ASSESSING KNOWLEDGE, ATTITUDES, AND PRACTICES OF BROILER CHICKEN FARMERS ON ANTIBIOTIC USE AND RESIDUES IN BLANTYRE, MALAWI

MASTER OF SCIENCE IN ENVIRONMENTAL HEALTH DISSERTATION

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UNIVERSITY OF MALAWI

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MASTER OF SCIENCE IN ENVIRONMENTAL HEALTH DISSERTATION

By

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(BSC. ANS)

A Dissertation Submitted to the Department Environmental Health, Faculty of Applied Sciences, in Partial Fulfilment of the Requirements for the Award of a Degree of Master of Science in Environmental Health

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DECLARATION

I, **Amon Abraham**, hereby declare that this thesis entitled "**assessing knowledge, attitudes, and practices of broiler chicken farmers on antibiotic use and residues in Blantyre, Malawi"** is my own original work and has not been submitted to any other institution for similar purposes. It has not been submitted for any degree or examination to any university or college.

Signature: …………………………………………

Date: ………………………………………….

WORD COUNT 21,326

CERTIFICATE OF APPROVAL

We, the undersigned, hereby certify that we have read and approved for acceptance by the University of Malawi, this thesis entitled **"assessing knowledge, attitudes, and practices of broiler chicken farmers on antibiotic use and residues in Blantyre, Malawi".**

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DEDICATION

I dedicate this to a family that has loved and supported me throughout my life and career. My mother Eunice teaches me to persist and never settle; not even when the going is good. To my brother, Ernest, who always knows when to be firm; and always reminds me that I can do better but also knows when to encourage. Lastly am so thankful to my lovely girlfriend Shira Hillary Likongwe for her support and encouragement.

ABSTRACT

Antibiotics are used in livestock farming for treatment, disease prevention, growth promotion, preventing outbreaks, and controlling disease outbreaks. Antibiotic use has become more common among farmers due to the high demand for poultry products. Across cross-sectional quantitative survey across ten urban and peri-urban areas of Blantyre was undertaken amongst 50 small, medium, and large-scale broiler farmers. The study's objectives were to assess farmers' knowledge, attitude, and practices (KAP) on antibiotic use in broiler production, analyze antibiotic residues in chicken meat samples, and examine correlations between farmers' KAP on the use of antibiotics and antibiotics residue accumulation. 40 meat samples were collected among the farmers and analyzed for ciprofloxacin, sulfamethoxazole, amoxicillin, and trimethoprim residues. To evaluate the farmer's KAP on antibiotic use a pretested and structured questionnaire was used to collect data through face-to-face interviews. Data were summarized using descriptive statistics, and chi-square tests were used to determine the association. Statistical significance was determined using a p-value of less than 0.05. All the farmers administered antibiotics to their flocks, and 46% (23/50) of them claimed to use antibiotics to treat any disease. 62% (31/50) of farmers have never attended any training on antibiotic use. 58% (29/50) of farmers had never heard of AMR. 66% (33/50) follow recommendations from other farmers. 82% (41/50) of farmers adhere to withdrawal periods, and 38% of farmers reported using antibiotics for a longer period in sick chickens. The High-Performance Liquid Chromatography (HPLC) screening showed that amoxicillin represents the highest residue percentage (92.5%), trimethoprim (32.5%), ciprofloxacin (10%), and least sulfamethoxazole (2.5%). Contaminated samples were higher, lower, and least in commercial, medium, and small-scale broiler management systems respectively. Amoxicillin residue ranged from 0.058926 - 5.138996 μg/kg, trimethoprim was 0.33909 – 3.25580 μg/g, sulfamethoxazole was 0.058926 - 5.138996 μg/g and ciprofloxacin was ND - 0.956627 μg/g. These residue levels were below the Maximum Residue Limit (MRL) recommended limit according to the European Union (EC). This study revealed that chicken samples collected from broiler farmers from urban and peri-urban areas of Blantyre contain antibiotic residues. Therefore, it is important to monitor antibiotic use in broiler chickens to ensure that residue levels remain below the Maximum Residue Limit (MRL) and to prevent the potential development of antibiotic resistance.

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ABBREVIATIONS AND ACRONYMS

AM	Antimicrobial
AMOX	Amoxicillin
AMR	Antimicrobial Resistance
AMU	Antimicrobial Use
CIP	Ciprofloxacin
CK	Chicken kidney
CL	Chicken liver
DAHLD	Department of Animal Health and Livestock Development
DNA	Deoxyribonucleic acid
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
GAP	Global action plan on antibiotic resistance
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-performance liquid chromatography
KAP	Knowledge, Altitude, and Practices
LC	Liquid chromatography
MALICO	Malawi Library and Information Consortium
MRL	The maximum residue limit
MS	Mass spectrum
MUBAS	Malawi University of Business and Applied Sciences
NCST	National Commission for Science and Technology

OIE	Office International des Epizooties
RNA	Ribonucleic acid
RSDV	Relative standard deviation
SDV	Standard deviation
SULF	Sulfamethoxazole
TRIM	Trimethoprim
U.S.	United States
UK	United Kingdom
WHO	World Health Organization
WS	Water soluble

CHAPTER 1: INTRODUCTION

There are several reasons why antibiotics are used in animal production. This includes therapeutic reasons which involve treating sick animals, Prophylaxis reasons which is the giving of antibiotics to animals at a high risk of infection who are otherwise healthy and in cases where no other animals in the herd or flock are afflicted with a disease, and metaphylaxis reasons which is the treating of a group of animals that don't show any symptoms of illness but are in close proximity to other animals that exhibit symptoms of an infectious condition. Additionally, it is commonly used for growth promotion (Gemeda et al., 2020; Tang et al., 2017). Antibiotics mainly help to support health and improve productivity in livestock through prevention, treatment of diseases, and weight gain (Van Boeckel et al., 2015). Unlike in developed countries, antibiotic use in low-income developing countries in food animals is still unregulated and unmonitored. Antibiotic resistance increases because of this abuse of antimicrobial medications. Globally 25 million pounds (11, 339, 809 kgs) of antimicrobial use for non-therapeutic purposes have been reported in animal food production (chicken, pig, and cow production) which is an alarming use as compared to 3 million pounds of antimicrobials reported to have been used in human medicine (World Health Organization, 2017). Lack of training on the usage of antibiotics and resistance, low access to information on antibiotics, and over-dependence on antibiotics are the contributing factors to the increase of the problem. Antibiotics are being utilized more and more frequently as growth stimulants on a global scale as a result of the expanding human population and the high demand for animal products (Van Boeckel et al., 2015). Overuse of antibiotics in animals can cause resistant strains of pathogens in humans (Burki, 2018). In accordance with WHO recommendations from 2017, the use of antibiotics as growth enhancers should end and antibiotics should only be used for disease treatment if a disease has been identified in other animals in the same group, only healthy animals should be given antibiotics to prevent it (World Health Organization, 2017). In Malawi, the use of antibiotics among chicken producers is suspected to be high amongst chicken producers due to an increase in demand for chicken products. Even though this is the case in Malawi, there is little evidence about the misuse of antibiotics let alone the quantity of consumable antibiotic residues available in the broiler. It is therefore against this background that the current study evaluates farmers' knowledge, attitudes, and practices on the use of antibiotics and also characterizes the antibiotic residues available in broiler chickens.

1.1 Problem statement

Antimicrobial resistance (AMR) is a growing global concern that poses a significant threat to public health. In Malawi, there is a concerning resistance to multiple first-line antibiotics, especially those used to treat bacteremia and other life-threatening conditions (Makoka et al., 2012). According to a study by the Institute for Health Metrics and Evaluation, 1.27 million deaths were directly caused by drug-resistant bacteria in 2019, with the highest death rate at all ages in western sub-Saharan Africa and the lowest in Australasia. Specifically in Malawi, there were 3,600 deaths attributable to AMR and 15,700 deaths associated with AMR. Additionally, Malawi had the 23rd highest age-standardized mortality rate per 100,000 population associated with AMR across 204 countries (Murray et al., 2022 and The Institute for Health Metrics and Evaluation, 2023). In Malawi, the burden of antimicrobial resistance (AMR) is further exacerbated by factors such as misuse and overuse of antimicrobials, lack of access to clean water, sanitation, and hygiene for both humans and animals, poor infection and disease prevention and control in healthcare facilities and farms, poor access to quality, affordable medicines, vaccines, and diagnostics, and lack of awareness, knowledge, enforcement, and legislation (MacPherson et al., 2023).

Broiler chicken is one of the dominant consumed chicken products that Malawi depends on as it supplies an affordable protein source to the population. However, poor animal husbandry practices in chicken production pose a threat to the human population. One of the common poor husbandry practices in chicken production is the heavy and inappropriate use of antibiotics which pose a great danger to the population. Ideally, chicken farmers are supposed to undergo intensive training in chicken production. Farmers are supposed to be taught and understand the available antibiotics, antibiotic use, and the serious impacts of antibiotic inappropriate use. Experience has shown that most of the farmers in Malawi use antibiotics without the necessary prescription from trained veterinary staff as most of them depend on information from veterinary shop staff (these staff are mostly not well-trained veterinary personnel). Some depend on information passed from other chicken farmers, and earlier farming experience while others depend on internet sources (Chah et al., 2022; Thi Huong-Anh et al., 2020). Consequently, this use of informal information leads to the abuse of antibiotics administered to chickens. This causes residue from antibiotics to build up in the chicken products, which eventually endangers the population of people who eat animal products. Previous research backs up the idea that the improper and excessive use of antibiotics on food-producing animals constitutes hazards to the human population since antibiotic residues are passed from animals to people, making people resistant to antibacterial drugs (Tufa et al., 2018).

Consequently, this study's goal was to determine how farmers' KAP impacts the use of antibiotics and its associated factors on broiler chickens in peri-urban and urban areas of Blantyre city.

1.2 Justification

Antibiotic resistance in human beings is now becoming a worldwide concern and meat consumption is one of the ways that human beings consume antibiotics (Spellberg et al., 2016; World Health Organization, 2020). This research is necessary to establish data on the extent of antibiotics found in the broiler meat consumed by Malawians, specifically in Malawi. This will provide the necessary information to the law enforcers, including government regulatory agencies such as the Ministry of Agriculture, the Malawi Bureau of Standards, and the Department of Animal Health and Livestock Development. These entities are responsible for formulating and enforcing regulations on antibiotic use in food animals.

Beyond law enforcers and researchers, this study has potential direct and indirect beneficiaries. Poultry farmers will directly benefit from the findings, as they offer guidance on responsible antibiotic use, influencing practices in chicken production. Consumers, as members of the public, will indirectly gain from heightened awareness about potential risks associated with antibiotic residues in broiler meat. This awareness empowers individuals to make informed dietary choices, contributing to improved food safety practices. Furthermore, policymakers can use the research to inform evidence-based policies and regulations related to antibiotic use in poultry production, contributing to broader public health and food safety objectives. Finally, the study’s findings will act as a benchmark or a base for other researchers to probe more into antibiotic use and antimicrobial resistance making significant contributions to the growing body of knowledge on antibiotic use and antimicrobial resistance, particularly in low-resource settings like Malawi.

1.3 Objectives

Broad objective

This project aimed to investigate the influence of farmers' Knowledge, Attitude, and Practices (KAP) on antibiotic use and associated factors in broiler chickens across peri-urban and urban areas of Blantyre city.

Specific objectives

The specific objectives are to,

I. assess farmers’ knowledge, attitudes, and practices on the use of antibiotics in chicken production.

II. characterize the amoxicillin, Trimethoprim, sulfamethoxazole, and ciprofloxacin antibiotic residues available in chicken meat samples.

III. examine the correlation between farmers' KAP on antibiotic use and the presence of antibiotic residues in the chicken samples.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview of chicken production in Malawi

Chickens make up 90% of the total poultry production in Malawi (Nandolo, 2021). The population of chickens *(Gallus gallus)* is estimated at 216,342,218 out of which 120,368,272 are broilers, 9,803,012 are layers, 83,615,970 are indigenous chickens, and 2,554,964 are Black Australorps. Nationally, the Department of Animal Health and Livestock Development (DAHLD) seeks to advance livestock production, establish animal product self-sufficiency, export surplus, and improve the welfare of Malawians through revenue-generating ventures. On the other hand, regarding disease control the Malawian policy mandate of animal health is to "promote increased and sustainable livestock production and productivity through the provision of animal health and production services and the protection of the public from zoonotic diseases (diseases transmitted between man and animals)" (*Policy Document on Livestock in Malawi, 2004*). In the earlier strategy on animal production, much effort was put into larger animals like cattle. However, recently due to the growing population and high demand for protein, poultry especially chickens have become extremely important to Malawians as a source of meat, eggs, income, and manure. The introduction of exotic breeds and increasing demand for indigenous chickens have seen the industry grow rapidly in Malawi. The poultry industry is expanding fast. Commercial breeds of broilers and layers are available from several commercial producers. The hardy Black Australorp (Mikolongwe) chickens are still being produced and are available from the poultry department's outlets at day old, six weeks, and fertile eggs (Nandolo, 2021). As a nation, Malawi envisions expanding and improving poultry production to expand the accessibility of poultry and poultry products. The aim is to promote the use of commercial breeds for meat and egg production and the conservation of indigenous breeds of chickens. The common available commercial breeds include broiler breeds such as Ross and Cobb 500 which are for meat production and have an average carcass yield of 1.5 to 2 kg. They are ready for consumption 6 weeks after hatching (Nandolo, 2021). The Hyline is a breed for egg production. It has a high genetic egg production potential of up to 300 eggs per year under good management. The Black Australorp is a breed raised for both producing meat and eggs. The average weight of an adult chicken ranges from 1.5 kg to 2.5kg. Hens will lay between 180 and 200 eggs per year under good management. This breed was brought into Malawi to enhance the number of eggs laid, improve egg size, and improve the meat weight of native chickens (Mussa et al., 2020).

2.2 Knowledge, Attitude, and Practices (KAP) of Farmers Regarding the Use of Antibiotics

Antibiotic use in livestock farms is expanding globally. Mostly, antibiotics are used for disease treatment and promotion of growth. The incorrect administration of antibiotics might result in antibacterial residues (toxic) accumulation in the food animals which in the end will have an impact on the consumer's health (Geta & Kibret, 2021a). The antibiotics used in the farm animals are influenced by many factors which include the farmer’s knowledge, attitude, practices on the use of the antibiotic, and the antibiotics' accessibility to the livestock farmers. A study conducted in Ethiopia by Tufa et al. (2018) reported that 80% of the respondents did not know antibiotics. These statistics are not extremely far from the results found by Geta & Kibret, (2021) who concluded that 90.1% of farmers were ignorant of the causes of the transmission of resistant bacteria to people and the effects of bacteria that is resistance to antibiotics on the wellness of humans and animals. Thi Huong-Anh et al. (2020) also revealed that 50.9% of farmers in Tay Ninh Province, Vietnam, said they lacked knowledge regarding how to use antibiotics properly. On the other hand, the studies reveal that there were poor practices among the farmers regarding the use of antibiotics as shown by the study conducted by Thi Huong-Anh et al. (2020) whose results showed that 83% of the farmers had bad practices, which include self-prescribing based on personal breeding experiences, adhering strictly to antibiotic package directions, seeking advice from drug sellers, relying on other farmers' experiences, persisting antibiotic use until shortly before selling chickens, and commonly mixing antibiotics with chicken food or drinking water. Additionally, Geta, reported that 52.75% of participants had poor practice in the Amhara region, northwestern Ethiopia. Even though this is the case, studies have shown that some of the farmers have a better attitude towards the use of antibiotics. Thi Huong-Anh et al. (2020) reported that 54.7% of the results had positive attitudes which were like those of Geta & Kibret, (2021) who found that 52.5% of animal farm owners and employees had positive perceptions of the appropriate use of antibiotics and resistance.

2.3 Antibiotics

Antibiotics are chemical substances produced by microorganisms, typically soil microorganisms, to control competing microorganism growth in a complex environment, preventing or treating diseases like bacteria and fungi (Waksman et al., 2023). The use of antibiotics has greatly improved food production, the well-being of animals, and human health. Antibiotics are the only treatment option available for treating serious bacterial infections in both people and animals. To avoid diseases from spreading and safeguard the rest of a herd or flock, antibiotics either eliminate hazardous germs or restrict their growth. Thus, antibiotics contribute to the well-being and health of animals, the safety of food, the halting of disease transmission, the saving of lives, and the support of farmers' livelihoods. Antibiotic use keeps animals safe from major infections, in addition to practices like immunization, biosecurity, good husbandry, and nutrition (Kasimanickam et al., 2021). Prophylaxis entails giving pharmaceuticals to a community of animals before any clinical symptoms of an illness appear, while meta-phylaxis involves giving medicines to groups of animals that are thought to be infected or disease susceptible to treatment and restrict the spread of disease among animals (Mora-Gamboa et al., 2022).

2.4 Commonly used antibiotics in farm animals and Antibiotic residues in chicken samples

Due to the increase in demand for animal products, antibiotic use for growth enhancement has increased significantly over the world. Antibiotic-resistant bacteria may develop because of antibiotic use in animals reared for food. These bacteria may then be transmitted to people through the eating of animal products and other vectors (Aidara-Kane et al., 2018). It has been observed or reported that most of the antibiotics used for food animal production are the same as those considered critically important antibiotics used in human beings (as shown in Table 1). Therefore, the World Health Organization established some recommendations regarding the application of antibiotics in food animal production to safeguard people (World Health Organization, 2019). According to WHO guidelines, the overall use of antibiotics should be reduced. The guidelines recommend that the application of antibiotics to improve growth and disease prevention in animals that are not considered to be at risk of getting sick should be completely restricted. The guidelines further recommend the restriction of antibiotics that have been determined to be vitally important for humans, to be used for disease treatment or control in food animals and exceptions should be made if the drug has proven to be the only treatment possibility through susceptibility testing (Aidara-Kane et al., 2018).

Nayiga et al. (2020) reported that oxytetracycline was the most frequently used antibiotic to treat and enhance the growth of livestock. This observation was similar to the results found from the studies conducted by Tufa et al. (2018) as well as a study by Geta & Kibret, (2021) in which both studies reported oxytetracycline as the most used antibiotic. Mohsin et al. (2019) reported medicines that are crucial for human health such as colistin, tylosin, doxycycline, and enrofloxacin were the most frequently utilized antimicrobials for preventive or therapeutic use with lincomycin reported being the often-used antibiotic in feed. Oxytetracycline amongst other antibiotics has been reported to be the most critical important antibiotics for use in human beings (Table 1) (World Health Organization, 2017).

Table 1. Withdraw periods of commonly used antibiotics in poultry

Antibiotic	**Withdrawal**
Oxytetracycline	**7 days**
Sulphadimidine	**5 days**
Ampicillin	**6 days**
Enrofloxacin	**10 days**
Ciprofloxacin	**10 days**
Sulfonamide	**4 days**
Chloramphenicol	**14 days**
Gentamycin	**14 days**
Doxycycline	**9 days**
Norfloxacin	**12 days**
Amoxicillin	**7 days**

(Thapa, 2021)

On the other hand, many studies on the residues found in chicken samples have also been conducted. Lee et al. (2018) conducted a study on "Prevalence of Antibiotic Residues and Antibiotic Resistance in Isolates of Chicken Meat in Korea", and reported that 45% of the chicken had antibiotic residues. Amoxicillin was present in significant concentrations (15%), enrofloxacin (12.1%), sulfamethoxazole (10.3%), and also *E. coli* strains were found in five chicken meat samples according to the study (Lee et al., 2018). A study in Lebanon reported that 77.5% of chicken muscles had antibiotic residues, out of which 53.75 percent of those samples were said to contain concurrent multidrug residues. From among the four antibiotic families screened, the study showed that there was 32.5 % of ciprofloxacin (quinolones), amoxicillin (β-lactams) (22.5%), and then tetracyclines (17.5%). The study also revealed that sarafloxacin, amoxicillin, and penicillin residue levels were above the Maximum Residue Limit (MRL) recommended by the European Union (Jammoul & El Darra, 2019)**.** Research undertaken in Turkey on the identification of quinolone antimicrobial agents in 127 chicken samples reported that 45.7 % of samples (58 chickens of 127 chicken samples) had quinolone residues (Er et al., 2013).

Table 2. Frequently used antibiotics in the production of chicken

Antibiotic	Class types of antibiotics	Mode of administration	Biological effects
Tetracyclines	Oxytetracycline Doxycycline Chlortetracycline	Oral and intramuscular	Bacteriostatic action against multiple protozoa, filariae, mycoplasmas, Gram-positive and -negative bacteria, as well as certain mycobacteria, mycoplasmas, and other microorganisms
Macrolides	Tylosin Tilmicosin	Oral	Antibacterial action towards pathogens like Gram-positive and Gram-negative bacteria
Lipopeptides	Polymyxins	Oral	Antibacterial activity against Gram-negative bacteria
Penicillin	Penicillin	Oral	Growth stimulant
Folate Pathway Inhibitors	Trimethoprim	Oral	Therapy for gastrointestinal and respiratory infections
Quinolones	Enrofloxacin Ciprofloxacin Danofloxacin	Oral	Growth-promoting and antimicrobial properties towards pathogens including Gram-positive and Gram-negative bacteria
Aminoglycosides	Neomycin Streptomycin	Oral	Gram-negative bacteria-specific antibacterial activity
Lincosamides	Lincomycin	Oral and intramuscular	antibacterial action towards Gram-positive bacteria

(Kumar et al., 2020).

2.5 Antibiotics use in animals

Antibiotic use in food animal production is increasing at an alarming rate. The use of antibiotics in food animals includes treating clinical illness, preventing and controlling common disease outbreaks, and promoting animal growth (McEwen & Fedorka-Cray, 2002). To promote healthy growth and lower animal mortality and morbidity, antibiotics are utilized for the prevention and treatment of bacterially caused illnesses. Antibiotics can also be used to stimulate growth because they improve the absorption of the feed that is consumed by destroying intestinal bacteria. Nonetheless, in some nations, this practice is prohibited (Davies, 2014; Mora-Gamboa et al., 2022). According to their purpose or use, three classes of antibiotics are distinguished including, therapeutic (used for the alleviating of bacterial illnesses), prophylactic (disease protection in animals that are thought to be in danger of infection), and promotion of growth (used for enhancement in growth rate or feed efficiency), (Food and Agriculture Organization & World Health Organization, 2015; Landfried et al., 2018; Mohsin et al., 2019). It has been reported by O'Neill, (2015) that animals consume two times more critically important antibiotics than human beings. The administration of antibiotics in animals, however, varies among species and is determined by a variety of circumstances, including the animal type, the reason it is kept in its health, and disease outbreaks. The population growth-related increase in demand for animal protein has hastened the usage of antibiotics as stimulants of growth. Antimicrobial use in food animal production was anticipated to be 63,151 tons worldwide in 2010 with an expected increase of 67% by 2030. The principal drivers of this increase are probably the transition to large-scale farms utilizing antibiotics and the surge in demand for animal products in middle-income nations (Van Boeckel et al., 2015).

2.6 Therapeutic use of antibiotics in animals

Antibiotics are mostly utilized to treat and prevent diseases in the aquaculture, livestock, poultry, and pig industries. Usually, they are given orally with feed and water or via injection. Antibiotics are administered therapeutically to animals, such as cattle, pigs, sheep, and horses, to treat a variety of infectious diseases. Antibiotics are used intravenously or orally in veterinary treatments to alleviate animal discomfort and minimize output losses. In cases of sick livestock or poultry, high doses are administered intermittently, using wide-ranging or combinations of antibiotics (Peng et al., 2014). Treatment is increasingly pathogen-specific, and there are three main patterns: prophylaxis, which aims to treat healthy animals before risk illnesses appear, meta-phylaxis, which entails treatment for severe clinical disorders, as well as extensive treatment for affected populations (Peng et al., 2014).

2.7 Use of antibiotics as growth promoters

In animal production, antibiotics such as oxytetracycline, bacitracin, chlortetracycline, tylosin, neomycin, avoparcin, and virginiamycin are frequently used to cure illnesses and stimulate development. Antibiotics as growth promoters are given as feed ingredients in small concentrations and for a long duration as compared to antibiotics prescribed for disease treatment. However, if the withdrawal period has not been followed when the antibiotics are being used for treatment purposes, there can be a problem of antibiotic residue accumulation in the chickens' body tissues, which affects humans (Muaz et al., 2018). To ensure the public safety of livestock products worldwide, regulatory authorities European Union, (2010), recommended practices for administering medications to animals, including the permissible residue level and drug withdrawal period (Kiiti et al., 2021). Globally, there is an estimate of an antimicrobial use surge of 67% (from 63, 151 to 105, 596 tones) between 2010 and 2030 due to an increase in demand for livestock products which will result in more food animals being raised intensively and using antibiotics, especially in middle-income countries (Van Boeckel et al., 2015). In low- and middle-income nations from 2000 to 2018, the fraction of antimicrobials demonstrating resistance above 50% increase in poultry, according to Kenya (Van Boeckel et al., 2019). The highest hotspots of resistance were found in China and India, with new hotspots emerging in Brazil and Kenya (Van Boeckel et al., 2019).

2.8 Antibiotic veterinary products used in poultry production in Malawi

Different poultry veterinary antibiotic drugs are available in veterinary shops in Malawi. The antibiotic drugs vary with different companies from different countries but most of them have the same ingredients (Table 3). The government imports medications, including antibiotics used in agriculture, in two ways. The first way is through collaboration with Non-Governmental Organizations (NGOs) that focus on animals, and the second way is through licenses issued by the Ministry of Trade, Commerce, and Industries (MTCI). The Pharmacy and Medicines Regulatory Authority (PMRA) and the Department of Animal Health and Livestock Development (DAHLD) provided a list of recommended medicines for importation, and licenses for importing medicines needed to reflect the recommended list (Mankhomwa et al., 2022).

Table 3: Country of origin for veterinary medicines found for sale in Malawi

Country	Number found
Netherlands	30
Tanzania	23
UK	12
Kenya	12
USA	4
France	2

(Mankhomwa et al., 2022)

The following list is some of the commonly available antibiotics in Malawi imported from the Netherlands.

Limovit WS

a water-soluble powder that effectively combines vitamins and antibiotics (oxytetracycline) to increase the production of eggs, growth, and feed utilization. In times of anxiety and sickness, it is also taken as a vitamin supplement. It protects against infections of the digestive tract, lungs, and urinary system in chickens among other animals.

Doxin-200 WS

A water-soluble powder called Doxin-200 WS comprises doxycycline and tylosin. It is in charge of treating gastrointestinal and respiratory infections brought on by microorganisms that are doxycycline- and tylosin-sensitive.

Gentadox WS

Animals with gastrointestinal and respiratory illnesses brought on by Bordetella, Campylobacter, Chlamydia, E. coli, Klebsiella, Haemophilus, Mycoplasma, Pasteurella, Rickettsia, Salmonella, Staphylococcus, and Streptococcus spp. are treated with Gentadox WS, a water-soluble powder containing gentamycin and doxycycline.

Doxin-200 WS

A water-soluble powder containing doxycycline and tylosin for treating gastrointestinal and respiratory infections caused by tylosin and doxycycline-sensitive micro-organisms in animals. Tylosin and doxycycline are complementary antibiotics that work as bacteriostatic agents against a wide range of bacteria, including *Chlamydia, Bordetella, Campylobacter, Escherichia coli, Mycoplasma Haemophilus, Salmonella, Pasteurella, Staphylococcus, Streptococcus, and Rickettsia spp*.

