	S	S	R	R	R	R	R	R	R	R
lers	<i>col</i>											
18	<i>i</i>											
broi	<i>E.</i>	R	S	S	R	R	R	R	R	R	R	R
lers	<i>col</i>											
19	<i>i</i>											
broi	<i>E.</i>	R	S	S	R	I	S	S	S	R	R	R
lers	<i>col</i>											
2	<i>i</i>											
broi	<i>E.</i>	R	I	S	R	R	I	R	R	R	R	R
lers	<i>col</i>											
20	<i>i</i>											
broi	<i>E.</i>	R	S	S	R	R	I	R	R	R	R	R
lers	<i>col</i>											
21	<i>i</i>											
broi	<i>E.</i>	R	S	R	R	R	I	S	R	R	R	S
lers	<i>col</i>											
22	<i>i</i>											
broi	<i>E.</i>	R	S	R	S	R	R	S	R	R	R	R
lers	<i>col</i>											
23	<i>i</i>											
broi	<i>E.</i>	R	S	S	S	R	R	S	R	R	R	R
lers	<i>col</i>											
24	<i>i</i>											

broi	<i>E.</i>	R	S	I	S	R	R	R	R	R	R	R
lers	<i>col</i>											
25	<i>i</i>											