Intertrim-480 WS

is a water-soluble powder that prevents gastrointestinal and respiratory infections in animals with compromised renal function, hepatic function, or blood dyscrasias. It contains sulfadiazine and trimethoprim.

Nemovit WS

A water-soluble powder with vitamins and antibiotics (Oxytetracycline and Neomycin) that efficiently promotes egg production, growth, and feed conversion. It aids in the prevention of infections of the gastrointestinal tract, respiratory system, and urinary system in animals such as calves, goats, sheep, chickens, and swine.

Aliseryl WS

A water-soluble powder with antibiotics (Colistin, Oxytetracycline, and Erythromycin) and vitamins, effectively promoting egg production, growth, and feed conversion. It protects against gastrointestinal, respiratory, and urinary infections in animals like cattle, goats, sheep, poultry, and swine.

Interflox Oral

An oral liquid that contains Enrofloxacin 10%. Responsible for gastrointestinal infections, respiratory infections, and urinary tract infections caused by enrofloxacin sensitive micro-organisms, like *Campylobacter*, *Escherichia coli, Haemophilus, Mycoplasma, Pasteurella,* and *Salmonella spp*. in calves, goats, poultry, sheep, and swine.

2.9 Common antibiotics used in poultry production in Malawi

The most frequently used antibiotics in Malawi's chicken production are aminoglycosides like gentamicin sulfate and Neomycin, tetracyclines like doxycycline and oxytetracycline, sulfadiazine and trimethoprim, polymyxins like colistin, and erythromycin. Sulfadiazine and trimethoprim.

trimethoprim and sulfadiazine

The combination of trimethoprim and sulfadiazine acts synergistic and usually bactericidal against many gram-positive and gram-negative bacteria like *Escherichia coli, Haemophilus, Pasteurella, Salmonella, Staphylococcus, and Streptococcus spp*. Both compounds affect bacterial purine synthesis differently, as a result, a double blockade is accomplished. They inhibit folic acid metabolism in bacteria. They are competitive inhibitors of dihydrofolate synthesis and dihydrofolate reductase enzyme. In contrast, a combination of sulfamethoxazole and trimethoprim is available for human usage. Sulfadiazine and trimethoprim are solely used in veterinary medicine. Sulfadiazine and sulfamethoxazole have been used with trimethoprim, and no reported variations in efficacy have been noted (Kester et al., 2012; Papich, 2016; Sykes & Papich, 2021).

Tetracycline

Tetracyclines are antibiotics effective against Mycoplasma, Chlamydia, and Gram-positive and -negative bacteria. They are bacteriostatic and resistant to many organisms, especially *S. aureus*. They have a synergistic effect with tylosin against Pasteurella (Varga, 2014). Oxytetracycline, a tetracycline, acts bacteriostatic against various bacteria, including *Bordetella, Campylobacter, Chlamydia Escherichia coli, Haemophilus, Mycoplasma, Pasteurella, Salmonella, Staphylococcus, and Streptococcus spp.* It inhibits bacterial protein synthesis and is primarily excreted in urine. Various bacterial-related infections of the skin, soft tissues, urinary tract, and respiratory system are treated with oxytetracycline. In pigs, it is frequently used to treat atrophic rhinitis, pneumonic pasteurellosis, and mycoplasma infections. It is also used in cattle to treat bovine respiratory illness. For Rickettsiae and Ehrlichiae in small animals, doxycycline is utilized. Oxytetracycline has been used to treat respiratory and soft tissue infections in horses, as well as equine piroplasmosis, Potomac fever, and other bacterial infections. To treat angular limb abnormalities in newborn horses, high doses are frequently used. This may be because juvenile tendons have less viscoelastic capacity (Papich, 2016; Varga, 2014).

Neomycin

Neomycin is an aminoglycoside with a bactericidal action against mainly Gram-negative bacteria like *Escherichia coli, Klebsiella, Pasteurella, and Salmonella spp.* Vitamins are essential for the proper operation of numerous physiological functions. Neomycin is frequently administered orally for the treatment of gastrointestinal bacteria, hepatic coma patients, and individuals with superficial infections, such as *Staphylococci* and gram-negative *Bacilli*. Additionally, it can lower ammonia in encephalopathic individuals with hepatic coma (Scholar, 2007).

Colistin

Colistin is a 50-year-old antibiotic, that was initially used against Gram-negative bacteria like *Pseudomonas aeruginosa.* Its rapid, concentration-dependent bactericidal activity was replaced by aminoglycosides in the 1970s. Colistin is an antibiotic from the group of polymyxins with a bactericidal action against Gram-negative bacteria like *Escherichia coli, Haemophilus, and Salmonella.* Since colistin is absorbed for a very small part after oral administration only gastrointestinal indications are relevant (Interchemie werken 'De Adelaar' B.V., 2023; Nation & Li, 2009; Poudyal et al., 2008).

Erythromycin

A macrolide that acts bacteriostatic against mainly Gram-positive bacteria like *Staphylococcus and Streptococcus spp.* Streptomycin is an aminoglycoside with a bactericidal action against mainly Gram-negative bacteria like *E. coli, Klebsiella, Pasteurella and Salmonella spp.*, and Mycoplasma. Erythromycin works by preventing protein synthesis, which stops the growth of bacteria. By adhering to the 23S ribosomal RNA molecule found on the 50S component of the bacterial ribosome, it hinders the synthesis of peptide chains. Due to its anti-inflammatory and immunomodulatory properties, erythromycin can prevent periodontal bone loss and lung inflammation (Farzam et al., 2023; Liang & Han, 2013).

Tylosin

Tylosin, a macrolide antibiotic, exhibits antimicrobial activity against Gram-positive and Gram-negative bacteria including *Campylobacter, Pasteurella, Staphylococcus, Streptococcus,* and *Treponema spp.,* as well as *Mycoplasma*. A 16-membered macrolide called tylosin is authorized for the treatment of several illnesses in pigs, cattle, dogs, and poultry. It stops protein synthesis and 50S ribosome binding in bacteria to prevent the growth of bacteria. Gram-positive aerobic bacteria that are resistant to *Salmonella and Escherichia coli* and vulnerable to *Clostridium and Campylobacter* are among the members of its spectrum (Papich, 2016). Tylosin and other macrolides are crucial for treating chronic respiratory conditions in poultry and enhancing feed conversion and growth, both of which help to prevent the development of erythromycin-resistant campylobacteriosis, a rare condition that can be treated in humans (Samanta & Bandyopadhyay, 2020).

Enrofloxacin

Enrofloxacin is a fluoroquinolone antibiotic that stops bacteria's ability to synthesize DNA and RNA by inhibiting DNA gyrase. In most animal species, it has bactericidal effects and is largely metabolized to ciprofloxacin. *Staphylococcus, Escherichia coli, Proteus, Klebsiella*, and *Pasteurella* are susceptible bacteria. *Streptococcus* and anaerobic bacteria are poorly affected by the antibiotic enrofloxacin (Papich, 2016).

2.10 The maximum residue limits

The maximum residue limit (MRL) is the highest level of residue that is permissibly present in a food product derived from an animal that has received veterinary treatment. Consumers must be protected from residues detected in food, and the amounts should be as small as feasible (European Union, 2010). To avoid antibiotic residues in foodstuffs of meat products, the EU established a maximum residue limit for important antibiotics used in livestock (Tables 4 and 5).

Table 4: Maximum residue limits of pharmacologically active substances available in Malawi

Drug	**Concentration µg/kg**			
	Chicken muscle	Chicken kidney	Chicken liver	Chicken fat
Sulfonamides	100	100	100	100
Gentamycin	-	-	-	-
Oxytetracycline	100	600	300	-
Doxycycline	100	600	300	-
Amoxicillin	50	50	50	50
Trimethoprim	50	50	50	50
Gentamycin	-	-	-	-
Neomycin	500	500	500	500
Colistin	150	200	150	150
Erythromycin	200	200	200	200

(European Union, 2010)

Table 5: The MRL of commonly used antibiotics in poultry production

Antibiotic type	**Concentration µg/kg**			
	Chicken fat	Chicken muscle	Chicken Liver	Chicken kidney
Oxytetracycline	-	-	-	-
Doxycycline	-	100	300	600
Chlortetracycline	-	-	-	-
Tylosin	100	100	100	100
Tilmicosin	75	75	1 000	250
Polymyxins	-	-	-	-
Penicillin	50	50	50	50
Trimethoprim	50	50	50	50
Enrofloxacin	-	-	-	-
Ciprofloxacin	100	100	200	300
Danofloxacin	50	100	200	200
Neomycin	500	500	500	5000
Streptomycin	-	-	-	-
Lincomycin	50	100	500	1500

(European Union, 2010)

2.11 Antibiotic use in human beings

Most of the antibiotics that are likely to be found in municipal wastewater discharges are those that are prescribed for human treatment. The human body naturally possesses the ability to fight disease, but over time, many disease-causing pathogens have evolved into resistant ones, leading to the introduction of various coping mechanisms, such as the use of antibiotics. Salvarsan, the primary antibiotic in recorded history, went into circulation in 1910. Since then, antibiotics have profoundly changed contemporary medicine and extended human life expectancy by 23 years in just over a century (Hutchings et al., 2019). Additionally, the discovery and development of penicillin by Sir Alexander Fleming in 1928 marked the beginning of the revolution of antibiotics (Fleming, 1929). For successful medical treatments, including organ transplantation and immunomodulatory therapy, antibiotics are indispensable (Ventola, 2015).

However, the achievements made during the antibiotic revolution are at risk due to antibiotic resistance, or bacteria's capacity to withstand the effects of drugs to which they were previously sensitive (Adedeji, 2016). After barely eight decades of antibiotic use, bacterial diseases that were formerly manageable are becoming incurable due to the rise in antimicrobial resistance and the drying up of the pipeline for new antibiotic research (MacGowan & Macnaughton, 2017). With the potential to affect not only the healthcare, veterinary, and agricultural sectors but also people at any stage of life, antimicrobial resistance poses a serious threat to public health on a global scale, killing in excess of 1.27 million people worldwide and being associated to over 5 million deaths in 2019 (Murray et al., 2022).

To reduce antibiotic resistance the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE) joined forces to create the Global Action Plan (GAP) to combat antibiotic resistance with the goal ensuring that infectious diseases are successfully treated and prevented with effective and safe medicines that are quality-assured, used responsibly, and accessible to all who need them (World Health Organization, 2018). WHO went one step further and classified the antimicrobials used on humans as critically important (table 6), highly important, and important antimicrobials (World Health Organization, 2018).

Table 6. List of critically important antimicrobial classes and examples of antimicrobial agents used in human medicine

Antimicrobial class	**Example of antimicrobials(s)**
Aminoglycosides	Gentamicin
Annamycin's	Rifampicin
Cephalosporins (3rd,4th and 5th generation)	Ceftriaxone, Cefepime, Cefazoline, Ceftobiprole
Glycopeptides	Vancomycin
Glycylcyclines	Tigecycline
Lipopeptides	Daptomycin
Macrolides and ketolides	azithromycin, Erythromycin, Telithromycin
Monobactams	Aztreonam
Oxazolidinones	Linezolid
Penicillin (antipseudomonal)	Piperacillin
Penicillin (aminopenicillins)	Ampicillin
Penicillin (aminopenicillin with beta-lactamase inhibitors)	amoxicillin-clavulanic-acid
Phosphonic acid derivatives	Fosfomycin
Polymyxins	Colistin
Quinolones	Ciprofloxacin
Drugs used solely to treat tuberculosis or other mycobacterial diseases	Isoniazid
Tetracyclines	Chlortetracycline, doxycycline, minocycline, oxytetracycline, and tetracycline

Source: World Health Organization (2019)

2.12 Strategies to cut back on animal use of antibiotics

Some countries have restrictions on the administration of different antibiotics, which has an impact on how widely they are used there to fight AMR. European nations have put regulations in place to minimize the utilization of antibiotics for farming purposes, with Scandinavian nation’s leading the way. All antibiotics used as growth promoters were outlawed by the EU in 2006, and food animals required a veterinarian's prescription. The effectiveness of these bans varied among European countries, with some in Northern Europe setting goals while others-maintained pre-ban levels. In the United States (US), the Food and Drug Administration (FDA) recently negotiated voluntary rules, although their success is still up for debate (The Pew Charitable Trusts, 2014). Monitoring farmer antibiotic use and usage is challenging when regulations are tightly specified. The usage of antibiotics should be restricted rather than their type to improve surveillance. More evident standards, rewards for animal sanitation, and separation of vet compensation and profits from antibiotic prescriptions are just a few examples of simpler laws that could lower the use of antibiotics (O'Neill, 2015). Taxing antimicrobial use encourages farmers to pay an additional cost for every antimicrobial they use, considering societal costs. This policy aligns farmers' incentives with societal goals and encourages alternative treatments like improved husbandry, vaccines, and diagnostics. For successful implementation, it is crucial to enhance the understanding of the demand curve for antimicrobials. The tax should discourage growth promotion and unnecessary prophylactic use while allowing adequate treatment for sick animals (O'Neill, 2015). However, concerns arise about farmers circumventing the tax by buying counterfeit or black-market drugs. Governments may prefer raising taxes over over-regulating, as raising taxes is more profitable and costs money. Interventions to minimize the use of antibiotics in agriculture include infection control, crop selection, vaccinations, diagnostics, surveillance, and behavioral changes. Farmers may be encouraged to cut back on use and seek alternatives through incentives like legislation or taxes (O'Neill, 2015).

2.13 The fate of antibiotics

Antibiotics can find their way into the environment through different means some of them include agricultural use (plant and animal production), human use, and industrial use. The emergence of AMR in both humans and animals is greatly impacted by environmental antibiotics. Antibiotic's presence and dispersion in the environment have drawn more attention as emerging pollutants because of the potential harm they may cause to human health and the ecosystem (Kulikova et al., 2022). The antibiotics used in human and animal health have the potential to enter the environment through the manufacturing process, excretion, disposal of surplus drugs, packaging materials, and materials used for treatment. As a result, there would be a significant contamination issue that might affect the delicate balance of ecosystems because both antibiotics found in human and animal excrement and those released into the environment directly would raise the likelihood of antibiotic residue accumulation (Christou et al., 2018). Antibiotic pollution in streams, sludges, and crop soils because of the overuse of antibiotics for human and animal health has caused an unanticipated rise in bacteria with multiple resistances, also known as antibiotic resistance, food contamination, and surface and underground water contamination (Akhil et al., 2021). To reduce selection pressures for antimicrobial resistance on diseases that affect both humans and non-human animals and plants, the human, animal, and plant sectors have a joint responsibility (World Health Organization, 2018).

2.14 Antimicrobial resistance, a growing threat to animal and human well-being

Antimicrobial resistance, which now accounts for most of the global mortality toll, is one of the greatest risks to modern healthcare. The danger to livelihoods, availability of food, human health, and animal health globally is escalating (Murray et al., 2022). Globally in 2019, According to reports, 1.3 million of the 4 million deaths associated with antimicrobial resistance in humans were brought on directly by resistant bacteria (World Organization for Animal Health, 2018).

2.15 Conceptual framework

The research approach was based on the following conceptual framework, relating demand for chicken, disease treatment, and use of antibiotics reflecting the possible outcomes. It covers a wide range of elements, such as farmers' knowledge, drug choices, administration routes, education levels, income levels, veterinary staff knowledge, farm practices, management systems, and antibiotic consumption in chicken strains. This framework offers a structured overview of the different factors contributing to antibiotic use in broiler chicken farming, serving as a foundation for understanding the complex dynamics at play in this agricultural context (figure 1).

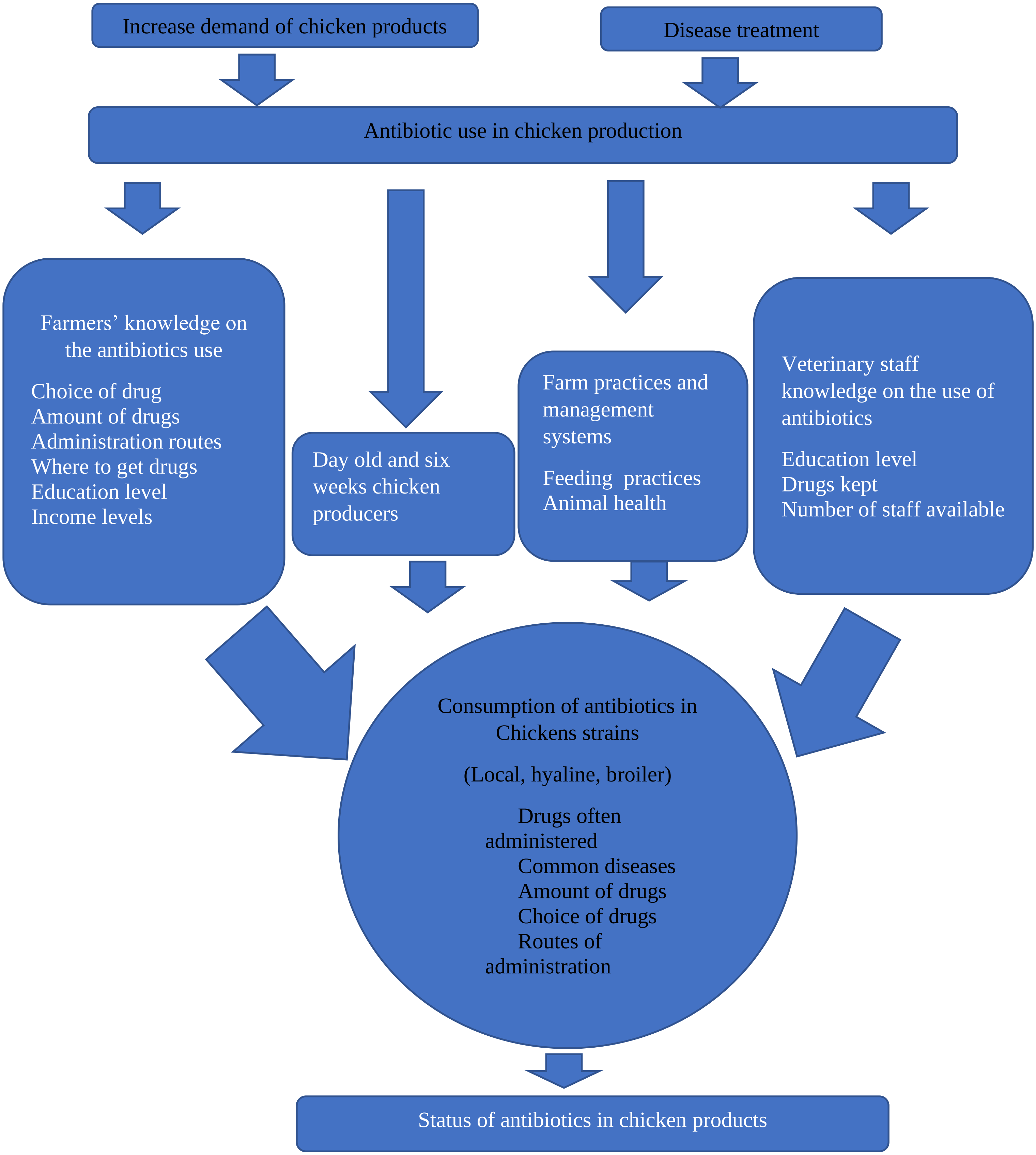

Figure 1: The conceptual framework.

CHAPTER 3: METHODS

3.1 Study design

A cross-sectional study was carried out in peri-urban and urban areas of Blantyre City areas in Malawi, from July 2022 to July 2023, targeting smallholder, medium, and commercial broiler chicken farmers. The study focused on factors affecting antibiotic use in broiler chickens, targeting small-scale farmers rearing 50-100 chickens, medium-scale farmers rearing 100-499 chickens, and commercial farmers rearing 500 and above broiler chickens. This categorization was based on guidance and recommendations made by the District Animal Health and Livestock Development Officer of Blantyre Agriculture Office.

3.2 Study area

Blantyre, founded in 1876, is Malawi's second-largest city. Located in the Shire highlands in the southern region of Malawi, the city covers an area of 220 km^2 and with a population of 1,251,484. Its land mix includes planned residential, unplanned, and semi-rural areas (Britannica, 2018; Malawi Plus, 2023; Millennium Cities Initiative, 2023). This study was conducted in 10 areas, six located within the government-defined city limits considered ‘urban’ (Kampala, Chilimba Zingwangwa, Chilobwe, Mkolokosa, and Ndirande) and four outside the city limits considered “peri-urban” (Mpemba, Chileka, Machinjiri, Chilomoni), (Mathanga et al., 2016). The study targeted peri-urban and urban areas in order to include a wide range of broiler chicken farming operations, taking into account differences in management systems, and resource access. This focused approach attempts to provide a thorough understanding of antibiotic use in various farming conditions. Furthermore, the choice of peri-urban and urban locations corresponds to the high concentration and intensity of broiler chicken production observed in these settings.

3.3 Sample size

The population of the broiler farmers in Blantyre was not known. Therefore, to decide the sample size for participants of the study Cochran’s formula for sample size calculation was employed.

Sample Size = $\mathrm{N} = [Z^2\,(P)\,(1 - P)]/e^2$

Where:

- N = population size
- Z = z-score
- e= margin of error

- p = standard of deviation

Z is the value of a normally distributed standard variate which for a 95% confidence interval takes on the value of 1.96. The value of P is not known (0.5 will be used);

Therefore;

$$N = ((1.96)^2 (0.5) (0.5)) / (0.05)^2 = 385.$$

Therefore, a random sample of 385 farmers that raise broiler chickens from our intended audience was adequate to offer the required levels of confidence. However, due to practical reasons limited resources, budget considerations, time to conduct, and long procedures in the lab a total of 50 farmers were interviewed, and a total of 20 chickens were used for antibiotic residue analysis in the laboratory. Despite the smaller sample size, the study aimed for a balance between statistical accuracy and the realities of field research. Even though our sample was smaller than the calculated value, it still offered enough data for meaningful insights and statistical analysis. This decision was a practical one, acknowledging the constraints we faced during the research. The choice of a small sample was also guided by literature. Over the past two decades, a common alternative for determining sample size has been Roscoe, (1975) set of guidelines. Roscoe suggested selecting a sample size of at least 30, but not more than 500, for the majority of behavioral experiments.

3.4 Sampling strategies

A random sampling of the farmers was employed whereby farmers in small-scale, medium-scale, and commercial broiler management were randomly selected. On the other hand, the chicken meat samples were randomly selected from the management systems (small, medium, and commercial broiler farmers) whereby 28 chicken meat samples were collected from small-scale and medium-scale (14 from each) and 12 meat samples from commercial farm management systems.

3.5 Data collection techniques

Data about the antibiotics use knowledge, attitude, and practices amongst the broiler farmers was collected using standardized questionnaires, while the broiler meat samples were analyzed for detection of amoxicillin, trimethoprim, sulfamethoxazole, ciprofloxacin residues in the laboratory using the High-Performance Liquid Chromatography (HPLC) antibiotics analysis method. The antibiotics selected for analysis were chosen based on their availability in the laboratory and also based on their prevalence in poultry production and their potential impact on human health.

3.6 Data collection process

To meet the objectives of the current study, a previously completed study's structured questionnaire was extensively modified (Geta & Kibret, 2021). We used the KoBoCollect app on smartphones to conduct the survey. Kobo Collect is an easy-to-use tool for gathering, summarizing, and evaluating field data. To interact with farmers, the questionnaire was translated into Chichewa. The first section of the questionnaire comprised five questions about demographic information and how long the farmers have been farming broilers. The second section included fourteen questions designed to assess farmers' broad knowledge regarding antibiotic use. The final section contained fifteen questions (13 closed-ended and 2 four-point Likert scale response alternatives) about farmers' attitudes toward antibiotic use. The fourth section comprised fourteen questions about farmers' antibiotic use practices. The questionnaire was piloted on a small group of farmers (10 small-scale and 10 medium-scale) from Chirimba and Machinjiri areas to assess its face validity and clarity. Based on the feedback, the questionnaire was revised and finalized for use in the study. Face-to-face surveys were used to administer the revised questionnaire to the farmers by skilled and experienced researchers. Farmers were interviewed to learn about their antimicrobial use and resistance knowledge, attitudes, and self-reported behaviors

3.7 Sample collection

Twenty samples of each type of tissue (liver and kidney) were taken for a total of 40 samples. The liver and kidney tissue samples were obtained from randomly chosen chicken broiler farmers in the peri-urban and urban areas of Blantyre. Additionally, some samples were obtained from the Blantyre market. This was due to the reason that some sampling units were inaccessible due to restrictions and challenges, so their chickens were sampled from the market through the use of product labels. A total of two chickens were collected from the market. Each sample was stored individually in an appropriately labeled, sterile plastic bag. All of the obtained samples were transported in an ice box to the pharmacy laboratory of Kamuzu University of Health Sciences, Blantyre, Malawi. Before further examination, these samples were kept in the refrigerator at a temperature of -20°C (Sarker et al., 2018; Thapa, 2021). The time between sample collection and arrival at the lab was approximately 30 minutes.

3.8 Sample preparation

After being thawed at room temperature, the frozen liver and kidney samples were crushed, and 2 grams were then weighed for each sample on a balanced scale (Pheonex instrument, max 2000g, d=0.01g, BTG-2002 model). Once the sample was prepared, the extraction process followed.

3.9 Chicken kidney and liver extraction procedure

The extraction procedure was conducted following a study by Abdelshakour et al. (2022) with some adjustments. The chicken sample (kidney and liver) (2 g of each) was crushed and weighed on an electrical balance, then the sample was homogenized and transferred to a 15 mL polypropylene centrifuge tube. The sample was mixed with 12 mL (0.2% formic acid in acetonitrile), vortexed for 5 min, sonicated for 5 min, followed by centrifugation for 5 min at 3200 rpm at ambient temperature. The supernatant was separated into 45 ml centrifuge tubes. The residue was mixed with 12 mL of methanol and vortexed for 5 min, sonicated for 5 min, followed by centrifugation for 5 min at 3200 rpm at ambient temperature. The supernatant was collected and mixed with the first extract in the same flask and evaporated to dryness. The residue was reconstituted in 1.5 mL methanol. The supernatant was filtered by a syringe nylon filter (0.22 um) into clear vials for analysis in the HPLC machine.

3.10 HPLC information and operating conditions

High-performance liquid chromatography (HPLC) analysis was performed using a Dr. Maisch GmbH Reprosil-Pur ODS-3 parking C*18 column (150 x 3.9 mm, 5 µM particle size). The column was heated to a maximum temperature of 90°C, and the temperature was uncontrolled. A pump (G4290A) with a 10-minute stop time limit and a maximum stop time of 15 minutes was used. The pressure bar was restricted to a 300-bar maximum, and 245 nm vibrate wavelength detector. The peak width was 0.1 minutes and 2 resp. time (5 Hz). An automatic liquid sampler injection mode was set to 10µL standard injection with 200 µL/min draw and injecting speeds, 0.0 nm draw location, and no time limit stop. Methanol (MeOH): distilled water (1:1) solvents were used to wash and rinse the column.

3.11 Ciprofloxacin HPLC analysis

3.11.1 Buffer and mobile phase preparation

1000 mL of distilled water was pulled into a 1000 mL glass sampling bottle. 2.88 mL of the water was replaced by 2.88 mL of orthophosphoric acid to make a 1000 mL water, orthophosphoric acid buffer. Trimethylamine was used to adjust the buffer's pH to 3. Then, 870 mL of buffer and 130 mL of acetonitrile were mixed to make the mobile phase A to be used in the HPLC. Mobile phase B was 100% acetonitrile.

3.11.2 Amoxicillin HPLC analysis

3.11.2.1 Buffer and mobile phase preparation

To make 1000 mL of buffer, 250 mL of 0.2M (6.8g) potassium dihydrogen orthophosphate was combined with 750 mL of distilled water. 2M (40g) sodium hydroxide (NaOH) was used to adjust the pH of the buffer to 5. To prepare for mobile phase A, a 1-volume acetonitrile: 99 volume buffer ratio of pH 5 was utilized. Mobile phase B was made using an acetonitrile-to-buffer (1:4, pH 5) volume ratio.

3.11.2.2 Sulfamethoxazole and trimethoprim

Sulfamethoxazole and trimethoprim, commonly co-administered antibiotics in veterinary and human medicine for their synergistic effects, were analyzed together in this study. Despite their distinct mechanisms of action, their combined use necessitates simultaneous analysis. By employing the same analytical methods for both compounds, consistency in results was ensured, reflecting their frequent concurrent use and the need for comprehensive monitoring of their presence in samples.

3.11.2.3 Mobile phase A preparation

For the mobile phase A preparation, 1.4g of sodium perchlorate was dissolved in 1000 mL of distilled water. The pH of the solution was carefully adjusted to 3.6 using orthophosphoric acid to optimize the separation of sulfamethoxazole and trimethoprim. In contrast, mobile phase B consisted of 100% methanol, serving as the eluent for the chromatographic analysis.

3.12 Standard preparation

To prepare a standard solution containing a concentration of 1 mg/mL, 5 mg of each antibiotic standard was carefully weighed and then dissolved in methanol and water (50:50 v/v). This standard solution served as the starting point for all subsequent dilutions.

3.12.1 Working standards

5 mL of different antibiotics standards were combined with mobile phase A to make a 1 mg/ mL stock solution concentration. Serial dilution was made in 50µg/mL, 100 µg/ mL, 200 µg/ mL, 300 µg/ mL, and 500µg/ mL to make concentrations of 0.05mg/mL, 0.1mg/mL, 0.2mg/mL, 0.3mg/mL, and 0.5µg/ mL respectively.

3.13 Identification and quantification of antibiotics

The retention time, the visible peak of a standard, and the sample extract were compared to identify the antibiotics. It was assumed that the sample extract contained the desired antibiotic residues if the peak appeared at the same retention time as a standard. A blank was utilized to distinguish between visible signal and noise (the noise-to-signal ratio was >3).

A standard calibration curve for the standard's successive dilutions on HPLC was used to determine the first concentrations of the discovered antibiotic residues. Plots were made between the peak areas of all the successive dilutions and the concentration (in µg/ mL). Utilizing the line's equation and R^2 value, the best-fit line was determined. The correlation coefficient was used to determine linearity. Calculations were made for the calibration curve's intercept, slope, and correlation coefficient. Linear regression was used to identify which data suited the model the best using the following equation:

$Y= mx +b$

Where, Y = Peak area, m = Slope, x = Concentration and b = Intercept

The concentration obtained using the equation is then used to calculate the second concentration using the dilution factor. So initially the sample was dissolved in 24 mL of supernatant then it was concentrated in 1.5 mL of methanol (this was injected in the HPLC machine). Then to find the concentration in 24 mL of the supernatant the following calculations were done.

Table 7: Dilution factor calculation

Initial solution	Second solution	Dilution factor
24 mL	1.5 mL	24 mL /1.5 mL =16

Then the first concentration was divided by the dilution factor (16) to get the second concentration. To get the final concentration in 2 g (sample which was prepared for analysis) the second concentration was divided by 2 g to find a mass-to-mass ratio of µg/g or divided by 1000 to find a concentration of µg/kg.

3.14 Data management

The data was extracted into an MS Excel spreadsheet for cleaning, processing, and further analysis. Each verified question was examined separately, and the responses were given a score of 1 (correct) or 0 (incorrect). The aggregate of each participant's replies in that section was calculated to examine how each farmer behaved overall in each category of knowledge, attitude, and practice. Those with more than 50% right responses on any portion of the questionnaire (or practices that inhibit the development of AMR) may have been evaluated as having positive knowledge, attitudes, or practices.

3.15 Data analysis

The Cronbach's alpha, which is a numerical coefficient to ascertain the internal consistency and reliability was assessed on a 3-point Likert scale: Agree = 1 point, neutral = 2 points, and disagree = 3 points. The alpha was determined as 0.54 for Altitudes. The data was processed with R software version 4.2.3. Categories and descriptive statistics such as mean frequency (%) and numerical standard deviation (SD) were used to summarize the data. The chi-square test (2) was used to determine the association between sociodemographic factors and the value of knowledge, attitude, and practice. A p-value less than 0.05 was used to determine statistical significance. The data from HPLC analysis was analyzed in Microsoft Excel.

3.16 Ethical considerations

The National Commission for Science and Technology (NCST) ethically certified the study to protect the public's safety. All farm owners/workers and all research respondents or participants provided written informed consent. This research was carried out following applicable guidelines/regulations.

CHAPTER 4: RESULTS

4.1 Socio-demographic data

The sociodemographic characteristics of the respondents are presented in Table 8. Of the 50 respondents, 58% were women. 52% of the farmers were from the urban areas. Ages ranged between 24 and 62 years, with most respondents being above 50 years (32 %). Most farmers had a secondary, diploma, or degree level of education while only 4% of the farmers were uneducated. A majority (66%) of the farmers were married. 52% of the farmers have raised broilers for 3 years and only 16 % of the farmers have raised for more than 9 years.

Table 8: Demographic data of broiler farmers who participated in the survey

Characteristic	**Farming scale**		
	Small, N = 20[1]	Middle, N = 20[1]	Large, N = 10[1]
Location			
Urban	12 (60%)	9 (45%)	5 (50%)
Peri-urban	8 (40%)	11 (55%)	5 (50%)
Gender			
Female	14 (70%)	11 (55%)	4 (40%)
Male	6 (30%)	9 (45%)	6 (60%)
Marriage status			
Married	14 (70%)	11 (55%)	8 (80%)
Single	2 (10%)	8 (40%)	2 (20%)
Widowed	4 (20%)	1 (5.0%)	0 (0%)
Education status			
Degree	4 (20%)	3 (15%)	2 (20%)
Diploma	5 (25%)	4 (20%)	2 (20%)
Post-graduate	2 (10%)	1 (5.0%)	0 (0%)
Primary	0 (0%)	4 (20%)	0 (0%)
Secondary	8 (40%)	7 (35%)	6 (60%)
Un educated	1 (5.0%)	1 (5.0%)	0 (0%)
Years spent as a farmer			
0-3	12 (60%)	11 (55%)	3 (30%)
4-6	4 (20%)	7 (35%)	2 (20%)
7-9	0 (0%)	1 (5.0%)	2 (20%)
>9	4 (20%)	1 (5.0%)	3 (30%)
Age categories			
0-30	5 (25%)	4 (20%)	3 (30%)
31-40	3 (15%)	5 (25%)	1 (10%)
41-50	6 (30%)	7 (35%)	0 (0%)
> 50	6 (30%)	4 (20%)	6 (60%)

[1]n (%)

4.2 Knowledge level of the farmers

The knowledge levels of the farmers on antibiotic use are presented in Tables 9 & Appendix 6. The usage of antibiotics in animals to increase weight was mentioned by 34% of the farmers. The usage of antibiotics for all kinds of animal diseases was reported by 46% of the farmers. According to 62% of respondents, antibiotics did not affect the good bacteria that exist inside the body, and antibiotics have side effects, according to 62% of respondents. 84% of respondents said that bacteria can develop resistance to drugs. Amongst the farmers, only 38% had ever participated in a training session on using antibiotics when raising poultry. Antibiotics can be utilized in both humans and animals, however, only 18% of farmers were aware of this. 30% of farmers said they used antibiotics to treat any ailment, 64% said they used antibiotics to treat bacterial diseases, just 2% said they used antibiotics to treat viral diseases, and 4% said they didn't know what diseases antibiotics were used to treat.

In regards to antibiotic resistance, 58% of farmers had never heard about antibiotic resistance (table 9). On the usage of antibiotics in animals for weight increase and on awareness of conditions for which antibiotics are used, there was a significant relationship between participants' education levels and their knowledge of antibiotics ($p < 0.05$) (Appendix 8). The level of farmers' knowledge regarding when to provide antibiotics to broilers was correlated with their age ($p = 0.062$), (Table 10). When asked about their understanding of the association between improper antibiotic usage and antimicrobial resistance, farmers' knowledge of antibiotics was found to be correlated with the size of their farms ($p = 0.028$), (Table 11). Farmers' understanding of antibiotic side effects was significantly ($p < 0.05$) correlated with their years of agricultural experience and their knowledge of antibiotic use (Appendix 9).

Table 9: Knowledge level of farmers on antibiotic use.

Characteristic	N = 50[1]
Antibiotics used for weight gain	17 (34%)
Antibiotics for types of diseases	23 (46%)
Improper use of antibiotics leads to AMR	36 (72%)
Antibiotics do not harm beneficial bacteria	31 (62%)
Antibiotics have side effects	31 (62%)
Bacteria can become resistant to antibiotics	42 (84%)
Knowledge of antibiotic resistance	43 (86%)
Training on antibiotic use in poultry	19 (38%)

[1]n (%)

Table 10: Association between broiler farmer's age and their knowledge of antibiotic use

	Age groups				
Characteristic	**0-30**, N = 12[1]	**31-40**, N = 9[1]	**41-50**, N = 13[1]	**> 50**, N = 16[1]	**p-value**[2]
Antibiotics are mostly used for					0.062
Disease treatment	8 (67%)	3 (33%)	6 (46%)	12 (75%)	
Don't know	0 (0%)	1 (11%)	0 (0%)	0 (0%)	
Prevention of infection	4 (33%)	1 (11%)	5 (38%)	2 (13%)	
Promoting growth	0 (0%)	1 (11%)	2 (15%)	1 (6.3%)	
Whenever I want to use	0 (0%)	3 (33%)	0 (0%)	1 (6.3%)	
[1]n (%)					
[2]Fisher's exact test					

Table 11:Association between farming scale and knowledge of farmers on antibiotic use

	Farming Scale			
Characteristic	**Small**, N = 20[1]	**Middle**, N = 20[1]	**Large**, N = 10[1]	**p-value**[2]
Improper use of antibiotics in animal farms can cause Anti-Microbial Resistance	11 (55%)	15 (75%)	10 (100%)	0.028
[1]n (%)				
[2]Fisher's exact test				

4.3 The attitude of farmers toward the use of antibiotics

The attitudes of the farmers towards antibiotic use are presented in Table 12 and Figure 2. When chickens exhibit any unusual illnesses or signs, 56% of the farmers said they believe their flock needs antibiotics the most. In response 11 (22%) respondents said they ask a drug seller at the vet shop, 10 (20%) said they ask the government veterinarian, and 8 (16%) said they self-medicate. To increase poultry output, 56% of respondents said that antibiotic use is essential. However, 44% of the farmers thought that limiting the use of antibiotics in broiler production would have more detrimental impacts than beneficial ones, while 10 (20%) respondents were unsure of what would happen. There was a connection between antibiotic use and the emergence of AMR, according to 76% of the farmers. Following 66% of farmers, prolonged drug use could result in the development of antibiotic resistance (AMR). In addition, 70% of the farmers agreed that non-therapeutic antibiotic use does contribute to AMR. 96 % of the farmers expressed interest in learning more about antibiotics and 45 people (90%) have confirmed that they can replace antibiotics with non-antibiotic alternatives. A drug withdrawal period should be followed, according to 92% of respondents, and buying antibiotics from a vet shop with a valid license is safe, according to 84% of respondents. Farmers' age and education level both had a significant association ($p < 0.05$) with attitudes toward long-term antibiotic use and the development of resistance to antimicrobials. Age was truncated into categories hence converting it from continuous data. Then a parametric statistic (Chi-square) was used to measure the association between the two categorical variables (age vs education), (Table 13 & Appendix 10).

Table 12: Attitude of farmers toward antibiotic use

Characteristic	N = 50[1]
Chicken needs antibiotics the most	
Guidance/suggestions from other people	3 (6.0%)
Have a disease	19 (38%)
Have any abnormal symptoms/signs	28(56%)
First thing done when chickens are showing signs of disease	
Ask drug seller	11(22%)
Ask Govt. veterinarian	10(20%)
Ask neighbor farmers	2 (4.0%)
Ask private veterinarian	8 (16%)
Isolation of sick bird outside the shed	8 (16%)
Isolation of sick birds within the shed	3 (6.0%)
Self-medication	8 (16%)
Interest in learning more about antibiotics	
Extremely interested	26(52%)
Interested	22(44%)
Neutral	1 (2.0%)
Not interested	1 (2.0%)
Antibiotics are necessary to improve the productivity of poultry	
Important	20(40%)
Less important	12(24%)
Neutral	5 (10%)
Not important	5 (10%)
Very important	8 (16%)
Veterinarian consultation before any use of antibiotics	37(74%)
Restricting the use of antibiotics in poultry will have more negative effects than positive ones	
Don’t know	10(20%)
No	18 (36%)
Yes	22 (44%)
Ability to substitute antibiotics with alternatives	
Don’t know	3 (6.0%)
No	2 (4.0%)
Yes	45 (90%)

[1]n (%)

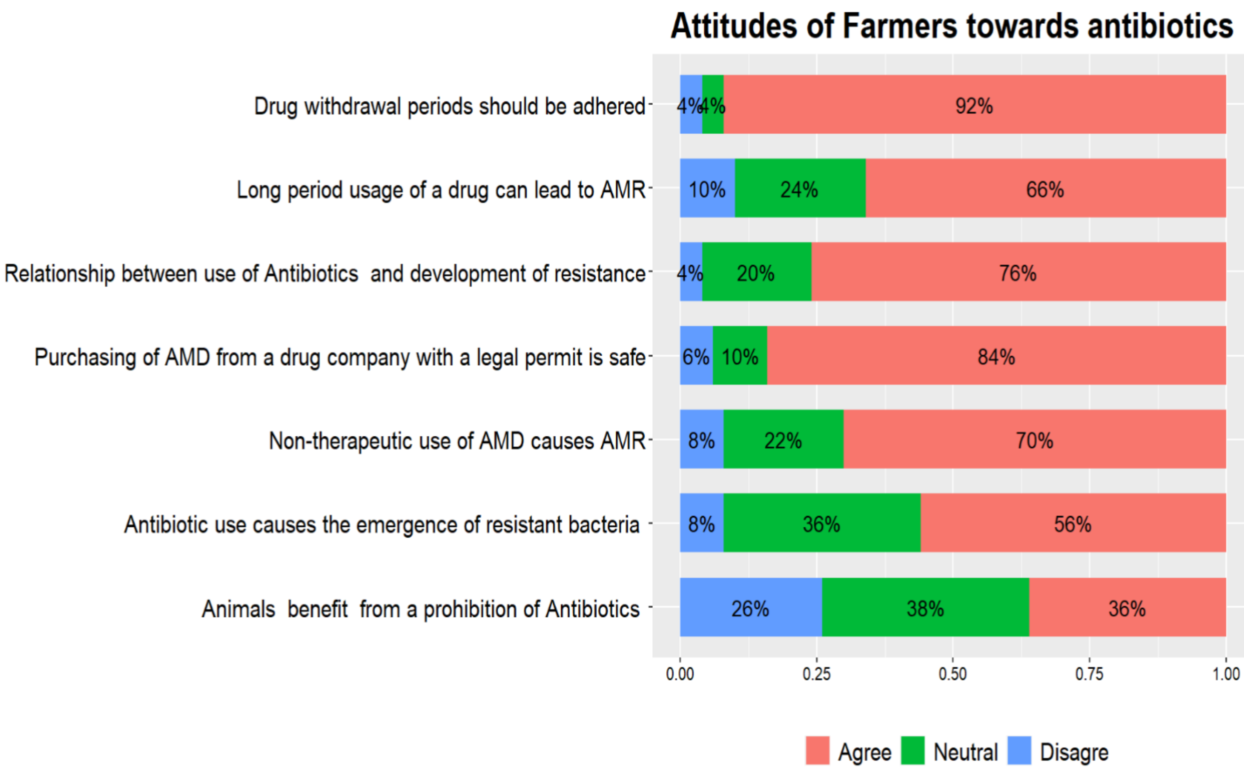

Figure 2: Attitudes of farmers toward antibiotic use

Table 13: Association between broiler farmer's age and their attitudes toward antibiotic use

	Age groups				
Characteristic	**0-30**, N = 12[1]	**31-40**, N = 9[1]	**41-50**, N = 13[1]	> **50**, N = 16[1]	**p-value**[2]
long period usage of a drug can lead to AMR					0.046
Agree	7 (58%)	3 (33%)	11 (85%)	12 (75%)	
Neutral	3 (25%)	6 (67%)	1 (7.7%)	2 (13%)	
Disagree	2 (17%)	0 (0%)	1 (7.7%)	2 (13%)	
[1]n (%)					
[2]Fisher's exact test					

4.4 The practice of farmers on antibiotics use

The practice of the farmers on antibiotics use is presented in Tables 14 & Appendix 7. 86% of farmers reported using drugs following the manufacturer's recommendations and for the duration specified on the label. Before giving antibiotics to chickens, 92% of farmers checked the expiry date, and when drugs expire, 94% of people stop using them. 48% said they chose drugs based on drug/feed/chicken seller recommendations, and 24% said they administer drugs to sick animals themselves. 66% of farmers followed other farmers' antibiotic-use recommendations. 38% reported increasing antibiotic doses and frequency of administration as long as animals show no signs of recovery, while 26% reported discontinuing antibiotics if animals feel better after the first day of treatment. 82% said they adhered to drug withdrawal periods before selling their chickens. 24% of the farmers reported that they would continue to use antibiotics in animals even if they knew they were harmful to public health. Both the level of education and the length of farming (years) had a significant association with farmers' practice of discontinuing antibiotic administration after the chickens improved after the first day of administration ($p < 0.05$) (Table 15 & Table Appendix 11).

Table 14: Practice of farmers on antibiotic use

Characteristic	**N = 50**[1]
Check the expiry date of antibiotics before use	46 (92%)
Increase antibiotic dose and frequency if animals don't show recovery	19 (38%)
Stop antibiotic treatment if animals show improvement	13 (26%)
Consider other farmers' recommendations on antibiotic use	33 (66%)
Continue using antibiotics even if harm public health	12 (24%)
Adhere to drug withdrawal periods before sending animals to slaughterhouse	41 (82%)

Table 15:Association between farmer's length of farming (years) and their practice on antibiotic use.

	Farming scale			
Characteristic	**Small**, N = 20[1]	**Middle**, N = 20[1]	**Large**, N = 10[1]	**p-value**[2]
If animals feel better after the first day of treatment, I stop giving the antibiotics	2 (10%)	9 (45%)	2 (20%)	0.038
[1]n (%)				
[2]Fisher's exact test				

4.5 HPLC analysis

Forty chicken samples (20 kidneys and 20 liver) were screened by High-Performance Liquid Chromatography (HPLC) to check for four different types of antibiotics (Ciprofloxacin, Sulfamethoxazole, Trimethoprim, and Amoxicillin). The HPLC analysis has shown that the amoxicillin standard had a peak at a retention time of 1.7 minutes on average (Figure 4). The amoxicillin standards serial dilutions of 50, 100, 200, 300, and 500 µg/ mL when plotted against their peak areas had a linear equation of $y = 25732x + 209719$ and R^2 value of 0.9992. Ciprofloxacin standard had a peak at a retention time of 1.4 minutes (Figure 5). The calibration curve of the cipro peaks against the serial dilutions had a linear equation $y=224239x+350941$ with an R^2 value of 1. Sulfamethazine and trim standards were run at the same time and trimethoprim had a peak area at 2.8 minutes while sulfamethazine had a peak at 3.3 minutes retention time (Figure 6). Trimethoprim had a linear equation of $y=16817x+70863$ with a R^2 value of 0.9994 while sulfamethazine had a linear equation of $y=42875x+95952$ with a R^2 value of 0.9998.

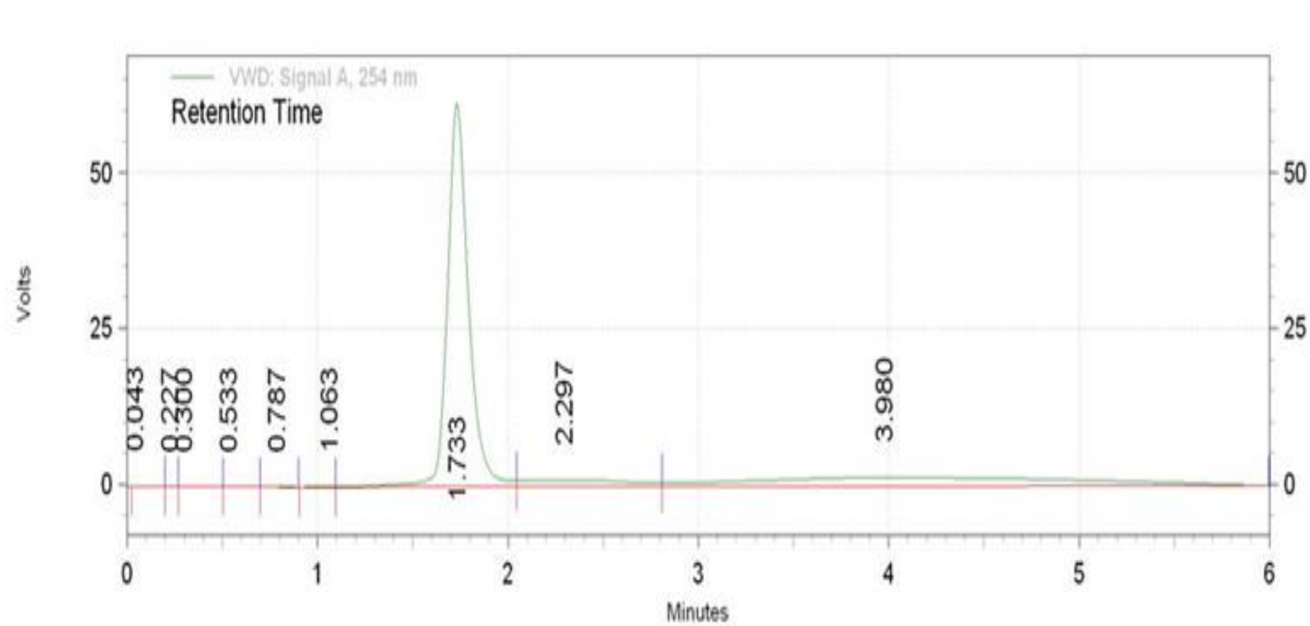

Figure 3:Chromatogram of Amoxil standard peak and retention time

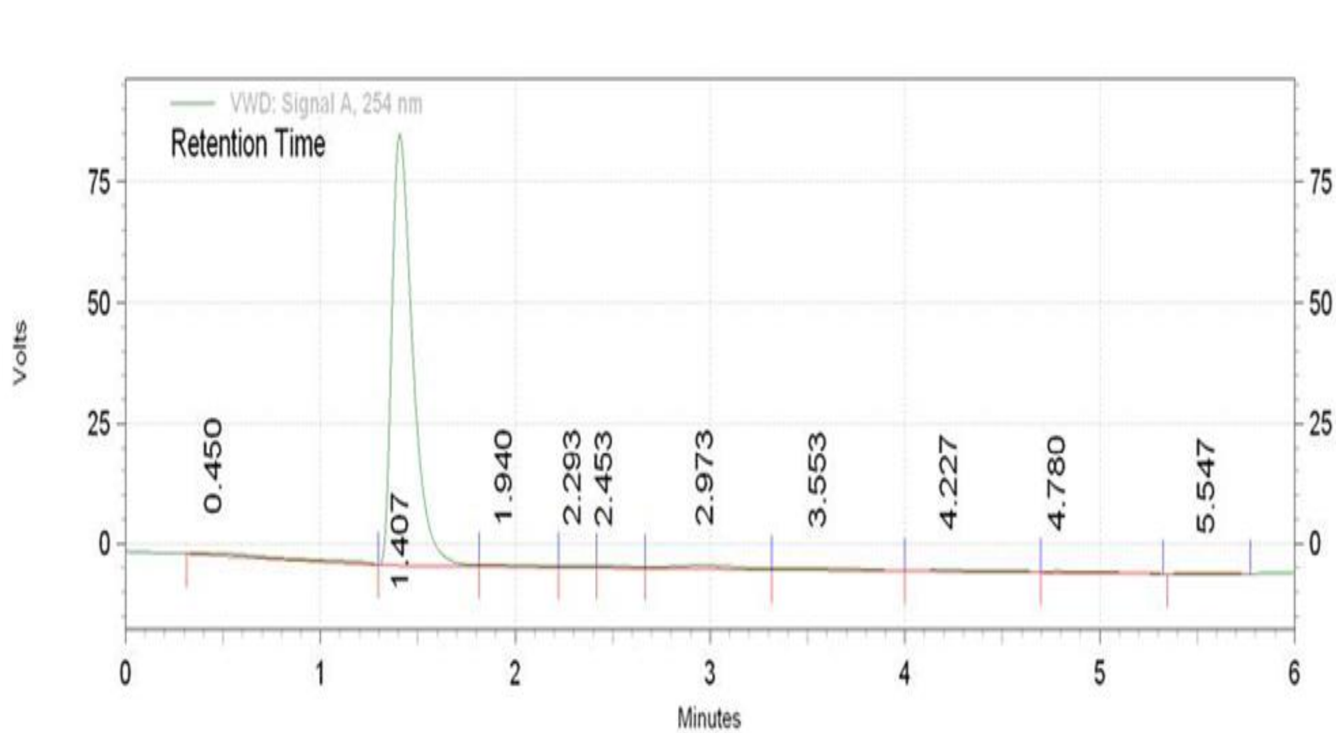

Figure 4: Chromatogram of ciprofloxacin standard peak and retention time

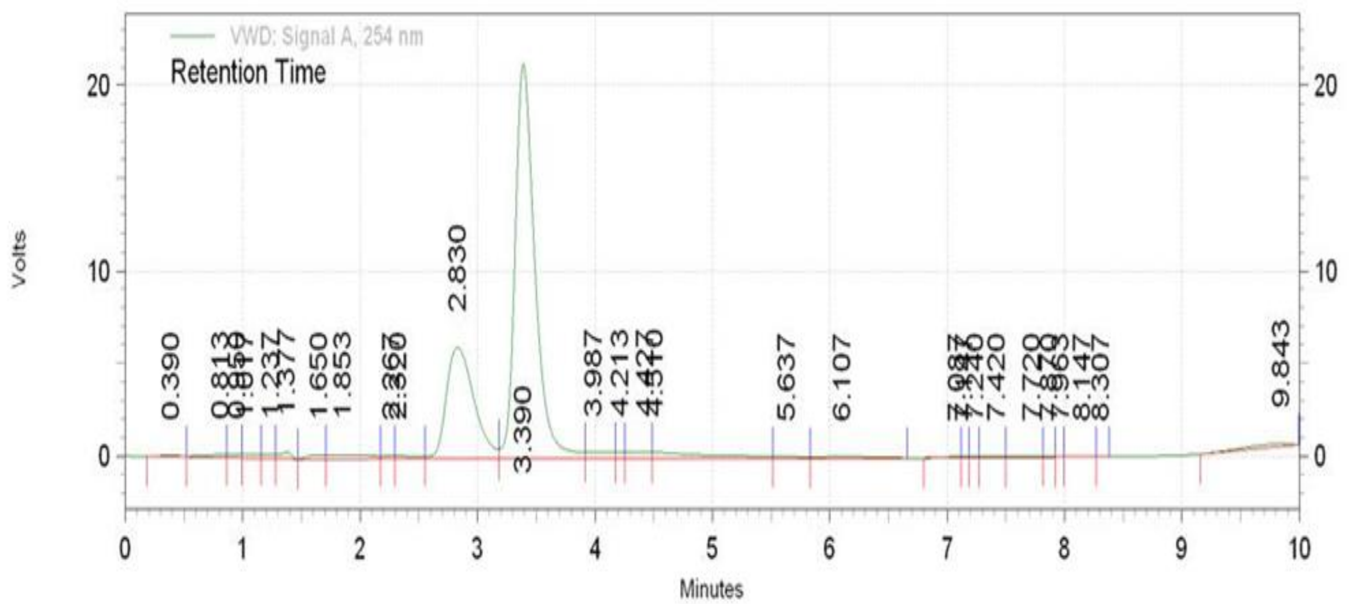

Figure 5: Chromatogram for sulfamethazine and trimethoprim standard peak and retention time

4.6 Antibiotics residues detected

Out of the 40 samples (20 kidney and 20 liver), Antibiotic residues were present in 37 samples (19 kidney and 18 liver samples), (Figure 6). Analysis of the chicken test's residual antibiotics showed that 7.5% (3/40) of the chicken sample samples had no detectable antibiotic residues. A minimum of one residue was present in 92.5% (37/40) of the samples. Out of the contaminated samples, 59.5% had one antibiotic residue, with 5.4% (2) containing only Trimethoprim contamination and 54.1% (20) containing only amoxicillin contamination. The presence of multiple antibiotic residues was detected in 40.5% of the samples, of which 24.3% (9) contained Trimethoprim, Sulfamethoxazole, and Amoxicillin; 10.8% (4) contained ciprofloxacin, sulfamethoxazole, and Amoxicillin; and 5.4% of the samples were contaminated with trimethoprim and amoxicillin (Table 24). Overall findings from individual analysis of the chicken samples (N=40) against each antibiotic revealed that 88% (35) of the samples were contaminated with amoxicillin, 30% (13) were contaminated with trimethoprim, 10% (4) were contaminated with ciprofloxacin, and 2% (1) were contaminated with sulfamethoxazole (Figure 6).

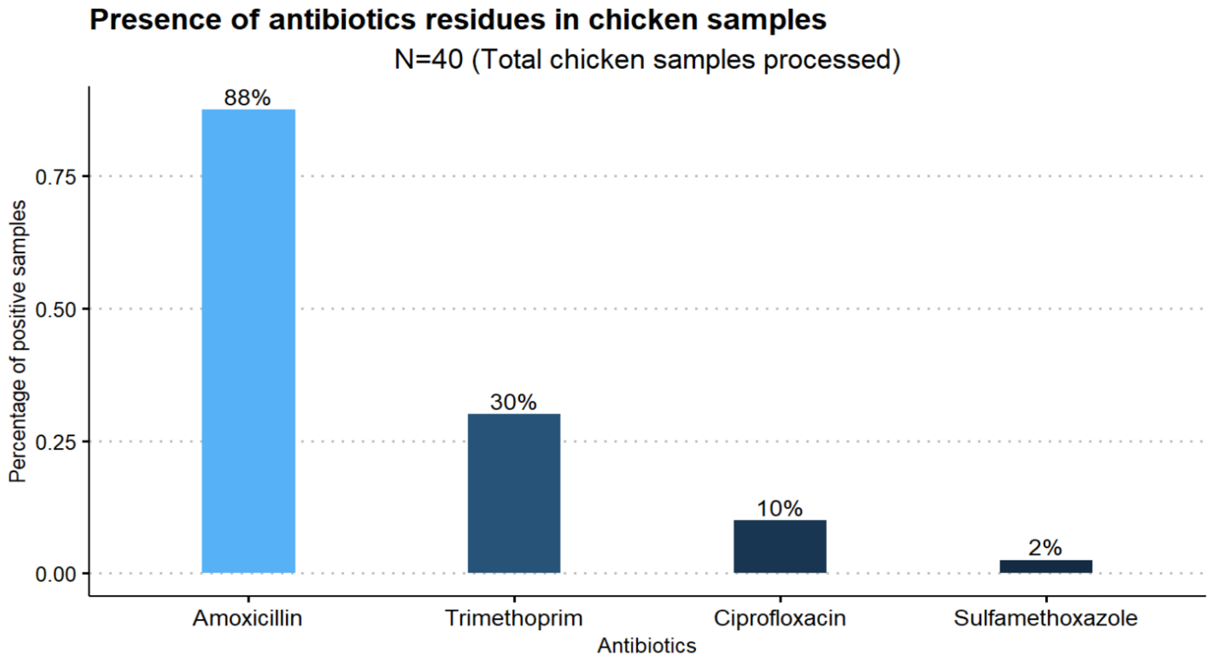

Figure 6: The presence of each antibiotic residue in all the chicken samples (N=40)

4.7 Antibiotic residues in chickens

Out of the twenty chickens, antibiotic residues were identified in 19 chickens. Specifically, in the kidneys, 64% of the samples showed the presence of amoxicillin, 21% exhibited trimethoprim residues, 11% contained ciprofloxacin, and 4% had sulfamethoxazole residues. In the liver, 71% of the samples had amoxicillin residues, 25% showed trimethoprim residues, and 11% exhibited ciprofloxacin residues. Notably, no sulfamethoxazole residues were detected in the liver samples (Figure 7).

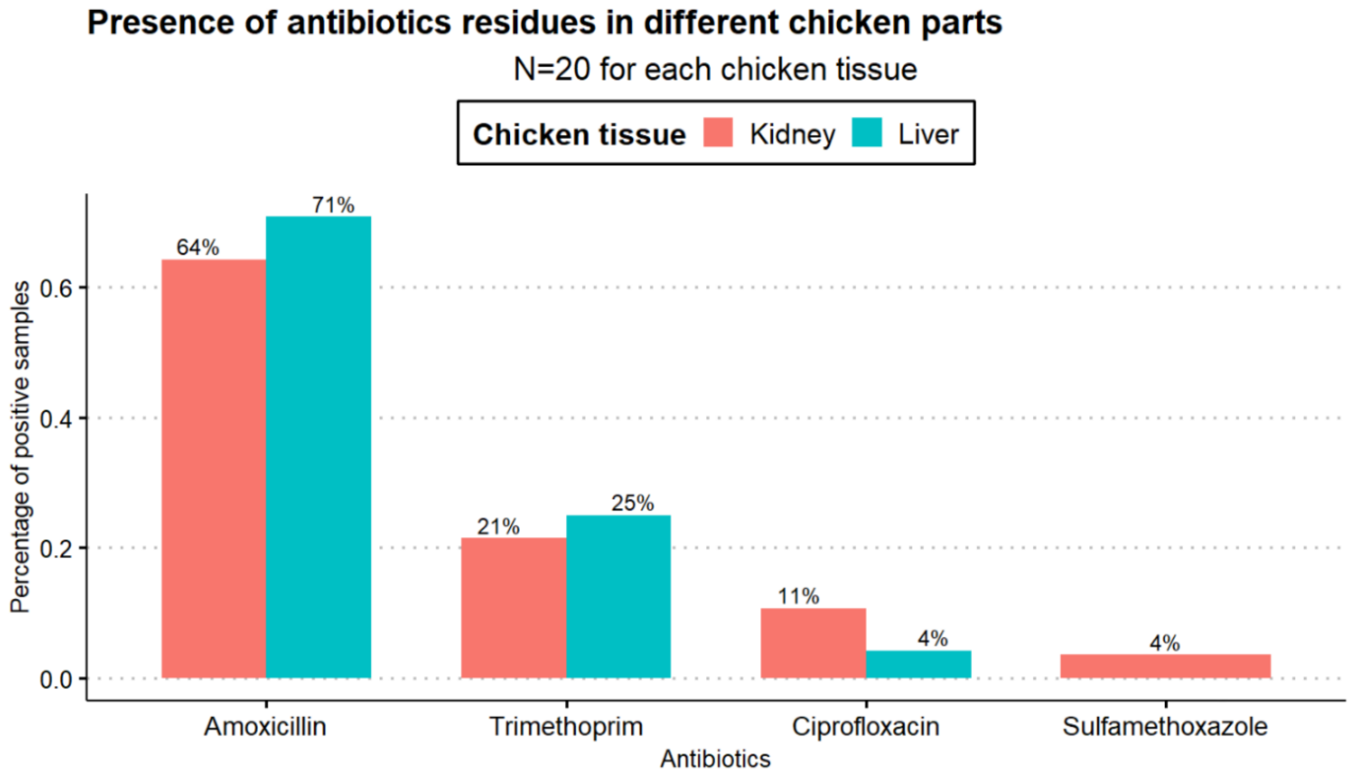

Figure 7: The detected antibiotic residues in kidney and liver chicken samples; CIP >Ciprofloxacin), TRIM > Trimethoprim, SULFER > Sulfamethoxazole, AMOX > Amoxicillin.

4.8 Antibiotic residues detected under different broiler farming management systems

Among the samples from the large-scale management system, antibiotic residues were detected in all six chickens (6 liver and six kidney samples) whereby 60% were contaminated with amoxicillin, 30% exhibited trimethoprim residues, and 10% had ciprofloxacin in their tissues. In medium-scale farm management systems, antibiotic residues were detected in all seven (7 liver and 7 kidney) chickens whereby 74%, 16%, 5%, and 5% of amoxicillin, trimethoprim, sulfamethoxazole, and ciprofloxacin residues, were present in the samples respectively. Under a small-scale farming system, antibiotic residues were detected in six chickens (5 livers and 6 kidneys) samples of the seven chickens tested where 69%, 23%, and 8% of the seven tested chickens had amoxicillin, trimethoprim, and ciprofloxacin residues, respectively (Figure 9).

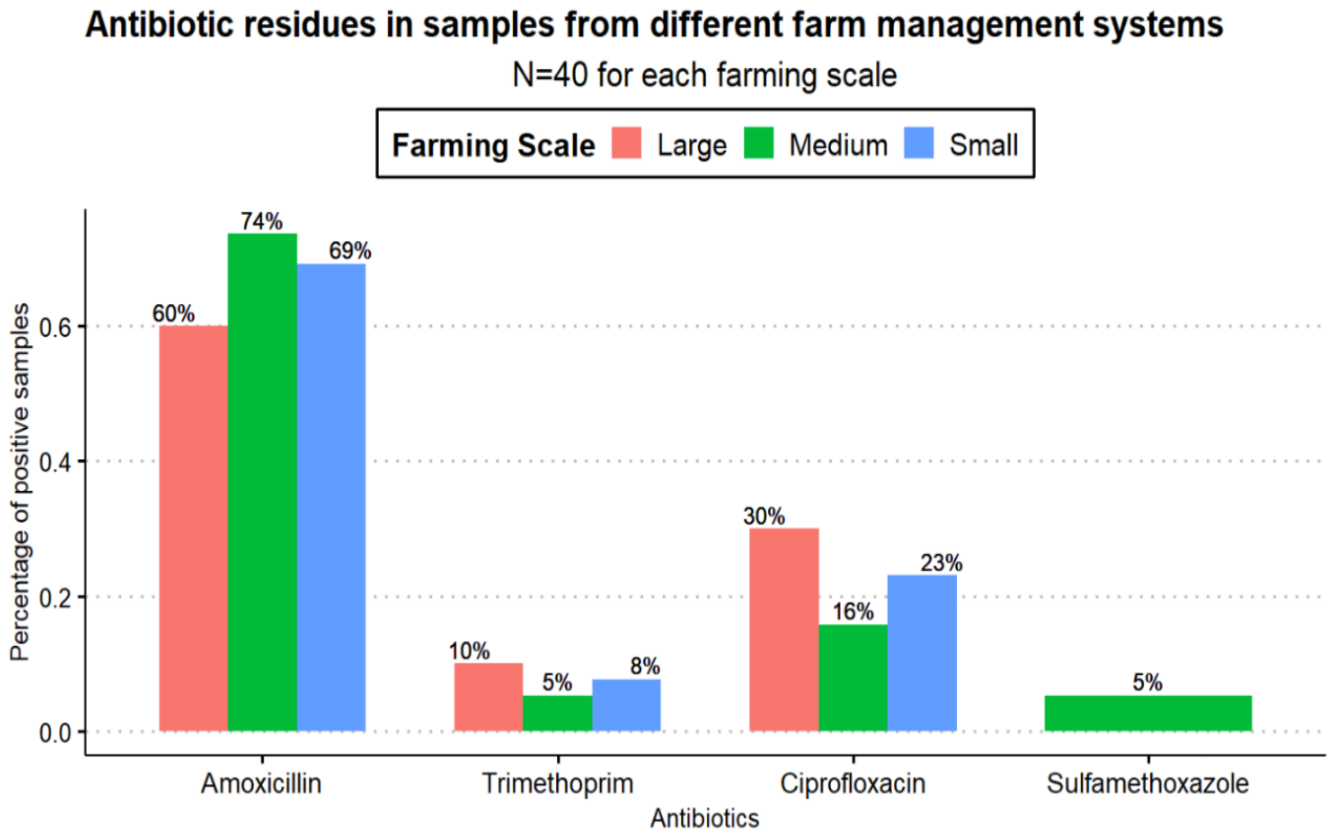

Figure 8: Presence of Ciprofloxacin, Sulfamethoxazole, Trimethoprim, and Amoxicillin antibiotic residues in chicken samples under different livestock management systems.

4.9 Antibiotics residues concentrations

The concentration of the residual antibiotics was determined using the calibration standards equation. The overall concentration of the antibiotic residues in the chicken samples amoxicillin was present in chicken samples (18 kidneys and 19 liver), with a concentration range of 0.204 µg/kg - 0.924 µg/kg. Ciprofloxacin was present in 4 chicken samples (2 kidneys and 2 livers) with a concentration range of 0.059 to 5.139 µg/g within the samples. Trimethoprim was found in 13 chicken samples (7 kidneys and 6 livers) with a concentration range of 0.3 to 3.526 µg/g within the samples. In one kidney sample, sulfamethoxazole was detected at a quantity of 0.957 µg/g (Table 17). Specifically in the kidney and liver tissues amoxicillin had a concentration range of 0.20 - 0.65 µg/Kg in the liver and 0.27 - 0.92 µg/Kg in the kidney. Ciprofloxacin had a concentration range of 0.06 - 0.18 µg/g in the liver and 0.13 - 5.13 µg/g in the kidney. Trimethoprim had a concentration range of 0.34 - 3.26 µg/g liver and 0.74 - 2.25 µg/g in the kidney. Sulfamethoxazole concentration ranged from ND - 0.96 µg/g in the liver and no kidney tissue was detected of sulfamethoxazole

(Table 18). The concentration of the kidney and liver samples under various broiler management systems revealed that amoxicillin had concentration ranges of 0.27 - 0.80 kidney and 0.20 - 0.53 µg/g in liver of small-scale system, 0.41 - 0.92 µg/g kidney and 0.26 - 0.59 µg/g liver of medium scale management system, and 0.28 - 0.69 µg/g kidney and 0.33 - 0.65 µg/g liver of large-scale management system. In a small-scale management system, trimethoprim concentrations ranged from 0.74 g/g to 2.25 g/g in the kidney and 0.34 g/g to 3.26 g/g in the liver. In a medium-scale management system, trimethoprim concentrations ranged from 0.75 g/g to 1.17 g/g in the kidney and 0.52 g/g to 1.15 g/g in the liver. In large-scale management, the concentration ranged from 0.84 µg/g - 1.30 µg/g kidney and a concentration of 3.12 µg/g in one liver. Ciprofloxacin was present in one kidney and one liver tissue under small-scale management with concentrations of 5.13 g/g and 0.18 g/g, respectively. It was also present in one liver sample under medium-scale management with a concentration range of 0.52 g/g - 1.15 g/g and in one kidney sample with a concentration of 0.06 g/g. Only one kidney sample contained sulfamethoxazole at a value of 0.96 g/g (Table 19).

Table 16: The concentration of the antibiotic residues in all the chicken tissue samples

Antibiotic	***Sample tested***	***positive samples n (%)***	***Conc of positive samples (Mean ± SE)***	***Range***
Amoxicillin	40	37, (88)	0.50±0.21 µg/Kg	0.204 - 0.924 µg/kg
Ciprofloxacin	40	4, (10)	1.38±6.99 µg/g	0.059 - 5.139 µg/g
Trimethoprim	40	11, (30)	1.019±1.38 µg/g	0.339 - 3.256 µg/g
Sulfamethoxazole	40	1, (2)	0.913 µg/g	ND - 096 µg/g

Table 17: The concentration of the antibiotic residues-positive kidney and liver chicken tissues

Antibiotic residues	**Chicken tissue**					
	Liver (N=18)			**Kidney (N=19)**		
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**
Amoxicillin	0.4µ µg/Kg	0.12	0.20 - 0.65 µg/Kg	0.59 µg/Kg	0.18	**0.27 - 0.92 µg/Kg**
Ciprofloxacin	0.12 µg/g	0.09	0.06 - 0.18 µg/g	2.63 µg/g	3.54	**0.13 - 5.13 µg/g**
Trimethoprim	1.56 µg/g	1.3	0.34 - 3.26 µg/g	1.22 µg/g	0.54	**0.74 - 2.25 µg/g**
Sulfamethoxazole	0.96 µg/g	ND	ND - 0.96 µg/g	ND	ND	**ND**

Table 18: The concentration of the antibiotic residues-positive kidney and liver chicken tissues under small-scale, medium-scale, and large-scale broiler management systems.

Characteristic	**Farm management system**					
	Large		**Medium**		**Small**	
	kidney, N = 6	**liver**, N = 6	**kidney**, N = 8	**liver**, N = 7	**kidney**, N = 5	**liver**, N = 5
Amoxicillin						
Mean µg/Kg (SD)	0.60 (0.23)	0.41 (0.13)	0.65 (0.14)	0.39 (0.11)	0.47 (0.16)	0.42 (0.13)
Range	0.27 - 0.80	0.20 - 0.53	0.41 - 0.92	0.26 - 0.59	0.28 - 0.69	0.33 - 0.65
Ciprofloxacin						
Mean µg/g (SD)	5.13 (NA)	0.18 (NA)	0.13 (NA)	ND	0.06 (NA)	ND
Range	ND - 5.13	ND - 0.18	ND - 0.13	ND	NA - 0.06	ND
Trimethoprim						
Mean µg/g (SD)	1.50 (0.76)	1.53 (1.53)	0.96 (0.30)	0.84 (0.45)	1.07 (0.32)	3.12 (ND)
Range	0.74 - 2.25	0.34 - 3.26	0.75 - 1.17	0.52 - 1.15	0.84 - 1.30	ND - 3.12
Sulfamethoxazole						
Mean µg/g (SD)	0.96 (NA)	ND	ND	ND	ND	ND
Range	0.96, 0.96	ND	ND	ND	ND	ND

CHAPTER 5: DISCUSSION

5.1 Farmer’s knowledge

All farmers reported using antibiotics on their farms, primarily to address diseases in chickens displaying undesirable signs. Notably, most of the farmers specifically mentioned using antibiotics for treating chicken diseases. According to a study by Phares et al. (2020), 94.7% of farmers administered antibiotics, and the majority of them (86.3%) did so to cure and prevent infections. The usage of antibiotics in animals to increase weight was mentioned by 34% of the farmers. According to research by Phares et al. (2020), 13% of farmers acknowledged employing antibiotics to promote growth. To reduce the cost of production most of the farmers reported the use of antibiotics without consulting the veterinarian and most of them prescribed the drugs themselves. This practice was associated with reasons such as farmer's experience and positive results, high treatment cost of veterinarian, poor veterinary services such as late response of veterinarian and some of the farmers considered some veterinarians to lack experience. One of the most significant factors encouraging farmers to use antibiotics without consulting a veterinarian is the cost of veterinary services (Ozturk et al., 2019). These results are consistent with a study by Geta & Kibret, (2021), who reported that owners and employees of livestock farms took antibiotics without seeing a veterinarian, and 72.5% bought them from private pharmacies without a prescription due to the reasons of cost serving to reduce the cost of production. According to a study by Islam et al. (2016) the majority of farmers (>60%) used antibiotics without a veterinarian's prescription due to farmers' claims of past success, the lack of veterinary services, and the higher expense of veterinary services. 40% of participants in a survey by Dyar et al. (2020), said they rarely consult veterinarians when their pigs are ill, even though the majority of participants had little experience keeping pigs. In the same manner, Sadiq et al. (2018), also reported that the majority of the farmers administered drugs to their livestock without a veterinarian prescription. Veterinarians must conduct clinical examinations, administer antimicrobial agents, when necessary, make appropriate choices based on experience, and provide detailed treatment protocols, including precautions and withdrawal times, for veterinary importance (World Organization for Animal Health, 2022).

The use of antibiotics by farmers without consulting a veterinarian can raise a broader public health concern. This practice is driven by factors such as personal experience, cost considerations, and dissatisfaction with veterinary services, indicating potential shortcomings in animal health management. This trend may lead to suboptimal treatment outcomes, which can aid the persistence and spread of diseases among chickens. Additionally, relying on antibiotics to address issues such as delayed veterinary responses and perceived lack of veterinary expertise reveals systemic challenges in accessing and ensuring the quality of veterinary services. These issues can compromise the health and well-being of livestock, impacting the safety of animal products in the food supply chain. Inadequate veterinary diagnosis may also affect the early detection of emerging diseases that have the potential to affect both animal and human health.

Even though a majority (64%) of the farmers said that they use antibiotics to treat bacterial diseases many of the farmers in the study did not fully understand what antibiotics are, what diseases can they be used, and when to use the antibiotics. Similarly Dyar et al. (2020), also reported that participants had little understanding of what antibiotics are, how and why they ought to be administered, and how antibiotic resistance works. Osei Sekyere, (2014) also reported that apart from using what they learned about clinical antibiotics, the farmers knew very little about the pharmacology of antibiotics. In the examined population of farmers, the ability to identify diseases was limited, yet they demonstrated proficiency in identifying associated symptoms. Among these symptoms, watery stools and respiratory difficulties emerged as the most frequently cited. The likelihood that the farmers were treating the chickens based on the symptoms rather than necessarily the real sickness is very high, which is another factor contributing to the development of resistance towards antibiotics in the chickens. Similarly, to these results, Osei Sekyere, (2014) reported that farmers treated diseases largely for their symptoms while disregarding their fundamental causes because they were unaware of the disease's name. The lack of knowledge regarding the specific indications, proper administration, and the concept of antibiotic resistance increases the risk of misuse and contributes to the development of antimicrobial resistance in broiler chickens. Treating chickens based only on observed symptoms, as highlighted in the study, indicates an important gap in disease diagnosis and targeted treatment. In addition to endangering animal welfare, this behavior raises the possibility of the emergence of antibiotic resistance.

The lack of knowledge has also been demonstrated by some farmers who reported that they use antibiotics to treat every disease on the farm. To avoid making losses in broiler production farmers will do anything to make profits and minimize costs, so the use of antibiotics works on most diseases according to some farmers, and some farmers are not even aware of other drugs apart from antibiotics. This needs to be taken into consideration and needs serious attention if we are to fight against antimicrobial resistance as these farmers are putting the public in danger through their lack of knowledge of antibiotic use. The majority of farmers' lack of expertise in livestock production and the usage of antibiotics is a contributing factor to the low levels of knowledge in this study. Farmers who lack expertise in livestock production and the usage of antibiotics may not be aware of the appropriate use of antibiotics, including the correct dosage, duration, and withdrawal periods. 62% of those surveyed said they have never received any training on how to raise chickens or use antibiotics. Similar to these findings, research by Phares et al. (2020), found that the majority (74%) of farmers had never been taught about antibiotics. The lack of understanding among farmers, as evidenced using antibiotics for various farm diseases without proper knowledge, raises immediate concerns for animal welfare and public health. Using antibiotics without understanding how to use them properly, and not considering other ways to treat illnesses, can be risky. The indiscriminate use of antibiotics without adequate knowledge can lead to ineffective treatments, prolonged illnesses, and economic losses. It is crucial to improve farmers' understanding of antibiotic use and resistance to ensure responsible antibiotic usage and effective disease management in animals.

The study found a significant ($p = 0.062$) correlation between the number of years spent farming and the use of antibiotics. This is because farmers who have been raising chickens for a long time have demonstrated a strong understanding of when to administer antibiotics to their flocks. Similarly, a study revealed that farmers with more than 10 years of farming expertise were more likely to give antibiotics than farmers with fewer than 10 years of experience (Phares et al., 2020). A correlation between farmers' knowledge of antibiotics and the size of their farms was also discovered when they were questioned about their comprehension of the link between improper antibiotic use and antimicrobial resistance ($p = 0.028$). Commercial farmers (100%) and middle-scale farmers (75%) demonstrated the highest levels of understanding of the connection. The relationship between farmers' experience and antibiotic use, as well as the connection between farm size and understanding the link between improper antibiotic use and antimicrobial resistance, has important implications for public health. Experienced farmers who demonstrate a strong understanding of antibiotic administration suggest the potential for responsible antibiotic practices, which can contribute to reduced antimicrobial resistance risks. On the other hand, the significant knowledge gap observed in smaller-scale farms indicates the need for targeted interventions and educational efforts in this demographic. It is essential to enhance knowledge among all farmers, regardless of experience or farm size, to promote responsible antibiotic use and mitigate the broader public health risks associated with antimicrobial resistance in poultry farming.

Farmers reported the use of antibiotics for all respiratory diseases and diarrhea in chickens. Antibiotics cure infectious diseases brought on by bacteria, although typical symptoms like coughing and diarrhea can also be brought by fungi, viruses, and parasites. Since viral illnesses, including viral respiratory infections, can be followed by bacterial ones, a proper diagnosis is essential before beginning treatment (Magnusson et al., 2019). Common antibiotics used in disease treatment by farmers based on available drugs in veterinary shops in Blantyre include tetracyclines (doxycycline Oxytetracycline), aminoglycoside (gentamicin and neomycin), polypeptides (colistin), micro ride (erythromycin), sulfonamides (sulfadiazine and trimethoprim). This is similar to a study by Aworh et al. (2021), who found that tetracycline (94.4%), sulfonamide (80.5%), penicillin (69.4%), aminoglycosides (61%), and colistin (52.8%) were the five most popular antimicrobials on the market. Sadiq et al. (2018) also claimed that medications such as cephalosporins, penicillin, tetracyclines, aminoglycosides, sulfonamides, and macrolides were often utilized in Malaysia. According to the World Organization for Animal Health all of these antibiotics apart from polypeptides are considered to be very important antibiotics in veterinary use (Diaz et al., 2014). Tetracyclines are classified as highly important antibiotics used in humans, while aminoglycoside, microlides, polypeptides, and sulfonamides have been listed as important antibiotics (World Health Organization, 2019). The Guideline Development Group (GDG) recommends a reduction in the use of medically important antimicrobials in food-producing animals for growth promotion and prevention of undiagnosed infectious diseases. It is inappropriate to utilize critically important antimicrobials for controlling the spread of clinically diagnosed infectious diseases within livestock or treating livestock with clinically confirmed transmissible diseases. However, veterinary professionals may use antimicrobials for disease prevention if they determine a high risk of spread (WHO Guideline Development Group et al., 2018). New legislation in the European Union bans routine antibiotic feeding to farm animals, limits antibiotic usage to sick, individual animals, and outlaws reimbursement for low welfare practices (World Animal Protection, 2023). On the other hand, the American Food and Drug Administration (FDA) introduced new rules in 2017 to stop the utilization of antibiotics as feed additives for the growth of livestock and poultry and to combat antibiotic resistance. The new regulations forbid the over-the-counter sale of critical human antibiotics to farmers.

The use of antibiotics by farmers to treat different diseases in chickens, such as respiratory issues and digestive problems, is causing concern about the accuracy of diagnosis and appropriate treatment methods. The reliance on farmers' judgment for antibiotic use can lead to unintended misapplication, including incorrect dosages, inappropriate treatment durations, and unnecessary use, ultimately contributing to antibiotic resistance, compromising animal health, and posing risks to public health. The popularity of certain types of antibiotics, like tetracyclines and aminoglycosides, reflects global trends and highlights the importance of using antibiotics carefully. Farmers must follow internationally recognized guidelines, such as those set by the World Organization for Animal Health, to ensure responsible use of antibiotics and prevent potential public health implications.

5.2 Farmer’s attitudes

All the farmers reported the purchase of antibiotics from registered shops and no farmer reported buying drugs from unauthorized vendors. According to the farmers buying drugs from such shops is safe as far as chicken production is concerned and buying from authorized personnel is considered risky. Farmers should purchase antibiotics from reputable, licensed retailers or well-established businesses (Magnusson et al., 2019). However, the purchase of drugs by farmers remains unregulated in Malawi as farmers buy drugs directly from the counter and without a prescription. Farmer access to antibiotics in Malawi was reportedly made simple by veterinary pharmacies, according to Mankhomwa et al. (2022). A study in Kenya by Kariuki et al. (2013) also reported that the majority of antimicrobials were bought over the counter or from animal health assistants, without consulting a veterinarian, as they were easily accessible. This is consistent with research conducted in Tanzania, Serbia, Ghana, Nigeria, India, Peru, and Malaysia where antimicrobials were sold over the counter and without a valid prescription (Aworh et al., 2021; Chauhan et al., 2018; Donkor et al., 2012; Horumpende et al., 2018; Horvat et al., 2017; Morgan et al., 2011; Redding et al., 2013; Sadiq et al., 2018). Normally the procedure was supposed to be that the farmers should report disease cases to veterinarians, who would letter on diagnose the animals, purchase the drugs, and administer the recommended drugs to the animals. Normally, the veterinary shops are supposed to sell the drugs to registered veterinarians and not directly to farmers. The use of antibiotics in the livestock industry varies significantly around the world, with low-income countries having limited access to them and unreliable or counterfeit pharmaceuticals being sold on unregulated markets for free. This allows for the use of antibiotics against the advice of veterinarians or other qualified animal health professionals (Magnusson et al., 2019).

The unrestricted purchase of antibiotics by farmers, without prescriptions and direct consultation with veterinarians, poses a potential threat to public health in several ways. Firstly, without proper diagnosis and guidance from veterinary professionals, there is a risk of misusing antibiotics, leading to ineffective treatments and potential harm to animal and human health. Secondly, unregulated antibiotic access may contribute to the emergence of antibiotic-resistant bacteria, compromising the effectiveness of these drugs in treating infections in both animals and humans. Additionally, the lack of oversight increases the likelihood of substandard or counterfeit antibiotics entering the market, posing further risks to public health. Although farmers in the study reported obtaining antibiotics from registered shops, the absence of regulatory oversight in drug acquisition contributes to unmonitored and possibly inappropriate use. The widespread availability of antibiotics over the counter, as observed in various countries, indicates a need for regulatory measures to ensure responsible antibiotic access.

Many farmers' choice of drugs was influenced mostly by the salesperson in the vet shop, based on fellow farmers' advice, based on experience, the price of the drugs, and also the availability of the drugs. The study has revealed that farmers who have much experience are more likely to influence other small farmers on where to buy the drugs and how to use them. Some farmers reported that other fellow farmers who are not veterinarians prescribe the drugs to their chickens. This practice just shows how prone we are to antibiotics due to misuse and poor handling of antibiotics by the farmers. These outcomes align with findings from prior research, for instance, a study in turkey by Ozturk et al. (2019), reported that when it came to using antibiotics to treat animal infections, a sizable portion of the participants in their survey favored following other farmers' advice. The inappropriate use of antibiotics in livestock raises concerns about food safety, as residues of these drugs may find their way into animal products, such as meat and eggs. Consumption of such products may contribute to the development of antibiotic-resistant bacteria in humans, posing risks to individuals and public health. The welfare of animals is also affected when antibiotics are misused, leading to inadequate treatment, unnecessary suffering, and the potential emergence of antibiotic-resistant infections in animals. Lastly, the misuse of antibiotics contributes to the global health crisis of antibiotic resistance. The spread of antibiotic-resistant bacteria through the food chain and the environment poses a direct threat to public health by limiting the effectiveness of antibiotics in treating bacterial infections.

According to the surveyed farmers, the study has found that farmers have a good attitude toward antibiotic use and antimicrobial resistance development. 65% of the farmers agreed that prolonged antibiotic use can lead to antibiotic resistance development and 70% of the farmers agreed that non-therapeutic antibiotic use does contribute to antimicrobial resistance. Despite this situation, 44% of farmers hold the belief that restricting antibiotic use in poultry farming would result in decreased productivity and increased suffering among the chickens. While to the contrary some farmers believe that if antibiotics can be replaced by other alternative drugs that are not antibiotics and can treat diseases like antibiotics then they can adopt the new drugs. Some farmers even went further to admit that they use the antibiotics because they don't have a choice of other drugs since antibiotics keep them in business, they don't have a choice but to use them. Contrary to this popular belief, according to the National Research Council Committee, (1980), responsible antibiotic use is a supplement to appropriate hygiene and animal husbandry methods rather than a replacement. He further argued that a ban on subtherapeutic feed additive antibiotics would require changes in disease control, including preventing exposure, treating outbreaks, and using immunological methods, that affect animal production (National Research Council Committee, 1980). However recent studies have shown that vaccines can be an alternative to antibiotics used in livestock. Antibiotic use in animal populations can be decreased with the use of vaccines and other alternative products, which will benefit animal agriculture in the long run. For vaccines to be widely utilized in animals that produce food, they must be secure, efficient, simple to administer, and affordable (Hoelzer et al., 2018). The second international conference on Options for Antibiotics was organized by the US Department of Agriculture and the World Organization for Animal Health, which focused on six important subjects: vaccines, probiotics, prebiotics, immunomodulatory products (antibodies, peptides, and cytokines), new-generation medicines, chemicals, and enzymes, as well as regulatory approaches for registering these alternatives (World Organization for Animal Health, 2019). The study revealed that many farmers recognize the risks of antibiotic resistance (AMR), but they still believed in the necessity of antibiotics for poultry productivity. This reliance on antibiotics, due to perceived limitations in alternative drugs, poses potential risks to public health by contributing to environmental contamination and antibiotic residues in food. Some farmers express openness to exploring alternative drugs, emphasizing the importance of comprehensive awareness and education on effective alternatives, such as vaccines. This shift toward alternatives can significantly impact food safety, reduce environmental risks, and enhance overall well-being in poultry production.

The age of the farmer was significantly correlated with knowledge and attitude usage. When compared to farmers between the ages of 31 and 40 and 18 to 30, those between the ages of 41 and 50 showed higher knowledge and a better attitude regarding the ideal time to use antibiotics. Comparable to the results of Hossain et al. (2022), who found that the farmers' ages had a substantial impact on their knowledge, beliefs, and conduct regarding AMU and AMR. 31-40-year-old farmers have better knowledge of AMU and AMR, with 1.281 times more attitude compared to 18-30-year-olds. Age has significant effects on farmers' knowledge, attitudes, and practices regarding antibiotic usage, according to research by Moffo et al. (2020) and Ozturk et al. (2019). Given their experience and exposure, older farmers are more likely than younger ones to have a better understanding, attitudes, and practices regarding the utilization of antibiotics in the broiler industry.

The study highlights the correlation between a farmer's age and their knowledge and attitudes toward antibiotic usage, which holds potential public health implications. Older farmers, particularly those between the ages of 41 and 50, exhibited higher levels of knowledge and more favorable attitudes regarding the appropriate use of antibiotics. This suggests that age-related factors may influence farmers' understanding and approaches to antibiotic practices in poultry farming. As older farmers tend to have more experience and exposure, addressing age-specific knowledge gaps through targeted educational programs could enhance overall awareness, promote responsible antibiotic use, and contribute to sustainable practices in the agricultural sector. By bridging generational knowledge disparities, such interventions may help mitigate risks associated with antibiotic misuse, thereby positively impacting food safety and public health.

5.3 Farmers practices

Under farmer’s practice on antibiotics use the survey found that the majority of the farmers reported that they follow drug admiration according to the instruction given by the manufacturer. Even though this is the case some of the farmers have reported continuous administration of drugs when no sign of recovery is being shown by the birds and also others reported the withdrawal of medication after a few days of signs of improvement in their birds. Famers reported that when the animal is not responding to the drugs, they buy other drugs and continue ad mistering. Similar to these findings Ozturk et al. (2019) concluded that 59% of farmers ceased administering antibiotics if animals showed indications of recovery a day after the initial administration of the treatment, whereas 45% continued larger doses in the absence of any recovery. Due to a lack of knowledge about antibiotics, farmers were observed to acquire various drugs under different brand names containing the same active ingredients. Consequently, they administered the same antibiotic over an extended period, thereby contributing to antibiotic resistance. Similarly, Osei Sekyere, (2014), reported that poor dosing practices in antibiotics led to incorrect dosages and abuse of different brands. Farmers lacked adequate measuring instruments, leading to varying antibiotic brands without improving disease conditions. This led to errors in antibiotic handling and administration on farms. Underdosing and continuous usage of antibiotics have been linked to the establishment of AMR, which has helped select and spread resistant bacteria in animals (Wall et al., 2016). Low dosages of antimicrobials contribute to resistance by promoting genetic and phenotypic variability in exposed bacteria, leading to selection bias and less effective killing than higher dosages (Andersson & Hughes, 2014; You & Silbergeld, 2014).

The practices reported by farmers in administering antibiotics reveal potential public health concerns. Continuous administration of drugs without signs of recovery and the withdrawal of medication after initial improvement may lead to inadequate treatment outcomes and potentially compromise the welfare of the chickens. The lack of knowledge about antibiotics and the tendency to switch between different drugs with similar ingredients raise concerns about the accuracy of dosing and potential errors in administration. Inconsistent dosing practices, coupled with a lack of proper measuring instruments, can result in incorrect dosages and contribute to the abuse of various antibiotic brands. Such practices may not only impact the effectiveness of treatment but also pose risks of ineffective disease control.

A majority of the farmers reported that they were aware of withdrawal periods and they followed them before selling their chickens. Similar to our findings, a study by Sadiq et al., (2018b), discovered that more than three-quarters (73%) of the farmers polled said they would not sell or butcher animals that had just received antimicrobial treatment to the market. This is however contrary to studies by Addah et al. (2009) and Osei Sekyere, (2014), who in their studies found that farmers' knowledge of withdrawal periods was low and led to mishandling of antibiotics. Similarly, in a study by Phares et al. (2020), only 16% of farmers consistently observed the withdrawal duration. The adherence to withdrawal periods by the farmers is an appositive finding as it indicates that farmers are taking steps to prevent antibiotic residues in animal-derived products, which can have direct or indirect impacts on human health. However, previous studies have reported low knowledge of withdrawal periods among farmers, leading to mishandling of antibiotics. Therefore, it is crucial to continue educating farmers on proper antibiotic use and withdrawal periods to prevent the spread of antibiotic resistance and safeguard public health.

Overall, a majority (76%) of the farmers were concerned about the public health impacts of antimicrobial resistance. This farmer reported that they are willing to adopt other drugs if antibiotics continue to pose a threat to the public. However, the 24 % of the farmers who were not concerned is still worrisome. These results are contrary to other studies. According to a study by Carter et al. (2016), although many people do not think that antibiotic overuse is a serious issue, they are aware that it contributes to antibiotic resistance. According to Sadiq et al. (2018), most farmers who were surveyed expressed little concern about how AMR will affect animals and the general public's health. Also, McDougall et al. (2017), reported that farmers had little knowledge regarding or concern for the risk of AMR, especially outside their farms, with only a small number of farmers completely agreeing that using antimicrobials on their farms would increase the risk of resistance in their herds and humans, respectively. The varying levels of concern among farmers regarding the public health impacts of antimicrobial resistance have potential implications for broader health education and awareness. Most farmers expressing concern signals a positive attitude towards public health consequences and a willingness to adopt alternative drugs. However, the significant proportion (24%) who lack such concern may indicate a need for targeted education and awareness campaigns to enhance understanding of the potential risks associated with antibiotic use. Addressing this diversity in awareness can contribute to a more comprehensive and unified approach to promoting health-conscious practices among farmers, aligning with broader public health goals.

5.4 Overall detection of antibiotics

The most frequently identified antibiotics were found to be amoxicillin and trimethoprim, followed by ciprofloxacin, and sulfamethoxazole was the least frequently found of all of them. These results are consistent with a research that Lee et al. (2018) conducted, and reported that amoxicillin was detected in most of the samples than the other antibiotics in the study. However, the Amoxicillin residues detected in this study were high as compared to previous studies. This can be attributed to the reasons for the high usage of antibiotics amongst the farmers, the use of different analytical methods during the antibiotics analysis, and different sample sizes among some of the contributing factors to different findings. These findings are contrary to a study by Sarker et al. (2018), who reported that Ciprofloxacin was higher in the samples than the other antibiotic residues, Amoxicillin being one of them. In a review study on "Antimicrobial Usage and Antimicrobial Resistance in Animal Production in Southeast Asia" published by Nhung et al. (2016), amoxicillin was listed as one of the most frequently used antibiotics in Asia (10/10 papers).

Following Thapa, (2021) findings, ciprofloxacin was present in 9% of the samples which is slightly lower than this study’s findings (10%). However, higher percentages of ciprofloxacin have been reported by other studies. A study by Ramatla et al. (2017) reported that ciprofloxacin was present in 32% of the samples. 39% of the chicken samples contained ciprofloxacin contamination, according to Sarker et al. (2018). According to research by Baghani et al. (2019) and Lee et al. (2018), respectively, chicken meat samples contained levels of ciprofloxacin as high as 65% and 95%.

Trimethoprim was the second most prevalent antibiotic residue found in the study, occurring in 32.5% of the chicken samples. According to Lee et al. (2018), antibiotic residues from the drug Trimethoprim were found in 41% of the chicken flesh samples. Trimethoprim has also been listed as one of the antibiotics that are often used in Asia (7/10 publications), according to a review paper by Nhung et al. (2016). Trimethoprim was discovered to be one of the frequently utilized antibiotic compounds in Malawi's broiler production, according to the survey conducted for this study, which supports these findings.

Only one kidney sample out of 40 tested positive for Sulfamethoxazole, making it the least common antibiotic with residues found in as few as 2.5% of the samples. This is due to the reason that sulfamethoxazole is not typically utilized in livestock medicines; instead, sulfadiazine is the sulfonamide component used in livestock medicine most frequently combined with trimethoprim. The antibacterial properties of trimethoprim are combined with a sulfonamide to prevent bacterial folic acid metabolism. They are dihydrofolate reductase inhibitors and competitive inhibitors of dihydrofolate production. There have been no observed variations in efficacy when trimethoprim has been used with sulfadiazine and sulfamethoxazole. Unlike trimethoprim + sulfamethoxazole, which is available for human use, trimethoprim + sulfadiazine is solely available as a veterinary preparation (Papich, 2016). 7.4% of sulfonamide antibiotic residues were found in the samples, according to Yamaguchi et al. (2015). In the study by Jammoul & El Darra, (2019), the least number of positive samples contained sulfonamides. There was no sulfonamide identified in chicken meat in a research by Wang et al. (2017).

Chicken kidney and liver had slightly varied levels of antibiotic residues, with the amoxicillin recording a high level of antibiotic residues in both tissues with higher levels in the liver. Trimethoprim antibiotic levels were also higher in the liver than in the kidney. Ciprofloxacin antibiotic residues were high in the kidney. The kidneys had all four antibiotic residues (amoxicillin, ciprofloxacin, sulfamethoxazole, and trimethoprim) under the study, whereas the liver samples were limited to three antibiotic residues. According to a study by Sarker et al. (2018), the liver had higher residue levels than the kidney, with amoxicillin residues discovered in the liver at 42% and ciprofloxacin residues at 42%. Our research identified elevated levels of amoxicillin in the river compared to the aforementioned study, and a greater quantity of ciprofloxacin was also observed in that investigation compared to our own. A study by Sarker et al. (2018b), also reported that liver tests were more frequently positive for antibiotic residues than other samples and he further justified that due to the liver's role as an organ for the detoxification of numerous metabolites, including antibiotics. Similar to this, Zhang et al. (2021), found that sheep livers had greater antibiotic detection rates than sheep kidneys, which in turn had higher rates than sheep muscles. According to Yang et al. (2020), there were significant amounts of antibiotic residues in chicken giblets (such as the liver and gizzard), with both the detection value and rate being high. Furthermore, he provided evidence that this might be connected to the physiological effects of chicken liver and chicken gizzard, given that the liver serves as a metabolic and detoxifying organ and that the gizzard is a crucial organ for digestion and absorption (Yang et al., 2020).

5.5 Antibiotics quantities

The quantification of the detected antibiotic residues revealed the presence of ciprofloxacin in the kidney and liver samples in the range of 0.059 - 5.136 µg/g, amoxicillin in the range of 0.204 - 0.923 µg/Kg, trimethoprim in the range of 0.339 - 3.256 µg/g, and sulfamethoxazole from a range of ND - 0.957 µg/g. According to the standards set by the European Union on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, the kidney and liver of all food-producing animals should not contain any more than 50 µg/kg of Amoxicillin and Trimethoprim; the liver and kidney of poultry should not contain more than 200 µg/kg and not more than 300 µg/kg of Ciprofloxacin respectively; and finally, All food producing species should contain no more than 100 µg/kg of all substances belonging to the sulfonamide group (sulfamethoxazole in this case). Different concentrations of antibiotic residues have been reported in several studies. In a study by Jammoul & El Darra, (2019), 3 out of 80 chicken samples had mean amoxicillin contamination levels that were higher than the MRL (50 µg/kg) (63, 62.5, and 77.5 µg/kg). Trimethoprim concentrations varied from ND to 1.16 µg/kg, fluoroquinolone detection peaked at 5.48 µg/kg, and sulfamethoxazole concentrations ranged from ND to 7.76 µg/kg, according to a study conducted in southern Xinjiang, China in 2021 (Zhang et al., 2021). Yamaguchi et al. (2015) showed high quantities of sulfonamide residues, ranging between 2500 and 2700 µg/kg. The concentrations of sulfamethoxazole in this study were higher than those reported by Cheong et al. (2010), who stated that the concentration of sulfonamides detected in samples from 11 states in Peninsular Malaysia ranged from 0.08-0.193 g/g in liver samples. According to a study by Yang et al. (2020) sulfonamide residue values ranged from 1.4 g/kg to 20.2 µg/kg. An earlier investigation by Er et al. (2013) reported that the mean levels of quinolone antibiotic residue in positive chicken samples were 30.81 0.45 µg/kg, which was higher than the amounts of ciprofloxacin detected in this study. The range of antibiotic residues in chicken giblets, however, was observed to be between 0.4 and 624.2 µg/kg in a study by Yang et al. (2020). All antibiotic residues found in this investigation varied from 0.058926 to 5.138996 µg/g, which is within the EU's maximum residue limits. This implies that the detected antibiotic levels in the examined poultry samples adhere to regulatory standards, affirming their suitability for consumption. This finding is reassuring for public health, indicating that the antibiotic residues present in these poultry products are within safe limits and do not pose immediate health concerns. Therefore, the study recommends periodic monitoring of the antibiotic residues in the chickens, and further study on antimicrobial resistance should be undertaken based on the confirmed presence of antibiotic residues detected.

5.6 Antibiotics detected under different broiler management systems

The presence of antibiotic residues concerning the broiler management systems in Malawi has demonstrated that more samples with antibiotic residues were under large or commercial management systems, followed by medium-scale management systems, and finally low levels have been detected in small-scale management systems. All of the samples in this investigation from which antibiotics were not found were collected from small-scale management systems. According to the study's survey, the majority of broiler farmers in Blantyre City are small-scale, intensive farmers who keep between 70 and 150 chickens. However, some medium-sized farmers are also becoming more prevalent, with most of them able to retain between 200 and 400 birds. Few farms can maintain 500 broiler chickens. Antibiotic usage in Malawi is still unregulated, and commercial farming in Malawi uses more veterinary drugs intensively than other agricultural systems, which may explain the high detection of antibiotic residues in samples of commercial chicken. These results, however, are in contrast to research by Mankhomwa et al. (2022), which found that antibiotic usage in animals was uncommon among households that did not use small-scale intensive agricultural techniques (Mankhomwa et al., 2022). The identification of antibiotic residues across different broiler management systems in Malawi carries implications for public health. The higher prevalence of antibiotic residues in large or commercial systems, followed by medium-scale systems, suggests a potential exposure risk for consumers. The intensive use of veterinary drugs in commercial farming, coupled with the lack of regulation on antibiotic usage in Malawi, raises concerns about the safety of chicken products for human consumption. Small-scale management systems, on the other hand, show lower levels of antibiotic residues, indicating a comparatively lower risk. This highlights the need for regulatory measures and awareness programs to ensure the safe and responsible use of antibiotics in poultry farming, safeguarding public health by minimizing potential health risks associated with antibiotic residues in chicken products.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the study highlights the insufficient awareness of antibiotic use in broiler production among farmers, who often struggle to explain the purpose of antibiotics and the diseases they treat. However, most farmers express a positive mindset and are open to adopting alternative methods to antibiotics. This indicates a significant gap in the use of antibiotics in broiler production, necessitating training in general broiler production, with a focus on animal health, antibiotics, storage, use, and waste.

The study emphasizes the importance of veterinary officers in maintaining animal health and combating antibiotic misuse. A strong relationship was found between education levels and antibiotic knowledge, with farming years influencing the appropriate timing for administering antibiotics to broilers. Education and farming years significantly impact attitudes toward long-term antibiotic use and resistance development. The study also found a significant relationship between education levels and antibiotic knowledge, with farming years related to the appropriate timing for administering antibiotics to broilers.

On the HPLC analysis of the samples and antibiotic residue detection, this study provides evidence for the presence of residues of ciprofloxacin, amoxicillin, trimethoprim, and sulfamethoxazole in chicken kidney and liver meat samples. The highest amount of amoxicillin residues was detected in the kidney and liver samples of broiler chickens. This can be attributed to the non-adherence of the withdrawal periods of drugs by the farmers. However, it was concluded that the levels of amoxicillin detected were lower than the MRL. The range of all the detected antibiotic residues was lower than MRL. The amounts of tested antibiotics were not high in the chicken kidney and liver samples, consequently, it is concluded that using chicken meat by consumers in Blantyre does not result in the consumption of high amounts of the antibiotics into the human body.

6.2 Recommendations

Based on the study's findings, the following recommendations are proposed

1. Promote responsible antibiotic use: Encourage farmers to follow appropriate hygiene and animal husbandry methods, and emphasize the importance of responsible antibiotic use as a supplement rather than a replacement for these practices

2. Encourage the use of alternative treatments: Promote the use of alternative treatments, such as vaccines, probiotics, and prebiotics, to reduce the reliance on antibiotics in animal production.
3. Implement regulations and guidelines: Establish and enforce regulations and guidelines for the use of antibiotics in animal production, ensuring that farmers adhere to these guidelines and understand the importance of responsible antibiotic use.
4. Monitor and surveillance: Regularly monitor antibiotic use and residue levels in the agricultural sector to identify trends and areas of concern, allowing for timely interventions and policy adjustments.
5. Collaborate with stakeholders: Encourage collaboration between farmers, veterinarians, government agencies, and other stakeholders to develop and implement effective strategies for promoting responsible antibiotic use and addressing antibiotic resistance.

REFERENCES

Abdelshakour, M. A., Mostafa, A. E., Hadad, G. M., Hamed, D. M., El-Darder, O. M., El-Gindy, A., Khinkar, R. M., Aldurdunji, M. M., Murshid, S. S., & Abdel Salam, R. A. (2022). Experimental Design Assisted HPLC/UV and LC-MS/MS for Simultaneous Determination of Selected Veterinary Antibiotics in Broiler Chicken. *Separations*, *9*(12), 427. https://doi.org/10.3390/separations9120427

Addah, W., Baah, J., Tia, S., & Okine, E. (2009). Knowledge and practices of smallholder farmers and herdsmen in the use of acaricides and gastrointestinal anthelminthes in Ghana. *Livestock Research for Rural Development*. https://www.semanticscholar.org/paper/Knowledge-and-practices-of-smallholder-farmers-and-Addah-Baah/79915da415654b3ddc0f54ab7c2ead1049577b1c

Adedeji, W. A. (2016). The treasure called antibiotics. *Annals of Ibadan Postgraduate Medicine*, *14*(2), 56–57.

Aidara-Kane, A., Angulo, F. J., Conly, J. M., Minato, Y., Silbergeld, E. K., McEwen, S. A., & Collignon, P. J. (2018). World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals. *Antimicrobial Resistance and Infection Control*, *7*, 7. https://doi.org/10.1186/s13756-017-0294-9

Andersson, D. I., & Hughes, D. (2014). Microbiological effects of sublethal levels of antibiotics. *Nature Reviews. Microbiology*, *12*(7), 465–478. https://doi.org/10.1038/nrmicro3270

Aworh, M. K., Kwaga, J. K. P., & Okolocha, E. C. (2021). Assessing knowledge, attitude, and practices of veterinarians towards antimicrobial use and stewardship as drivers of inappropriate use in Abuja, Nigeria. *One Health Outlook*, *3*(1), 25. https://doi.org/10.1186/s42522-021-00058-3

Baghani, A., Mesdaghinia, A., Rafieiyan, M., Soltan Dallal, M. M., & Douraghi, M. (2019). Tetracycline and ciprofloxacin multiresidues in beef and chicken meat samples using indirect competitive ELISA. *Journal of Immunoassay and Immunochemistry*, *40*(3), 328–342. https://doi.org/10.1080/15321819.2019.1597735

Britannica. (2018). *Blantyre | Safari Tours, Wildlife & Culture | Britannica*. https://www.britannica.com/place/Blantyre-Malawi

Burki, T. K. (2018). Tackling antimicrobial resistance in food-producing animals. *The Lancet Respiratory Medicine*, *6*(2), 93–94. https://doi.org/10.1016/S2213-2600(18)30017-1

Carter, R. R., Sun, J., & Jump, R. L. P. (2016). A Survey and Analysis of the American Public's Perceptions and Knowledge About Antibiotic Resistance. *Open Forum Infectious Diseases*, *3*(3), ofw112. https://doi.org/10.1093/ofid/ofw112

Chah, J. M., Nwankwo, S. C., Uddin, I. O., & Chah, K. F. (2022). Knowledge and practices regarding antibiotic use among small-scale poultry farmers in Enugu State, Nigeria. *Heliyon*, *8*(4), e09342. https://doi.org/10.1016/j.heliyon.2022.e09342

Chauhan, A. S., George, M. S., Chatterjee, P., Lindahl, J., Grace, D., & Kakkar, M. (2018). The social biography of antibiotic use in smallholder dairy farms in India. *Antimicrobial Resistance & Infection Control*, *7*(1), 60. https://doi.org/10.1186/s13756-018-0354-9

Cheong, C. K., Hajeb, P., Jinap, S., & Ismail-Fitry, M. R. (2010). Sulfonamides determination in chicken meat products from Malaysia. *International Food Research Journal*, *17*, 885–892.

Christou, A., Michael, C., Fatta-Kassinos, D., & Fotopoulos, V. (2018). Can the pharmaceutically active compounds released in agroecosystems be considered as emerging plant stressors? *Environment International*, *114*, 360–364. https://doi.org/10.1016/j.envint.2018.03.003

Davies, J. (2014). Antibiotic Resistance in and from Nature. In *One Health* (pp. 183–194). John Wiley & Sons, Ltd. https://doi.org/10.1128/9781555818432.ch12

Davies, J. (2017). *Antibiotic resistance in and from nature*.

Diaz, D. F., Scientific, O., & Leboucq, D. N. (2014). *OIE List of Antimicrobial Agents of Veterinary Importance and OIE Standards and Activities*. http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/

Donkor, E. S., Tetteh-Quarcoo, P. B., Nartey, P., & Agyeman, I. O. (2012). Self-Medication Practices with Antibiotics among Tertiary Level Students in Accra, Ghana: A Cross-Sectional Study. *International Journal of Environmental Research and Public Health*, *9*(10), 3519–3529. https://doi.org/10.3390/ijerph9103519

Dyar, O. J., Zhang, T., Peng, Y., Sun, M., Sun, C., Yin, J., Ding, L., Sun, C., Wang, Y., Sun, Q., Greko, C., & Stålsby Lundborg, C. (2020). Knowledge, attitudes and practices relating to antibiotic use and antibiotic resistance among backyard pig farmers in rural Shandong province, China. *Preventive Veterinary Medicine*, *175*, 104858. https://doi.org/10.1016/j.prevetmed.2019.104858

Er, B., Onurdağ, F. K., Demirhan, B., Özgacar, S. Ö., Öktem, A. B., & Abbasoğlu, U. (2013a). Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey. *Poultry Science*, *92*(8), 2212–2215. https://doi.org/10.3382/ps.2013-03072

Er, B., Onurdağ, F. K., Demirhan, B., Özgacar, S. Ö., Öktem, A. B., & Abbasoğlu, U. (2013b). Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey. *Poultry Science*, *92*(8), 2212–2215. https://doi.org/10.3382/ps.2013-03072

European Union. (2010). *Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin*. *470*.

Farzam, K., Nessel, T. A., & Quick, J. (2023). Erythromycin. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK532249/

Fleming, A. (1929). *On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenze.*

Food and Agriculture Organization & World Health Organization. (2015). *Codex Texts on Foodborne Antimicrobial Resistance*. FAO/WHO. https://www.fao.org/publications/card/en/c/7209750e-2c7a-4694-a0fe-8d7f0050acae/

Gemeda, B. A., Amenu, K., Magnusson, U., Dohoo, I., Hallenberg, G. S., Alemayehu, G., Desta, H., & Wieland, B. (2020). Antimicrobial Use in Extensive Smallholder Livestock Farming Systems in Ethiopia: Knowledge, Attitudes, and Practices of Livestock Keepers. *Frontiers in Veterinary Science*, *7*, 55. https://doi.org/10.3389/fvets.2020.00055

Geta, K., & Kibret, M. (2021a). Knowledge, attitudes and practices of animal farm owners/workers on antibiotic use and resistance in Amhara region, north western Ethiopia. *Scientific Reports*, *11*(1), 21211. https://doi.org/10.1038/s41598-021-00617-8

Geta, K., & Kibret, M. (2021b). Knowledge, attitudes and practices of animal farm owners/workers on antibiotic use and resistance in Amhara region, north western Ethiopia. *Scientific Reports*, *11*(1), 21211. https://doi.org/10.1038/s41598-021-00617-8

Hoelzer, K., Bielke, L., Blake, D. P., Cox, E., Cutting, S. M., Devriendt, B., Erlacher-Vindel, E., Goossens, E., Karaca, K., Lemiere, S., Metzner, M., Raicek, M., Collell Suriñach, M., Wong, N. M., Gay, C., & Van Immerseel, F. (2018). Vaccines as alternatives to antibiotics for food producing animals. Part 2: New approaches and potential solutions. *Veterinary Research*, *49*(1), 70. https://doi.org/10.1186/s13567-018-0561-7

Horumpende, P. G., Sonda, T. B., van Zwetselaar, M., Antony, M. L., Tenu, F. F., Mwanziva, C. E., Shao, E. R., Mshana, S. E., Mmbaga, B. T., & Chilongola, J. O. (2018). Prescription and non-prescription antibiotic dispensing practices in part I and part II pharmacies in Moshi Municipality, Kilimanjaro Region in Tanzania: A simulated clients approach. *PloS One*, *13*(11), e0207465. https://doi.org/10.1371/journal.pone.0207465

Horvat, O. J., Tomas, A. D., Paut Kusturica, M. M., Savkov, A. V., Bukumirić, D. U., Tomić, Z. S., & Sabo, A. J. (2017). Is the level of knowledge a predictor of rational antibiotic use in Serbia? *PloS One*, *12*(7), e0180799. https://doi.org/10.1371/journal.pone.0180799

Hossain, M. T., Rafiq, K., Islam, M. Z., Chowdhury, S., Islam, P., Haque, Z., Samad, M. A., Sani, A. A., Ferdous, Most. R. A., Islam, M. R., Ahmed, N., Hossen, M. I., Khasruzzman, A. K. M., Bhuiyan, M. K. J., & Hossain, M. T. (2022). A Survey on Knowledge, Attitude, and Practices of Large-Animal Farmers towards Antimicrobial Use, Resistance, and Residues in Mymensingh Division of Bangladesh. *Antibiotics*, *11*(4), 442. https://doi.org/10.3390/antibiotics11040442

Interchemie werken ‘De Adelaar’ B.V. (2023). *Interchemie -Netherlands*. https://www.interchemie.com/limovit-ws/

Islam, K. B. M. S., Shiraj-Um-Mahmuda, S., & Hazzaz-Bin-Kabir, M. (2016). Antibiotic Usage Patterns in Selected Broiler Farms of Bangladesh and their Public Health Implications. *Journal of Public Health in Developing Countries*, *2*(3), 276–284.

Jammoul, A., & El Darra, N. (2019a). Evaluation of Antibiotics Residues in Chicken Meat Samples in Lebanon. *Antibiotics*, *8*(2), 69. https://doi.org/10.3390/antibiotics8020069

Jammoul, A., & El Darra, N. (2019b). Evaluation of Antibiotics Residues in Chicken Meat Samples in Lebanon. *Antibiotics*, *8*(2), 69. https://doi.org/10.3390/antibiotics8020069

John J. Mussa (last), James L. Banda, & L. Chikomola. (2020). Animal Health and Livestock Development. In *Guide to Agriculture Production and Natural Resource management in Malawi* (Revised, pp. 353–392). Agriculture Communication Branch,Department of Agriculture Extension Services, Ministry of Agriculture, P.O. Box30145,Lilongwe, Malawi.

Kariuki, S., Onsare, R., Mwituria, J., Ng'Etich, R., Nafula, C., Karimi, K., Karimi, P., Njeruh, F., Irungu, P., & Mitema, E. (2013). FAO/WHO project report: Improving food safety in meat value chains in Kenya. *Food Protection Trends*, *33*(3), 172–179.

Kasimanickam, V., Kasimanickam, M., & Kasimanickam, R. (2021). Antibiotics Use in Food Animal Production: Escalation of Antimicrobial Resistance: Where Are We Now in Combating AMR? *Medical Sciences*, *9*(1), 14. https://doi.org/10.3390/medsci9010014

Kester, M., Karpa, K. D., & Vrana, K. E. (2012). 4—Treatment of Infectious Diseases. In M. Kester, K. D. Karpa, & K. E. Vrana (Eds.), *Elsevier's Integrated Review Pharmacology (Second Edition) (Second Edition)* (pp. 41–78). W.B. Saunders. https://doi.org/10.1016/B978-0-323-07445-2.00004-5

Kiiti, R. W., Komba, E. V., Msoffe, P. L., Mshana, S. E., Rweyemamu, M., & Matee, M. I. N. (2021). Antimicrobial Resistance Profiles of Escherichia coli Isolated from Broiler and Layer Chickens in Arusha and Mwanza, Tanzania. *International Journal of Microbiology*, *2021*, 6759046. https://doi.org/10.1155/2021/6759046

Kulikova, N. A., Solovyova, A. A., & Perminova, I. V. (2022). Interaction of Antibiotics and Humic Substances: Environmental Consequences and Remediation Prospects. *Molecules (Basel, Switzerland)*, *27*(22), 7754. https://doi.org/10.3390/molecules27227754

Kumar, H., Bhardwaj, K., Kaur, T., Nepovimova, E., Kuča, K., Kumar, V., Bhatia, S. K., Dhanjal, D. S., Chopra, C., Singh, R., Guleria, S., Bhalla, T. C., Verma, R., & Kumar, D. (2020). Detection of Bacterial Pathogens and Antibiotic Residues in Chicken Meat: A Review. *Foods*, *9*(10), 1504. https://doi.org/10.3390/foods9101504

Landfried, K. L., Barnidge, E., Pithua, K. P., Lewis, D. R., Jacoby, A. J., King, C. C., & Baskin, R. C. (2018). Antibiotic Use on Goat Farms: An Investigation of Knowledge, Attitudes, and Behaviors of Missouri Goat Farmers. *Animals*, *8*(11), Article 11. https://doi.org/10.3390/ani8110198

Lee, H.-J., Cho, S.-H., Shin, D., & Kang, H.-S. (2018). Prevalence of Antibiotic Residues and Antibiotic Resistance in Isolates of Chicken Meat in Korea. *Korean Journal for Food Science of Animal Resources*, *38*(5), 1055–1063. https://doi.org/10.5851/kosfa.2018.e39

Liang, J.-H., & Han, X. (2013). Structure-activity relationships and mechanism of action of macrolides derived from erythromycin as antibacterial agents. *Current Topics in Medicinal Chemistry*, *13*(24), 3131–3164. https://doi.org/10.2174/15680266113136660223

MacGowan, A., & Macnaughton, E. (2017). Antibiotic resistance. *Medicine*, *45*(10), 622–628. https://doi.org/10.1016/j.mpmed.2017.07.006

MacPherson, E. E., Mankhomwa, J., Dixon, J., Pongolani, R., Phiri, M., Feasey, N., O'Byrne, T., Tolhurst, R., & MacPherson, P. (2023). Household antibiotic use in Malawi: A cross-sectional survey from urban and peri-urban Blantyre. *PLOS Global Public Health*, *3*(8), e0001946. https://doi.org/10.1371/journal.pgph.0001946

Magnusson, U., Sternberg, S., Eklund, G., & Rozstalnyy, A. (2019). *Prudent and efficient use of antimicrobials in pigs and poultry: A practical manual*. FAO. https://doi.org/10.4060/CA6729EN

Makoka, M. H., Miller, W. C., Hoffman, I. F., Cholera, R., Gilligan, P. H., Kamwendo, D., Malunga, G., Joaki, G., Martinson, F., & Hosseinipour, M. C. (2012). Bacterial infections in Lilongwe, Malawi: Aetiology and antibiotic resistance. *BMC Infectious Diseases*, *12*(1), 67. https://doi.org/10.1186/1471-2334-12-67

Malawiplus. (2023, February 14). *Blantyre District in Malawi / Malawi Travel and Business Guide*. Malawi Travel and Business Guide. https://malawiplus.com/blantyre/

Mankhomwa, J., Tolhurst, R., M'biya, E., Chikowe, I., Banda, P., Mussa, J., Mwasikakata, H., Simpson, V., Feasey, N., & MacPherson, E. E. (2022a). A Qualitative Study of Antibiotic Use Practices in Intensive Small-Scale Farming in Urban and Peri-Urban Blantyre, Malawi: Implications for Antimicrobial Resistance. *Frontiers in Veterinary Science*, *9*, 876513. https://doi.org/10.3389/fvets.2022.876513

Mankhomwa, J., Tolhurst, R., M'biya, E., Chikowe, I., Banda, P., Mussa, J., Mwasikakata, H., Simpson, V., Feasey, N., & MacPherson, E. E. (2022b). A Qualitative Study of Antibiotic Use Practices in Intensive Small-Scale Farming in Urban and Peri-Urban Blantyre, Malawi: Implications for Antimicrobial Resistance. *Frontiers in Veterinary Science*, *9*, 876513. https://doi.org/10.3389/fvets.2022.876513

Mathanga, D. P., Tembo, A. K., Mzilahowa, T., Bauleni, A., Mtimaukenena, K., Taylor, T. E., Valim, C., Walker, E. D., & Wilson, M. L. (2016). Patterns and determinants of malaria risk in urban and peri-urban areas of Blantyre, Malawi. *Malaria Journal*, *15*(1), 590. https://doi.org/10.1186/s12936-016-1623-9

McDougall, S., Compton, C., & Botha, N. (2017). Factors influencing antimicrobial prescribing by veterinarians and usage by dairy farmers in New Zealand. *New Zealand Veterinary Journal*, *65*(2), 84–92. https://doi.org/10.1080/00480169.2016.1246214

McEwen, S. A., & Fedorka-Cray, P. J. (2002). Antimicrobial Use and Resistance in Animals. *Clinical Infectious Diseases*, *34*(s3), S93–S106. https://doi.org/10.1086/340246

Millennium Cities Initiative. (2023). *Blantyre Maps—Millennium Cities Initiative*. http://mci.ei.columbia.edu/millennium-cities/blantyre-malawi/blantyre-maps-and-population-data/

Moffo, F., Mouliom Mouiche, M. M., Kochivi, F. L., Dongmo, J. B., Djomgang, H. K., Tombe, P., Mbah, C. K., Mapiefou, N. P., Mingoas, J.-P. K., & Awah-Ndukum, J. (2020). Knowledge, attitudes, practices and risk perception of rural poultry farmers in Cameroon to antimicrobial use and resistance. *Preventive Veterinary Medicine*, *182*, 105087. https://doi.org/10.1016/j.prevetmed.2020.105087

Mohsin, M., Van Boeckel, T. P., Saleemi, M. K., Umair, M., Naseem, M. N., He, C., Khan, A., & Laxminarayan, R. (2019). Excessive use of medically important antimicrobials in food animals in Pakistan: A five-year surveillance survey. *Global Health Action*, *12*(sup1), 1697541. https://doi.org/10.1080/16549716.2019.1697541

Mora-Gamboa, M. P. C., Rincón-Gamboa, S. M., Ardila-Leal, L. D., Pedroza-Rodríguez, A. M., & Quevedo-Hidalgo, B. E. (2022). *Antibiotic Use in Livestock Farming*.

Morgan, D. J., Okeke, I. N., Laxminarayan, R., Perencevich, E. N., & Weisenberg, S. (2011). Non-prescription antimicrobial use worldwide: A systematic review. *The Lancet. Infectious Diseases*, *11*(9), 692–701. https://doi.org/10.1016/S1473-3099(11)70054-8

Muaz, K., Riaz, M., Akhtar, S., Park, S., & Ismail, A. (2018). Antibiotic Residues in Chicken Meat: Global Prevalence, Threats, and Decontamination Strategies: A Review. *Journal of Food Protection*, *81*(4), 619–627. https://doi.org/10.4315/0362-028X.JFP-17-086

Murray, C., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet, 399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet*, *399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Nandolo, W. (2021). *Livestock in Malawi—Statistics*. https://livestockinmalawi.page.tl/Statistics.htm

Nation, R. L., & Li, J. (2009). Colistin in the 21st Century. *Current Opinion in Infectious Diseases, 22*(6), 535–543. https://doi.org/10.1097/QCO.0b013e328332e672

National Research Council Committee. (1980). *The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feeds*. National Academies Press (US). http://www.ncbi.nlm.nih.gov/books/NBK216513/

Nayiga, S., Kayendeke, M., Nabirye, C., Willis, L. D., Chandler, C. I. R., & Staedke, S. G. (2020). Use of antibiotics to treat humans and animals in Uganda: A cross-sectional survey of households and farmers in rural, urban and peri-urban settings. *JAC-Antimicrobial Resistance, 2*(4), dlaa082. https://doi.org/10.1093/jacamr/dlaa082

Nhung, N., Cuong, N., Thwaites, G., & Carrique-Mas, J. (2016). Antimicrobial Usage and Antimicrobial Resistance in Animal Production in Southeast Asia: A Review. *Antibiotics, 5*(4), 37. https://doi.org/10.3390/antibiotics5040037

O'Neill, J. (2015). *Antimicrobials in agriculture and the environment: Reducing unnecessary use and waste / the Review on Antimicrobial Resistance*. Wellcome Collection. https://wellcomecollection.org/works/rwmqsevu

Osei Sekyere, J. (2014). Antibiotic Types and Handling Practices in Disease Management among Pig Farms in Ashanti Region, Ghana. *Journal of Veterinary Medicine*, *2014*, e531952. https://doi.org/10.1155/2014/531952

Ozturk, Y., Celik, S., Sahin, E., Acik, M. N., & Cetinkaya, B. (2019). Assessment of Farmers' Knowledge, Attitudes and Practices on Antibiotics and Antimicrobial Resistance. *Animals*, *9*(9), 653. https://doi.org/10.3390/ani9090653

Papich, M. G. (2016a). Enrofloxacin. In M. G. Papich (Ed.), *Saunders Handbook of Veterinary Drugs (Fourth Edition)* (pp. 287–289). W.B. Saunders. https://doi.org/10.1016/B978-0-323-24485-5.00240-0

Papich, M. G. (2016b). *Saunders Handbook of VETERINARY DRUGS Small and Large Animal*. https://www.academia.edu/46116971/Saunders_Handbook_of_VETERINARY_DRUGS_Small_and_Large_Animal_M_Papich

Papich, M. G. (2016c). Trimethoprim + Sulfamethoxazole. In M. G. Papich (Ed.), *Saunders Handbook of Veterinary Drugs (Fourth Edition)* (pp. 820–822). W.B. Saunders. https://doi.org/10.1016/B978-0-323-24485-5.00576-3

Papich, M. G. (2016d). Tylosin. In M. G. Papich (Ed.), *Saunders Handbook of Veterinary Drugs (Fourth Edition)* (pp. 826–827). W.B. Saunders. https://doi.org/10.1016/B978-0-323-24485-5.00579-9

Peng, M., Salaheen, S., & Biswas, D. (2014). Animal Health: Global Antibiotic Issues. In *Encyclopedia of Agriculture and Food Systems* (pp. 346–357). Elsevier. https://doi.org/10.1016/B978-0-444-52512-3.00187-X

Phares, C. A., Danquah, A., Atiah, K., Agyei, F. K., & Michael, O.-T. (2020). Antibiotics utilization and farmers' knowledge of its effects on soil ecosystem in the coastal drylands of Ghana. *PLOS ONE*, *15*(2), e0228777. https://doi.org/10.1371/journal.pone.0228777

Poudyal, A., Howden, B. P., Bell, J. M., Gao, W., Owen, R. J., Turnidge, J. D., Nation, R. L., & Li, J. (2008). In vitro pharmacodynamics of colistin against multidrug-resistant Klebsiella pneumoniae. *The Journal of Antimicrobial Chemotherapy*, *62*(6), 1311–1318. https://doi.org/10.1093/jac/dkn425

Ramatla, T., Ngoma, L., Adetunji, M., & Mwanza, M. (2017). Evaluation of Antibiotic Residues in Raw Meat Using Different Analytical Methods. *Antibiotics*, *6*(4), 34. https://doi.org/10.3390/antibiotics6040034

Redding, L. E., Barg, F. K., Smith, G., Galligan, D. T., Levy, M. Z., & Hennessy, S. (2013). The role of veterinarians and feed-store vendors in the prescription and use of antibiotics on small dairy farms in rural Peru. *Journal of Dairy Science*, *96*(11), 7349–7354. https://doi.org/10.3168/jds.2013-7045

Roscoe, J. T. (1975). Fundamental research statistics for the behavioral sciences. In *Fundamental research statistics for the behavioral sciences* (2d ed.). Holt, Rinehart and Winston.

Sadiq, M. B., Syed-Hussain, S. S., Ramanoon, S. Z., Saharee, A. A., Ahmad, N. I., Mohd Zin, N., Khalid, S. F., Naseeha, D. S., Syahirah, A. A., & Mansor, R. (2018a). Knowledge, attitude and perception regarding antimicrobial resistance and usage among ruminant farmers in Selangor, Malaysia. *Preventive Veterinary Medicine*, *156*, 76–83. https://doi.org/10.1016/j.prevetmed.2018.04.013

Sadiq, M. B., Syed-Hussain, S. S., Ramanoon, S. Z., Saharee, A. A., Ahmad, N. I., Mohd Zin, N., Khalid, S. F., Naseeha, D. S., Syahirah, A. A., & Mansor, R. (2018b). Knowledge, attitude and perception regarding antimicrobial resistance and usage among ruminant farmers in Selangor, Malaysia. *Preventive Veterinary Medicine*, *156*, 76–83. https://doi.org/10.1016/j.prevetmed.2018.04.013

Samanta, I., & Bandyopadhyay, S. (2020). Chapter 3—The emergence of antimicrobial-resistant bacteria in livestock, poultry and agriculture. In I. Samanta & S. Bandyopadhyay (Eds.), *Antimicrobial Resistance in Agriculture* (pp. 19–27). Academic Press. https://doi.org/10.1016/B978-0-12-815770-1.00003-1

Sarker, Y., Hasan, M., Paul, T., Rashid, S., Alam, M., & Sikder, M. (2018a). Screening of antibiotic residues in chicken meat in Bangladesh by thin layer chromatography. *Journal of Advanced Veterinary and Animal Research*, *5*(3), 140. https://doi.org/10.5455/javar.2018.e257

Sarker, Y., Hasan, M., Paul, T., Rashid, S., Alam, M., & Sikder, M. (2018b). Screening of antibiotic residues in chicken meat in Bangladesh by thin layer chromatography. *Journal of Advanced Veterinary and Animal Research*, *5*(3), 140. https://doi.org/10.5455/javar.2018.e257

Scholar, E. (2007). Neomycin. In S. J. Enna & D. B. Bylund (Eds.), *xPharm: The Comprehensive Pharmacology Reference* (pp. 1–5). Elsevier. https://doi.org/10.1016/B978-008055232-3.62258-5

Spellberg, B., Hansen, R. G., Kar, A., Cordova, D. C., Price, B. L., & Johnson, R. J. (2016). *Antibiotic Resistance in Humans and Animals* [Editorial brifing]. McGraw Hill's AccessScience; McGraw Hill. https://doi.org/10.1036/1097-8542.BR0125171

Sykes, J. E., & Papich, M. G. (2021). 10—Antibacterial Drugs. In J. E. Sykes (Ed.), *Greene's Infectious Diseases of the Dog and Cat (Fifth Edition)* (pp. 103–126). W.B. Saunders. https://doi.org/10.1016/B978-0-323-50934-3.00010-0

Tang, K. L., Caffrey, N. P., Nóbrega, D. B., Cork, S. C., Ronksley, P. E., Barkema, H. W., Polachek, A. J., Ganshorn, H., Sharma, N., Kellner, J. D., & Ghali, W. A. (2017, March). *Table 1, Definitions for terms used in the classification scheme for interventions* [Text]. World Health Organization. https://www.ncbi.nlm.nih.gov/books/NBK487950/table/annexa_1.3.t1/

Thapa, S. (2021). *Antibiotic Residues in Broiler Meat Sold in Dharan*.

The Institute for Health Metrics and Evaluation. (2023). *The burden of antimicrobial resistance (AMR) in Malawi*. https://www.healthdata.org/sites/default/files/2023-09/Malawi.pdf

The Pew Charitable Trusts. (2014, November 30). *Gaps in FDA's Antibiotics Policy*. http://bit.ly/1uElZLZ

Thi Huong-Anh, N., Van Chinh, D., & Thi Tuyet-Hanh, T. (2020). Antibiotic Residues in Chickens and Farmers' Knowledge of Their Use in Tay Ninh Province, Vietnam, in 2017. *Asia Pacific Journal of Public Health*, *32*(2–3), 126–132. https://doi.org/10.1177/1010539520909942

Tufa, B., Takele, Feraol, G., Beyi, A. F., Hogeveen, H., Beyene, T. J., Ayana, D., Woldemariyam, F. T., Hailemariam, E., Gutema, F. D., & Stegeman, J. A. (2018). *Veterinary medicinal product usage among food animal producers and its health implications in Central Ethiopia | SpringerLink*. https://link.springer.com/article/10.1186/s12917-018-1737-0

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(18), 5649–5654. https://doi.org/10.1073/pnas.1503141112

Van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G., Gilbert, M., Bonhoeffer, S., & Laxminarayan, R. (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science*, *365*(6459). https://doi.org/10.1126/science.aaw1944

Varga, M. (2014). Therapeutics. In *Rabbit Medicine* (pp. 137–177). Elsevier. https://doi.org/10.1016/B978-0-7020-4979-8.00003-0

Ventola, C. L. (2015). The Antibiotic Resistance Crisis. *Pharmacy and Therapeutics*, *40*(4), 277–283.

Waksman, A., Thomas, L., & Selman, H. (2023, July 5). *Antibiotic | Definition, Types, Side Effects, Resistance, Classification, & Facts | Britannica*. https://www.britannica.com/science/antibiotic

Wall, B. A., Mateus, A. L. P., Marshall, L., Pfeiffer, D. U., Lubroth, J., Ormel, H. J., Otto, P., & Patriarchi, A. (2016). Drivers, dynamics and epidemiology of antimicrobial resistance in animal production. *Food and Agriculture Organization of the United Nations*.

Wang, H., Ren, L., Yu, X., Hu, J., Chen, Y., He, G., & Jiang, Q. (2017). Antibiotic residues in meat, milk and aquatic products in Shanghai and human exposure assessment. *Food Control*, *80*, 217–225. https://doi.org/10.1016/j.foodcont.2017.04.034

Word Health Organisation. (2020). *Antibiotic resistance*. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

World Animal Protection. (2023). *EU bans the routine use of antibiotics in farmed animals*. https://www.worldanimalprotection.org/news/eu-bans-antibiotic-overuse-farmed-animals

World Health Organization. (2017). *Antibiotic resistance*. http://www.who.int/foodsafety/ areas_work/antimicrobialresistance/ cia_guidelines/en/

World Health Organization. (2018). *WHO report on surveillance of antibiotic consumption: 2016-2018 early implementation*. World Health Organization. https://apps.who.int/iris/handle/10665/277359

World Health Organization. (2019). *Critically important antimicrobials for human medicine* (6th rev.). World Health Organization. https://apps.who.int/iris/handle/10665/312266

World Health Organization, Aidara-Kane, A., Angulo, F. J., Conly, J. M., Minato, Y., Silbergeld, E. K., McEwen, S. A., & Collignon, P. J. (2018). World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals. *Antimicrobial Resistance & Infection Control*, *7*(1), 7. https://doi.org/10.1186/s13756-017-0294-9

World Organization for Animal Health. (2018). *Antimicrobial resistance*. WOAH - World Organisation for Animal Health. https://www.woah.org/en/what-we-do/global-initiatives/antimicrobial-resistance/

World Organization for Animal Health. (2019). 2nd OIE Global Conference on Antimicrobial Resistance. *WOAH Bulletin*. https://bulletin.woah.org/?officiel=2019-1-marrakesh-amr-conf-en

World Organization for Animal Health. (2022, October 8). *Terrestrial animal health code. Chapter 6.10. Responsible and Prudent Use of Antimicrobial Agents in Veterinary Medicine*. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahc/2018/en_chapitre_antibio_use.htm

Yamaguchi, T., Okihashi, M., Harada, K., Konishi, Y., Uchida, K., Do, M. H. N., Bui, H. D. T., Nguyen, T. D., Nguyen, P. D., Chau, V. V., Dao, K. T. V., Nguyen, H. T. N., Kajimura, K., Kumeda, Y., Bui, C. T., Vien, M. Q., Le, N. H., Hirata, K., & Yamamoto, Y. (2015).

Antibiotic Residue Monitoring Results for Pork, Chicken, and Beef Samples in Vietnam in 2012–2013. *Journal of Agricultural and Food Chemistry*, *63*(21), 5141–5145. https://doi.org/10.1021/jf505254y

Yang, Y., Qiu, W., Li, Y., & Liu, L. (2020). Antibiotic residues in poultry food in Fujian Province of China. *Food Additives & Contaminants: Part B*, *13*(3), 177–184. https://doi.org/10.1080/19393210.2020.1751309

You, Y., & Silbergeld, E. K. (2014). Learning from agriculture: Understanding low-dose antimicrobials as drivers of resistome expansion. *Frontiers in Microbiology*, *5*, 284. https://doi.org/10.3389/fmicb.2014.00284

Zhang, Y., Lu, J., Yan, Y., Liu, J., & Wang, M. (2021). Antibiotic residues in cattle and sheep meat and human exposure assessment in southern Xinjiang, China. *Food Science & Nutrition*, *9*(11), 6152–6161. https://doi.org/10.1002/fsn3.2568

APPENDICES

Appendix 1: Informed consent form (English)

I (respondent name) …………………………… voluntarily agree to take part in this study. I understand that this I understand this work is for academic purposes. I also understand that the researcher, hereby named Amon Abraham is a student of the Malawi University of Business and Applied Sciences, studying for a master of Science in Environmental Health.

I understand that the title of the research is ***assessing knowledge, attitudes, and practices of broiler chicken farmers on antibiotic use and residues in Blantyre, Malawi.***

I also understand that I waive any claim for copyright to this material should the researchers ever publish it in a scholarly journal or electronic format online.

I also understand that the researcher will maintain my anonymity regarding my response to the questionnaire item,

I hereby give my permission in the form of a signature below

Name …………………………….. Signature…………… Date………………

Contacts of the Researchers:

Amon abraham - +265881209311 /+2650994545487

Appendix 2: Informed consent (Chichewa)

Ine (dzina la oyakha) …………………………. Ndikuvomeleza mosakakamizidwa kutengapo mbali pa kafukufukuyu ndipo ndikuvetsetsa zoti cholinga cha kafukufukuyu ndi kuthandizira maphunziro basi. Ndikumvetetsaso kuti a Amon Abraham mwini wa kafukufukuyu ndi ophunzira wa zaumoyo wapa sukulu ya ukachenjede ya Malawi University of Business and Applied Sciences. Ndikuvesetsa zoti kafukufukuyu mutu wake ndi kufufuza za mphamvu ya mzeru, mmene, timaonera kapena kumvera ndinso machitidwe a alimi oweta nkhuku za mtundu wa nyama mmadera akumidzi ndi m'matauni mu mzinda wa kabula (Blantyre) pa kagwilitsidwe ntchito ka mankhwala otetezera ku tizilombo toyambitsa matenda ta bakiteliya ndi zotsatila zake za mankhwalawa mu Nkhuku.

Ndikumvesesaso kuti nditha kukasumila munthu yemwe angagwilitse ntchito zotsatila za kafukufukuyu ngati zake zikazatsindikizidwa mma pepala oyika pa tsamba la intaneti.

Ndikumvesesaso kuti ofufuza adzasunga ndikubisa mayakho anga podzatsindikiza zotsatilazi Machoncho ndikupeleka chiloleza potsindika dzina langa mmusimu

Dzina……………………….. Kusainila…………………………. Tsiku……………………….

Mutha kulumikizana ndi ofufuza poimbila lamya pa nambala zili mmusimu

Amon abraham +265881209311/+265994545487

Appendix 3: Participant information sheet (English)

Research Project Title: The impact of knowledge, attitude, and practices on antibiotic use and its associated factors in broiler chickens in peri-urban and urban areas of Blantyre city.

Invitation

You are invited to participate in a research study that intends to study the impact of knowledge, attitude, and practices on antibiotic use and its associated factors in broiler chickens in peri-urban and urban areas of Blantyre city. The study is being conducted by a Malawi University of Business and Applied Sciences postgraduate student pursuing a Master of Science in Environmental Health**,** namely Amon Abraham.

About the study

The study aims at evaluating the impact of farmers' Knowledge, Attitude, and Practice on antibiotic use and its associated factors on broiler chickens in peri-urban and urban areas of Blantyre city. Furthermore, the study explores the possibility of having antibiotic residues in broiler chickens. Specifically, the study will focus on the assessment of farmers' knowledge, attitude, and practices regarding the use of antibiotics in chicken production and then characterize the penicillin, tetracyclines, sulfonamides, and quinolones antibiotic residues available in chicken meat samples in Blantyre.

Why Have I Been Invited to Participate in this Study? Do I Have to Take Part?

You have been invited to take part in the study where you will only be required to respond to a one-on-one interview using a questionnaire, it will only take less than 25 minutes to respond to the questionnaire. The questionnaire contains questions that are related to the objectives of the study explained above. Provided that you are a broiler farmer raising more than 50 broiler chickens in one of the peri-urban and urban areas of Blantyre City Malawi, you are eligible to respond to the questionnaire.

Your participation in this study is voluntary. While I would be pleased to have you participate, I respect your right to decline. If you would decide not to participate, it will not affect you in any way. If you do decide to take part, you will be given this information sheet to keep and will be asked to sign a consent form.

What are the Possible Risks and Benefits of Participation?

There are no physical, emotional, or psychological risks associated with your participation in the study. Your participation will help us understand the level of knowledge, attitude, and practice on antibiotic use and its associated factors among broiler farmers in Blantyre peri-urban and urban city areas.

Confidentiality

The interview will be one on one using a structured questionnaire. Your name and identifying information will not be associated with any part of the written report of the research. All your information and interview responses will be kept confidential. The researcher will not share your responses or any results from the laboratory with anyone other than the research supervisor. You are encouraged to ask questions or raise concerns at any time about the nature of the study or the methods being used. Please contact me at any time at the telephone number listed above.

Appendix 4: Participant information sheet (chichewa)

Mutu wa kafukufuku: kufufuza za mphamvu ya mzeru, mmene timaonera kapena kumvera ndinso machitidwe a alimi oweta nkhuku za mtundu wa nyama mmadera akumidzi ndi m'matauni a mu mzinda wa kabula (Blantyre) pa kagwilitsidwe ntchito ka mankhwala otetezera ku tizilombo toyambitsa matenda ta bakiteliya ndi zotsatila zake za mankhwalawa mu Nkhuku..

Kuitana

Mukupemphedwa kutenga nawo mbali mu kafukufuku wofufuza momwe mphamvu ya mzeru, mmene timaonera kapena kumvera ndinso machitidwe a alimi oweta nkhuku za mtundu wa nyama mmadera akumidzi ndi m'matauni a mu mzinda wa kabula (Blantyre) pa kagwilitsidwe ntchito ka mankhwala otetezera ku tizilombo toyambitsa matenda ta bakiteliya ndi zotsatila zake za mankhwalawa mu Nkhuku. Kafukufukuyu akuchitidwa ndi wophunzira waku pa sukulu ya ukachenjede ya Malawi University of Business and Applied Sciences yemwe akuchita maphunziro apamwamba a zaumoyo **,** omwe ndi a Amon Abraham.

Kodi Phunziroli Likukhudza Chiyani?

Cholinga cha kafukufukuyu ndikuwunika momwe alimi amakhudzidwira pakugwiritsa ntchito mankhwala opha tizilombo toyambitsa matenda komanso zotsatira zake pa nkhuku za nyama m'madera a m'mphepete mwa tawuni ndi m'tauni ya Blantyre. Kuphatikiza apo, kafukufukuyu azayang'ana kuyesa zotsalira za kupezeka kwa makhwala otetezela ku tilombo ta bakiteliya mu nyama ya khuku. Makamaka, kafukufukuyu ayang'ana kuunika kwa chidziwitso cha alimi, malingaliro, ndi machitidwe awo pakagwilitsidwe ntchito ka makhwala otetezela ku tilombo ta bakiteliya poweta nkhuku ndipo kachiwiri kuwonetsetsa za kupezeka kwa penicillin, tetracyclines, sulfonamides ndi quinolones amene ali ena mwa mitundu ya makhwala otetezela ku tizilombo ta bakiteliya mu nyama ya nkhuku.

N'cifukwa chiyani Ndapemphedwa Kuti Nditengeko Nawo gawo mu kafukufukuyu? Kodi Ndiyenera Kutenga Mbali?

Mwaitanidwa kuti mutenge nawo mbali mu phunziroli komwe mudzangofunika kuyankha kuyankhulana kwa munthu payekha pogwiritsa ntchito mafunso, zidzangotenga mphindi zosakwana makumi awiri ndi zisanu (25) kuti muyankhe mafunsowo. Mafunsowa ali ndi mafunso okhudzana ndi zolinga za kafukufuku zomwe zafotokozedwa pamwambapa. Mukuyenera kutenga nawo gawo pokhapokha kuti ndinu mlimi woweta nkhuku za mtundu wa nyama zoposa makumi asanu (50) m'dera lina la m'mphepete mwa tawuni ndi m'tauni ya kabula (Blantyre) ,ndipo ndinu oyenerera kuyankha mafunsowa.

Kutenga nawo mbali mu kafukufukuyu ndi mwakufuna kwanu. Ngakhale ndingasangalale kuti mutenge nawo mbali, ndikulemekeza ufulu wanu wokana. Ngati mungaganize kusatenga nawo mbali, sizingakhudze inu mulimonse. Ngati mwasankha kutenga nawo mbali, mudzapatsidwa pepala ili kuti musunge ndipo mudzafunsidwa kudinda dzina lanu pa pepala yololeza.

Kodi Zowopsa Zomwe Zingatheke Ndi Ubwino Wotengapo Mbali Ndi Chiyani?

Palibe zowopsa zakuthupi, zamalingaliro, kapena zamaganizidwe zomwe zikugwirizana ndi kutenga nawo gawo mu kafukufukuyu. Kutengapo gawo kwanu kungatithandize kumvetsetsa momwe chidziwitso, malingaliro, ndi machitidwe ogwiritsira ntchito mankhwala oteteza ku tizilombo ta baketeliya amagwiritsidwira ntchito komanso momwe alimi a nkhuku za mtundu wa nyama a mmadela mwa m'mphepete mwa tawuni ndi m'tauni ya mzimda waukulu wa kabula .

Kusunga Chinsinsi

Kuyankhulana kudzakhala kwa modzi-modzi pogwiritsa ntchito mafunso okonzedwa. Dzina lanu ndi chidziwitso chanu sizidzalumikizidwa ndi gawo lililonse la zotsatila zolembedwa za kafukufukuyu. Zambiri zanu zonse ndi mayankho anu oyankhulana azisungidwa mwachinsinsi. Wofufuzayo sagawana mayankho anu payekha kapena zotsatira za labotale ndi wina aliyense kupatula woyang'anira kafukufukuyo.

Mukulimbikitsidwa kufunsa mafunso kapena kudzutsa nkhawa nthawi ina iliyonse yokhudza mtundu wa kafukufukuyu kapena njira zomwe ndikugwiritsa ntchito. Chonde nditumizireni nthawi iliyonse uthenga kapena kuimba phone pa manambala a lamya omwe atchulidwa pamwambapa.

Appendix 5: Questionnaire (english)

Assessing knowledge, attitudes, and practices of broiler chicken farmers on antibiotic use and residues in Blantyre, Malawi

Section A: Demographic characteristics of the farmers and common characteristics of the farms

1. Name of the respondent / Dzina la oyakha……………………………………………...
2. Sex: Male/Mwamuna ☐ Female/ Mkazi Age/ Dzaka ☐ …………...
3. Marital status/Banja
    a) Married/ Okwatira
    b) Single/Osakwatira
    c) Divorced/Banja linatha
4. Education level/Maphunziro
    a) Uneducated/Osaphunzira
    b) Primary level/Pulayimale
    c) Secondary level/Sekondale
    d) Diploma/mwaphunziro a ukukadaulo
    e) Degree/ maphunziro a ukachenjede
    f) Postgraduate/Maphunziro oposera a ukachenjede
5. For how long have you been a broiler farmer (years)/ Ndi kwa nthawi yaitali bwanji mwakhala mukuweta Nkhuku za mtundu wa nyama? ………………

Section B: knowledge of the farm owners / Upangili wa eni ake a Nkukhu

6. Antibiotics can be used in animals for weight gain/ Makhwala ophela tizilombo ta bakiteliya atha kugwilitsidwa ntchito ngati okuzila msinkhu wa Nkhuku?
    a) Yes/inde
    b) No/Ayi
7. Antibiotics can be used for all types of diseases in animals/ makhwala ophela tizilombo ta bakiteliya atha kugwilitsidwa ntchito pofuna kuthana ndi matenda aliwonse omwe amagwira khuku?
    a) Yes/inde
    b) No/Ayi

8. Improper use of antibiotics in animal farm can cause Anti-Microbial Resistance/ kusagwiritsa bwino ntchito makhwala ophera tizilombo ta bakiteliya kutha kuchititsa kuti tizilombo ta bakiteliyati tisiye kungonjetsetwa ndi makhwalawa?
   a) Yes/inde
   b) No/Ayi
9. Antibiotics are not harmful for beneficial bacteria living in the body/ Makhwala ophera tizilombo ta baketeliya sioopsya kwa tizilombo ta bakiteliya tokhala mkati mwa thupi tomwe ndi topindulila mthupi?
   a) Yes/inde
   b) No/Ayi
10. Antibiotics have side effects/ Makhwala ophela tizilombo ta bakiteliya ali ndi zotsatira zina zosakhalabwino?
    a) Yes/inde
    b) No/Ayi
11. Bacteria can become resistant to antibiotics/ Kachilombo ka bakiteliya katha kukhala kosapheka/kopilila ku makhwala ophela tizilombo ta bakiteliya
    a) Yes/inde
    b) No/Ayi
12. Have you ever heard about antibiotic resistance? / Munayamba mwamvapo za kupilila kapena kusapheka kwa tizilombo ta bakiteliya ku makhwala ophela tizilomboti?
    a) Yes/Inde
    b) No/Ayi
13. Have you ever attended any training on using antibiotics in poultry production? / Munayamba mwakhalapo pa maphunziro a kagwilitsidwe ntchito ka makhawala ophera tizilombo ta bakiteliya?
    a) Yes/Inde
    b) No/Ayi
14. Do you think all antibiotics can be used in both humans and animals? / Kodi mukuganiza kuti makhawala ophela tizilombo ta bakiteliyawa amagwilitsa ntchito pochiza anthu ndi nyama zomwe?
    a) Yes/Inde
    b) No/Ayi

c) Don’t know/Sindikudziwa

15. When do you mostly use antibiotics? / Kodi makhwala ophela tizilombo ta bakiteliyawa mumagwilitsa ntchito kwambiri nthawi iti?
    a) Disease treatment/Pochiza matenda
    b) Prevention of infection /Popewa matenda
    c) Promoting growth/ Pokuza chiweto
    d) Whenever I want to use/ Thawi iliyonsde ndingafune kugwilitsa ntchito
    e) don’t know/Sindikudziwa
16. Which diseases are antibiotics used for? / Kodi ndi matenda ati amene makhwala ophera tizilombo ta bakiteliya amachiza?
    a) Bacterial diseases/Matenda oyambitsidwa ndi kachilombo ka bakiteliya
    b) Viral disease/ Matenda oyambitsidwa ndi kachilombo ka vairasi
    c) Any Disease/ Nthenda iliyonse
    d) Don’t know/ Sindikudziwa
17. What is your understanding of the word antibiotic resistance? / kodi kumvetsetsa kwanu ndi Kotani pa mawu oti kukhala opilira ku makhwala ophela tizilombo ta bakiteliya?
    a) It causes poor response to treatment/Kubweretsa zotsatila zosayembekezeleka tikapeleka makhwala pofuna ndi cholinga chochiza
    b) It causes treatment failure/ Kuyambitsa kusachizika
    c) It is dangerous but I do not know how to describe it /Ndizoopsya ndithu koma sindikudziwa kuti ndingafotokoze bwanji
    d) Others/Zina zoonjezera ……………..
18. What do you think about the use of antibiotics in animal feed? /Kodi maganizo anu ndi otani pa kugwilitsa ntchito makhwala ophela tizilombo ta bakiteliya popangila chakudya cha nyama?
    a) All ready feed has antibiotics/Chokudya chonse chopangidwa pofuna kugulitsa kwa alimi chimakhala kale ndi makhwala ophela tizilombo ta bakiteliya
    b) Some ready feed has antibiotics/ Chokudya china chopangidwa pofuna kugulitsa kwa alimi chimakhala kale ndi makhwala ophela tizilombo ta bakiteliya
    c) None of the feed has any antibiotics/ Palibe chakudya chomwe chimakhala kale ndi makhwala ophera tizilombo ta bakiteliya
    d) I don’t know/ Sindikudziwa

19. Antibiotic use in feed is prohibited/ kugwilitsa ntchito makhawala ophera tizilombo ta bakiteliya mu chakudya cha ziweto ndikoletsedwa?
    a) I have no idea/ Sindikudziwapo kanthu
    b) No antibiotic used in feed/ Palibe kugwilitsa ntchito makhwalawa mu chakudya cha ziweto
    c) No antibiotic allowed to use in feed and if used, should follow the withdrawal period/Makhwala ophera tizilombo ta bakiteliya sayenera kugwilitsidwa ntchito mu chakudya, koma ngati agwilitsidwa ntchito pamafunika kutsatila ndondomeko yolondola chiwetocho pa nthawi yomwe chiweto chikaphedwe

Section C: Attitude of the farmers/ Malingaliro a alimi

20. When do you think your chicken need antibiotics the most? / Kodi mukuganiza kuti Nkhuku zanu zimafunika makhawala ophera tilombo tophera bakiteliya kwambiri pa nthawi iti?
    a) Have a disease/Pamene yadwala
    b) Have any abnormal symptoms/signs/ Pamene ikuonetsa zizindikilo zachilema
    c) Guidance/suggestion from other people/ Kutengela malangizo kwa anthu ena
21. What would be the first thing you do when your chickens are showing signs of disease? /Kodi ndi chithu chiti choyambilila chomwe mumachita mukaona zoti khuku zanu zikuonetsa zizindikilo zoti zagwidwa ndi matenda?
    a) Ask a private veterinarian/ Kuwafunsa madokotala a ziweto omwe sali a boma
    b) Ask Govt. veterinarian/ Kuwafunsa adokotala a ziweto a boma
    c) Ask neighbor farmers/ Kuwafunsa alimi azanthu oyandikana nawo
    d) Self-medication/ Kupeleka makhwala ku nkhuku pandekha
    e) Ask drug seller/ Kufunsa kwa anthu amene amagulitsa makhwala
    f) Isolation of sick bird outside the shed/Kuchotsa khuku zodwala mu khola
    g) Isolation of sick bird within the shed/ Kuika khuku zodwala mbali ina ya khola
    h) Other (specify)/ zina zoonjezera ………………………………………………...
22. Please rate your interest in learning more about antibiotics/ Kodi chidwi chanu ndi chotani pofuna kudziwa zambili za makhwala ophera tizilombo ta bakiteliya?
    a) Extremely interested/ Ndili ndi chidwi kwambili
    b) Interested/ Ndili ndi chidwi
    c) Neutral/ Ndili pakatikati

d) Not interested/ Ndilibe chidwi

23. Use of antibiotics is necessary to improve the productivity of poultry/ kugwilitsa ntchito makhwala ophera tizilombo ta bakiteliya ndi kofunika pofuna kuchulutsa phindu mu ulimi wa Nkhuku?
    a) Particularly important/Ndiofinika ndithu
    b) Important/Ndiofunika
    c) Neutral/Ndiofunika chikatikati
    d) Less important/ Ndi osafunika kwenikwei
    e) Not important/ Ndi osafunika konse
24. Do you think you should always consult with a veterinarian before any use of antibiotics? / Kodi mukuganiza kuti ndikoyenera kufunsa kwa dokotala kapena mulangizi wa ziweto musanagwilitse ntchito makhwala ophere tizilombo ta bakiteliya?
    a) Yes/Inde
    b) No/Ayi
25. Do you believe that restricting the use of antibiotics in poultry will have more negative effects than positive ones? /Kodi mukuganiza kuti kuletsa kugwilitsa ntchito makhwala ophela tizilombo ta bakiteliya mu ulimi wan nkhuku za mazira kutha kukhala koopya kusiyana ndi kugwilitsa ntchito makhwalawa?
    a) Yes/ Inde
    b) No/Ayi
    c) I don't know/ Sindikudwiwa
26. If you use antibiotics on your farm, do you want to substitute them with alternatives that are not antibiotics? / Kodi mungakonde kugwilitsa ntchito njira zina mmalo mogwilitsa ntchito makhawala ophela tizilombo ta bakiteliya?
    a) Yes/inde
    b) No/ayi
    c) I don't know/sindikudziwa
27. Antibiotic resistance in animals is not important for public health/ kupilila kapena kusapheka kwa tizilombo ta bakiteliya si kofunikila kapena sikungakhudze anthu?
    a) Agree/ Ndikuvomeleza
    b) Neutral / Chikatikati
    c) Disagree/ Sindikuvomeleza

28. There is a relationship between antibiotic use in animals and the development of resistance/ Pali kugwilizana pakati pa kagwilitsidwe ntchito ka makhwala ophela tizilombo ta bakiteliya ndi kuyambitsa kupilila kapena kusapheka kwa tizilombo ta bakiteliya ndi makhwalawa?
    a) Agree/ Ndikuvomeleza
    b) Neutral / Chikatikati
    c) Disagree/ Sindikuvomeleza
29. The use of antibiotics in livestock causes the emergence of resistant bacteria which cause diseases in humans. / Kugwilitsa ntchito makhwala ophela tizilombo ta bakiteliya kutha kuyambitsa kusapheka kwa tizilombo ta bakiteliya tomwe timayambitsa matenda mu anthu?
    a) Agree/ Ndikuvomeleza
    b) Neutral / Chikatikati
    c) Disagree/ Sindikuvomeleza
30. Animals will benefit more from a prohibition on antibiotic use than they will suffer from it. / Kuletsa kugwilitsa ntchito makhwala ophelatizilombo ta bakiteliya kutha kutha kukhala kopindulitsa kwambiri?
    a) Agree/ Ndikuvomeleza
    b) Neutral / Chikatikati
    c) Disagree/ Sindikuvomeleza
31. Usage of the same antimicrobial drug (AMD) for a prolonged period can lead to antimicrobial resistance (AMR)/ Kugwilitsa ntchito makhwala ophela tizilombo ta bakiteliya a mtundu umodzi kwa thawi yaitali kutha kuyambitsa kupilila kapena kusapheka kwa tizilombo ta bakiteliya?
    a) Agree/ ndikuvomeleza
    b) Neutral / chikatikati
    c) Disagree/ sindikuvomeleza
32. Usage of AMD for non-therapeutic reasons lead to AMR/ kugwilitsa ntchito makhwala ophela tizilombo ta bakiteliya posafuna kuteteza matenda zitha kuyambitsa kusapheka kapena kupililakwa bakiteliya ku makhwalawa?
    a) Agree/ ndikuvomeleza
    b) Neutral / chikatikati

c) Disagree/ sindikuvomeleza

33. Purchasing AMD from a drug company or cooperative with a legal permit is safe/ kugula mankhwala kwa anthu kapena ma shopu ovomelezeka ndi koyenera?
    a) Agree/ ndikuvomeleza
    b) Neutral / chikatikati
    c) Disagree/ sindikuvomeleza
34. Drug withdrawal periods should be adhered to as per the prescription to avoid drug residues in meat or animal products/ kulondoloza ndondomeko ya masiku okwanira pamene chiweto chomwe chapatsidwa mankhwala chikukaphedwa ndikudyedwa ngati nyama ndi koyenera kukutsatila
    d) Agree/ Ndikuvomeleza
    e) Neutral / Chikatikati
    f) Disagree/ Sindikuvomeleza

Section D: Farmers practices/ Zochita za alimi

35. Do you check the expiry date of antibiotics before using it for your chicken? / Kodi mumaona tsiku limene makhwala opangidwa asiye kugwira ntchito yake musanapeleke makhwalawa ku Nkhuku zanu?
    a) Yes/ inde
    b) No/ ayi
36. I increase the dose of antibiotics and frequency of administration if animals do not show any signs of recovery. / Ndimapeleka makhwala pafupi pafupi pamene chiweto changa sichikonetsa kuchira kulikonse
    a) Yes/ inde
    b) No/ ayi
37. if animals feel better after the first day of treatment, I stop giving the antibiotics/ Chiweto chikapeza bwino patsiku loyamba lomwe chapatsidwa makhwala ndimasiya kupekleka makhwalawo?
    a) Yes/ inde
    b) No/ ayi

38. I consider the recommendations of other farmers about antibiotic use/ Ndimagwilitsa ntchito malangizo a alimi amzanga pa zakagwilitsidwe ntchito ka makhwala ophela tizilombo ta bakiteliya?
    a) Yes/ inde
    b) No/ ayi
39. I would continue to use antibiotics in animals even if I knew they would harm public health. / Ngakhale nditadziwa kuti kugwilitsa ntchito makhwala ophera bakiteliya mu ziweto ndi koopsya kwa anthu sindingasiye kugwilitsa ntchito makhwalawa.
    a) Yes/ inde
    b) No/ ayi
40. I adhere to specified drug withdrawal periods before sending animals to the slaughterhouse/ ndimatsatila ndondomeko ya nthawi yomwe ndapeleka mankhwala kwa chiweto ndi nthawi yomwe chikupita kukaphedwa.
    a) Yes/ inde
    b) No/ ayi
41. Where do you get treatment for your sick Chicken? / kodi ndi kwa ndani komwe mumatengako malangizo Nkhuku zanu zikadwala?
    a) Govt. veterinary physician/ Mlangizi wa ziweto boma
    b) Private veterinary physician/ Mlangizi wa ziweto woti siwaboma
    c) Feed/chick/medicine company representative/ Kwa anthu omwe amaimilira ma fakitole opannga makhwalawa
    d) Feed/chick/medicine seller/ Kwa ogulitsa chakudya kapena anapiye a Khuku
    e) Veterinary paraprofessional/ Dokotala wa ziweto
    f) Self-medication/ Ndimapeleka ndekha makhwala
    g) Family/friends/ Kwa abale komanso abwemzi
    h) Other animal raisers/ Kwa alimi amzanga
42. Why do you use antibiotics for your boiler chicken? / Kodi mumagwilitsa ntchito mankhwala ophela tizilomo ta bakiteliya ku Nkhuku zanu chifukwa chani?
    a) For treatment of diseases/ Kuchiza matenda
    b) For prevention of infection/ Kupewa matenda
    c) For growth promotion/ Pofuna kukulitsa Nkhuku mwachangu
    d) Others/ zina zoonjezera..

43. Where do you buy/collect antibiotics? / Kodi makhwala mumagula kuti/
    a) From animal drug store/ Mma shopu ogulitsira makhwala a ziweto
    b) From salesperson of animal health company/ Ku fakitole ya anthu omwe adaphunzira za umoyo wa ziweto
    c) Others/zina zoonjezera …..
44. How do you use the antibiotics? / Kodi makhwala ophela tizilombo ta bakiteliya mmagwilitsa ntchito bwanji?
    a) Following the manufacturer instruction/ Kutsatila ndondomeko za opanga makhwala
    b) Following earlier experiences/ Kutsatila zomwe ndikudziwakale
45. What factors do you prioritize when you buy antibiotics? / Kodi ndi ziti zina zomwe mmatsatira mukugula makhwala ophela tizilombo ta bakiteliya
    a) Expired date/ Tsiku losiya kugwira makhwala
    b) Based on recommendations of the drug/feed/chick seller/ Kutengela malangizo a opanga makhwala
    c) Certain brand/trademark/ Kutsatila dzina la opanga makhwala
    d) Trusted drug stores/ Ku shop kogula makhwala kodalilika
46. If the antibiotic was expired, what would you do? / Ngati makhwala apitilila masiku ake ogwila ntchito mumatani?
    a) Stop using/ Kusiya kugwilitsa ntchito
    b) No answer/don't know/ Sindikudziwa
    c) Other /zina zoonjezera……………………………………………………….
47. Do you use suggested/recommended doses of antibiotics? / kodi mumagwilitsa ntchito mlingo weniweni oyenela kagwilitsidwe ka mankhwala?
    a) Yes/ Inde
    b) No, I used higher dose/ Ayi, ndimagwilitsa ntchito mulingo okwera
    c) No, I used lower dose/ Ayi ndimagwilitsa ntchito mulingo otsika
    d) Do not know/ Sindikudziwa
48. Do you administer antibiotics for the suggested duration to your chicken? / kodi mumagwilitsa ntchito makhwala pa masiku oyenelela kugwilitsa ntchito makhwalawa?
    a) Yes/ Inde
    b) No, I used it for a longer duration/ Ndimagwilitsa ntchito masks opitilila

c) No, I used for shorter duration/ Ndimagwilitsa ntchito masiku ochepela

d) Do not know/ Sindikudziwa

Thank you for your participation.

Appendix 6: Knowledge level of farmers on antibiotic use

Characteristic	N = 50[1]
All antibiotics can be used in both humans and animals	
Don't know	**20 (40%)**
No	**21 (42%)**
Yes	**9 (18%)**
Antibiotics are mostly used for	
Disease treatment	**29 (58%)**
Don't know	**1 (2.0%)**
Prevention of infection	**12 (24%)**
Promoting growth	**4 (8.0%)**
Whenever I want to use	**4 (8.0%)**
Diseases which antibiotics are used for	
Any disease	**15 (30%)**
Bacterial diseases	**32 (64%)**
Don'**t know**	**2 (4.0%)**
Viral diseases	**1 (2.0%)**
Thoughts on antibiotic use in animal feed	
All ready feed has antibiotics	**10 (20%)**
Don'**t know**	**12 (24%)**
None of the feed has any antibiotics	**6 (12%)**
Some ready feed has antibiotics	**22 (44%)**
Antibiotic use in feed is prohibited	
I have no idea	**35 (70%)**
No antibiotic is allowed to be used in feed and if used, should follow the withdrawal period	**10 (20%)**
No antibiotic is used in the feed	**5 (10%)**

[1]**n (%)**

Appendix 7: Practice of farmers on antibiotic use

Characteristic	N = 50[1]
Treatment source	
Family/friends	2 (4.0%)
Feed/chick/medicine company representative	7 (14%)
Feed/chick/medicine seller	3 (6.0%)
Govt. veterinary physician	10 (20%)
Private veterinary physician	13 (26%)
Self-medication	12 (24%)
Veterinary paraprofessional	3 (6.0%)
Antibiotic use	
Any disease	15 (30%)
Bacterial diseases	32 (64%)
Don't know	2 (4.0%)
Viral diseases	1 (2.0%)
Priorotise_when_buying	
Based on recommendations of the drug/feed/chick seller	24 (48%)
Certain brand/trademark	3 (6.0%)
Expired date	17 (34%)
Trusted drug stores	6 (12%)
Action if expired	
No answer/Don't know	3 (6.0%)
Stop using	47 (94%)
Recommended dose	
Don't know	1 (2.0%)
No, I used a higher dose	5 (10%)
No, I used a lower dose	1 (2.0%)
Yes	43 (86%)
Suggested duration	
No, I used a higher dose	2 (4.0%)
No, I used a lower dose	5 (10%)
Yes	43 (86%)

[1]n (%)

Appendix 8: Relationship between farmers' knowledge of antibiotic use and educational level

	Level of Education						
Characteristic	**Degree**, N = 9[1]	**Diploma**, N = 11[1]	**Post-graduate**, N = 3[1]	**Primary**, N = 4[1]	**Secondary**, N = 21[1]	**Uneducated**, N = 2[1]	**p-value**[2]
Antibiotic use for weight gain	3 (33%)	4 (36%)	3 (100%)	1 (25%)	4 (19%)	2 (100%)	0.034
Diseases which antibiotics are used for							0.044
Any disease	1 (11%)	3 (27%)	1 (33%)	4 (100%)	4 (19%)	2 (100%)	
Bacterial diseases	7 (78%)	8 (73%)	2 (67%)	0 (0%)	15 (71%)	0 (0%)	
Don't know	1 (11%)	0 (0%)	0 (0%)	0 (0%)	1 (4.8%)	0 (0%)	
Viral diseases	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.8%)	0 (0%)	
[1]n (%)							
[2]Fisher's exact test							

Appendix 9: Association between farmer's length of farming (years) and their knowledge of antibiotic use

	Farming scale				
Characteristic	**0-3**, N = 26[1]	**4-6**, N = 13[1]	**7-9**, N = 3[1]	>**9**, N = 8[1]	**p-value**[2]
Antibiotics have side effects	12 (46%)	9 (69%)	2 (67%)	8 (100%)	0.027
[1]n (%)					
[2]Fisher's exact test					

Appendix 10: Association between broiler farmer's level of education and their attitudes toward antibiotic use

	Level of Education						
Characteristic	**Degree**, N = 9[1]	**Diploma**, N = 11[1]	**Post-graduate**, N = 3[1]	**Primary**, N = 4[1]	**Secondary**, N = 21[1]	**Uneducated**, N = 2[1]	**p-value**[2]
long period usage of a drug can lead to AMR							0.006
Agree	7 (78%)	5 (45%)	0 (0%)	1 (25%)	18 (86%)	2 (100%)	
Neutral	2 (22%)	4 (36%)	1 (33%)	3 (75%)	2 (9.5%)	0 (0%)	
Disagree	0 (0%)	2 (18%)	2 (67%)	0 (0%)	1 (4.8%)	0 (0%)	
[1]n (%)							
[2]Fisher's exact test							

Appendix 11: Association between broiler farmer's level of education and their practice on antibiotic use

	Level of Education						
Characteristic	**Degree**, N = 9[1]	**Diploma**, N = 11[1]	**Post-graduate**, N = 3[1]	**Primary**, N = 4[1]	**Secondary**, N = 21[1]	**Uneducated**, N = 2[1]	**p-value**[2]
Stop antibiotic treatment if animals show improvement	0 (0%)	4 (36%)	1 (33%)	3 (75%)	4 (19%)	1 (50%)	0.041

[1]n (%)

[2]Fisher's exact test

Appendix 12: Calculations for serial dilution

The serial dilutions were made using the following formula

$Mass_1 Volume_1 = Mass_2 Volume_2$

For 0.5 mg/ mL

5 mL of stock solution was combined with 5 mL of mobile phase A

For 0.3 mg/ mL

0.5 mg × Volume1 = 0.3mg×1000 mL

$Volume_1$=0.3mg×1000 mL /0.5 mg

$Volume_1$=600 mL

Therefore, to make a 0.3 mg/mL concentration 600 mills of a standard solution of o.5 mg/mL was combined with 400 mills of mobile phase A.

For 0.2 mg/mL

0.3 mg × $Volume_1$ = 0.2 mg×1000 mL

$Volume_1$=0.3 mg×1000 mL /0.3 mg

$Volume_1$=666.7 mL

Therefore, to make a 0.2 mg/mL concentration 666.7 mills of standard solution of o.3 mg/mL was combined with 400 mills of mobile phase A.

For 0.1 mg/mL

0.2 mg × $Volume_1$ = 0.2 mg×1000 mL

$Volume_1$=0.1 mg×1000 mL /0.2 mg

$Volume_1$=500 mL

Therefore, to make a 0.1 mg/mL concentration 500 mL of a standard solution of o.5 mg/mL was combined with 400 mL of mobile phase A.

For 0.05 mg/mL

0.1 mg × $Volume_1$ = 0.05 mg×1000 mL

$Volume_1$=0.05 mg×1000ml/0.1 mg

$Volume_1$=500 mL

Therefore, to make a 0.1 mg/mL concentration 500 mills of a standard solution of o.5 mg/mL was combined with 400 mills of mobile phase A.

Appendix 13: Calibration curves

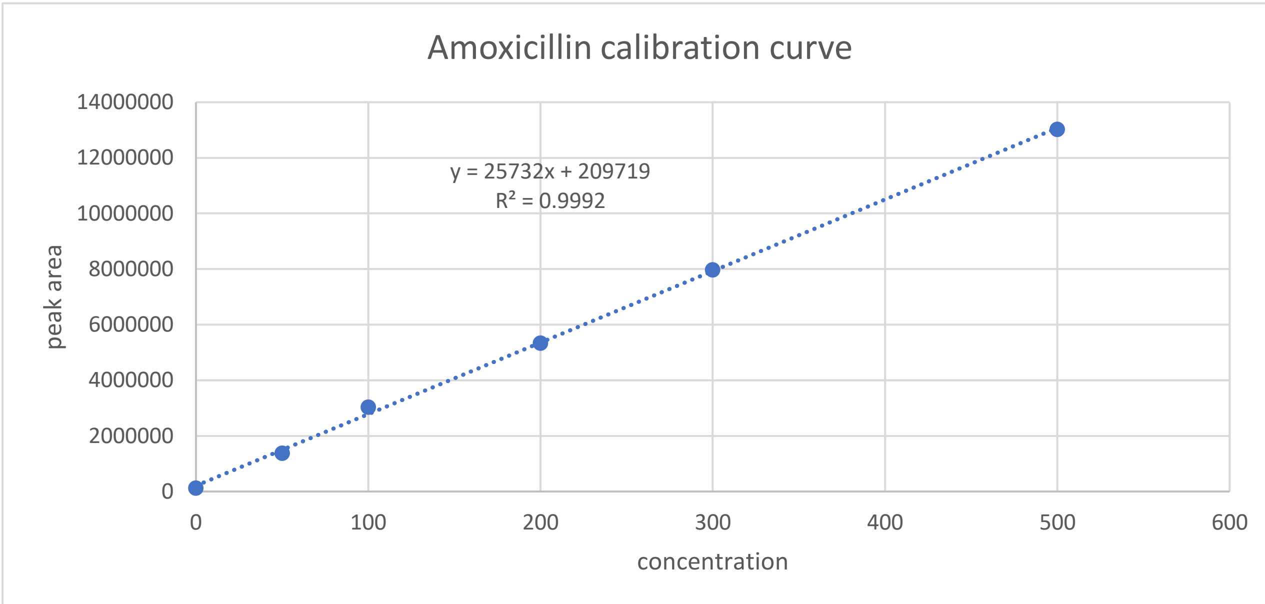

Figure 9: Calibration curve of Amoxil standards serial dilutions of 50 µg/ mL,100 µg/ mL,200 µg/ mL,300 µg/ mL, and 500 µg/ mL

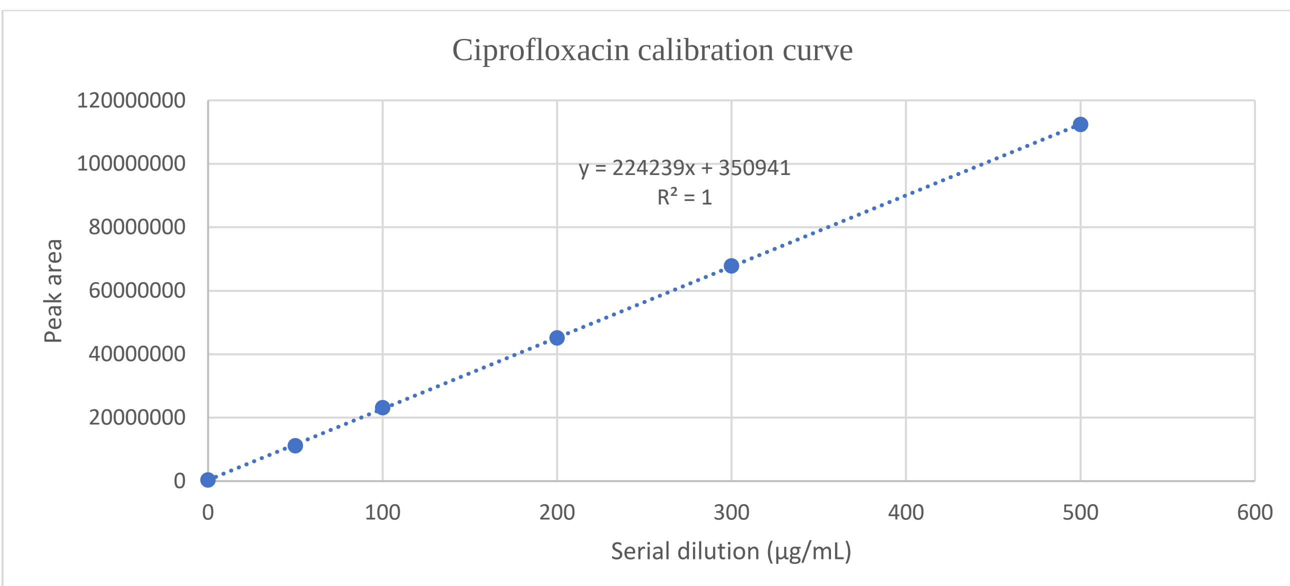

Figure 10: Calibration curve of cipro standards serial dilutions of 50 µg/ mL,100 µg/ mL,200 µg/ mL,300 µg/ mL, and 500 µg/ mL

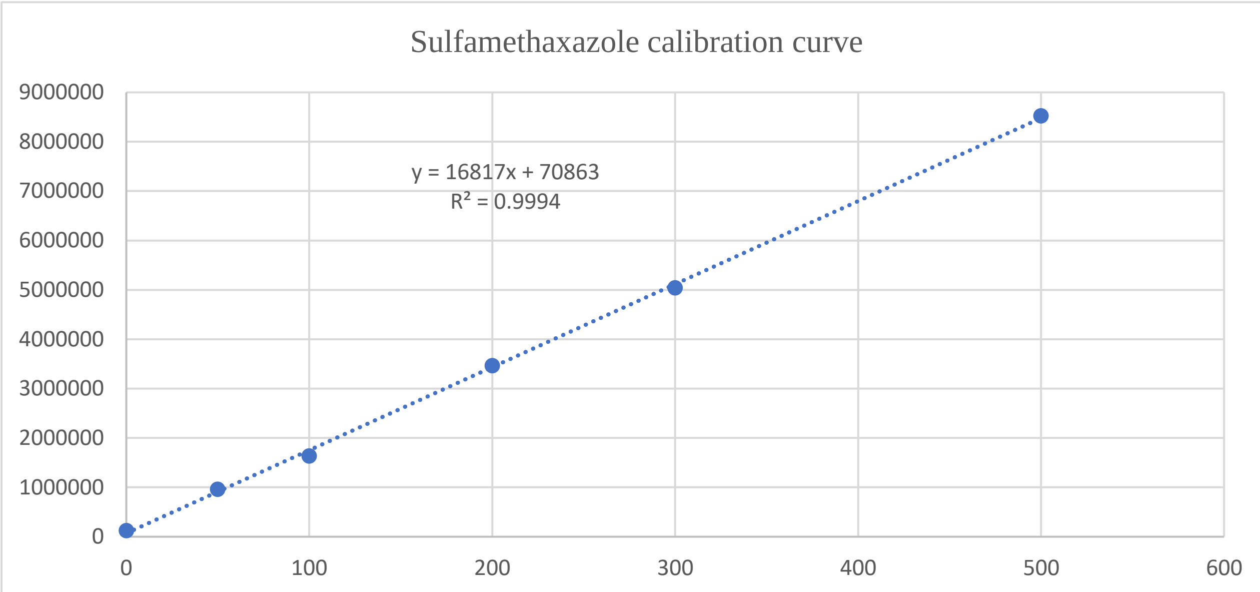

Figure 11: Showing a calibration curve of sulfamethazine standards serial dilutions of 50 µg,100 µg/ mL,200 µg/ mL,300 µg/ mL, and 500 µg/mL.

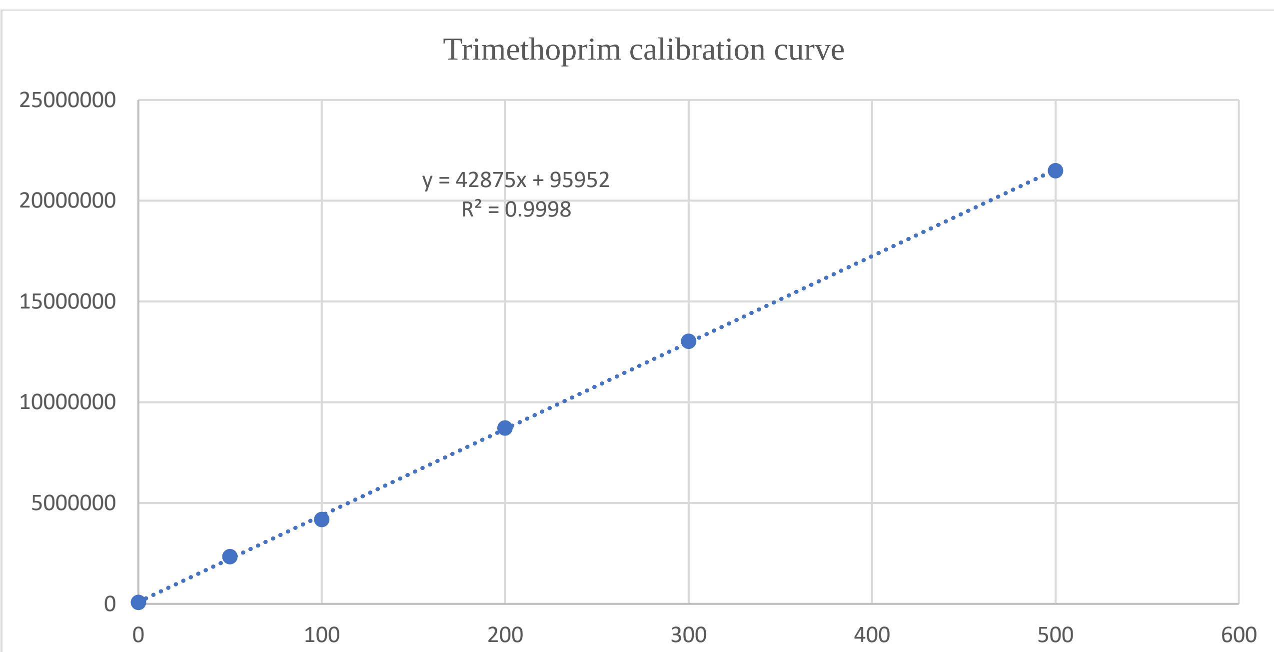

Figure 12: Calibration curve of trimethoprim standards serial dilutions of 50 µg/mL,100 µg/mL,200 µg/mL,300 µg/mL, and 500 µg/mL.

Appendix 14: The average retention time and average peak area of all the antibiotic residues

Antibiotic	Standard Calibration curve equation	R^2	Average retention time (Minutes)	Average peak area
Amoxicillin	y = 25732x + 209719	0.9992	1.76	205357220.8
Ciprofloxacin	y = 224239x + 350941	1	1.443	10214835.33
Sulfamethoxazole	y = 16817x + 70863	0.9994	3.36	1408444.333
Trimethoprim	y = 42875x + 95952	0.9998	2.783	1991511.487

Appendix 15: Conditions for HPLC for each antibiotic analysis

Antibiotics	Flow rate	Temperature	wavelength	Injection volume	Run time	Solvent's ratio	
						A	B
Ciprofloxacin	1.5ml/min	40	278 Nanometer	5 µL	6 minutes	55	45
Amoxicillin	1ml/min	Room temperature	254 Nanometer	10µL	10 minutes	55	45
Sulfamethoxazole and trimethoprim	1ml/min	Uncontrolled	254 Nanometer	10µL	10 minutes	35	65

Appendix 16: The presence of each antibiotic residue in all the chicken samples

Antibiotic type	Kidney and liver chicken samples positive (N=40)					
	Positive	%	Negative	%	Total samples positive	%
Ciprofloxacin	4	10	36	90	37	92.5
Trimethoprim	13	32.5	27	67.5		
Sulfamethoxazole	1	2.5	39	97.5		
Amoxicillin	35	87.5	5	12.5		

Appendix 17: Presence of antibiotic residues in chicken samples under different livestock management systems

Farming management system	Antibiotic type (N=40)			
	CIPRO	TRIM	SUFFER	AMOX
Small	1, (2.5%)	3, (7.5%)		9, (22.5%)
Medium	1, (2.5%)	4, (10%)	1, (2.5%)	12, (30%)
Large	2, (5%)	6, (15%)		14, (35%)

Appendix 18: The concentration of the amoxicillin-positive kidney and liver chicken tissues

Sample ID	Farm management system	Retention time	Peak area	Conc 1 (µg/mL)	Conc 2 (µg/mL)	Conc in 1 Kg (µg/Kg)	Mean (SD)	Range	95% CI[1]	p-value[2]
Kidney	Small	1.74	284325428	11041.3	690.084	0.69008	0.59 (0.18)	0.27 - 0.92	0.50, 0.68	0.002
Kidney	Small	1.77	265571334.3	10312.5	644.532	0.64453				
Kidney	medium	1.77	241022174.3	9358.48	584.905	0.58491				
Kidney	Medium	1.77	380555928.7	14781.1	923.816	0.92382				
Kidney	Large	1.77	324404937	12598.9	787.432	0.78743				
Kidney	Large	1.77	249739210	9697.24	606.078	0.60608				
Kidney	Large	1.77	330822310	12848.3	803.019	0.80302				
Kidney	Large	1.77	318899064.3	12384.9	774.059	0.77406				
Kidney	Medium	1.77	256339926	9953.76	622.11	0.62211				
Kidney	Small	1.77	215005666.7	8347.43	521.714	0.52171				
Kidney	Medium	1.76	282630113.3	10975.5	685.966	0.68597				
Kidney	Small	1.76	207017592	8036.99	502.312	0.50231				
Kidney	Medium	1.75	168779503.3	6550.98	409.436	0.40944				
Kidney	Large	1.75	160188582	6217.12	388.57	0.38857				
Kidney	Small	1.75	151053234.3	5862.1	366.381	0.36638				
Kidney	Medium	1.75	274454668.3	10657.7	666.109	0.66611				
Kidney	Medium	1.75	267555119.7	10389.6	649.351	0.64935				
Kidney	Small	1.75	114537530	4443.02	277.689	0.27769				

Kidney	Large	1.75	111164659.3	4311.94	269.496	0.2695				
Liver	Small	1.75	265822605	10322.3	645.142	0.64514	0.40 (0.12)	0.20 - 0.65	0.35, 0.46	
Liver	Medium	1.77	244653259	9499.59	593.725	0.59372				
Liver	Medium	1.77	175972645.7	6830.52	426.907	0.42691				
Liver	Large	1.76	219616926	8526.63	532.914	0.53291				
Liver	Large	1.77	198892979	7721.25	482.578	0.48258				
Liver	Large	1.77	128419403.7	4982.5	311.406	0.31141				
Liver	Large	1.78	159138064.7	6176.29	386.018	0.38602				
Liver	Medium	1.77	146228834.7	5674.61	354.663	0.35466				
Liver	Small	1.77	162221568	6296.12	393.508	0.39351				
Liver	Medium	1.76	131840456.7	5115.45	319.716	0.31972				
Liver	Small	1.76	138037179.3	5356.27	334.767	0.33477				
Liver	Medium	1.75	176429529	6848.27	428.017	0.42802				
Liver	Large	1.75	219207696.7	8510.73	531.92	0.53192				
Liver	Small	1.75	151723100	5888.13	368.008	0.36801				
Liver	Medium	1.75	136475808.7	5295.59	330.974	0.33097				
Liver	Medium	1.75	109066363.7	4230.4	264.4	0.2644				
Liver	Small	1.75	146105460	5669.82	354.364	0.35436				
Liver	Large	1.75	84298309	3267.86	204.241	0.20424				

Appendix 19: The concentration of the ciprofloxacin-positive kidney and liver chicken tissues

Sample ID	**Sample**	**Retention time**	**Peak area**	**Conc 1** µg/mL	**Conc 2** µg/mL	**Conc in 2g** (µg/g)	**Mean (SD)**	**Range**	**95% CI[1]**	**p-value[2]**
Medium	Kidney	1.403	1251841	4.119	0.257	0.129	2.63 (3.54)	0.13, 5.13	-29, 34	0.7
Large	Kidney	1.423	3.7E+07	164.445	10.278	5.139				
Small	Liver	1.477	750879	1.886	0.118	0.059	0.12 (0.09)	0.06, 0.18	-0.67, 0.91	
Large	Liver	1.467	1642053	5.859	0.366	0.183				

Appendix 20: The concentration of the trimethoprim-positive kidney and liver chicken tissues

Sample	Retention time	Peak area	Conc 1μg/mL	Conc 2 μg/mL	Conc in 2g (μg/g)	Mean (SD)	Range	95% CI[1]	p-value[2]
Kidney	2.823	1252561	26.97630321	1.68601895	0.843009475	1.22 (0.54)	0.74, 2.25	0.72, 1.7	>0.9
Kidney	2.807	1879911	41.60837318	2.600523324	1.300261662				
Kidney	2.75	1123134	23.95759767	1.497349854	0.748674927				
Kidney	2.813	1701989	37.45858892	2.341161808	1.170580904				
Kidney	2.84	3185734	72.0648863	4.504055394	2.252027697				
Kidney	2.733	2147324	47.84541108	2.990338192	1.495169096				
Kidney	2.737	1106925	23.57954519	1.473721574	0.736860787				
Liver	2.807	4377363	99.85798251	6.241123907	3.120561953	1.56 (1.30)	0.34, 3.26	0.20, 2.9	
Liver	2.753	1680418	36.95547522	2.309717201	1.154858601				
Liver	2.823	804884	16.53485714	1.033428571	0.516714286				
Liver	2.727	1439600	31.33872886	1.958670554	0.979335277				
Liver	2.807	562309	10.8771312	0.6798207	0.33991035				
Liver	2.76	4567426	104.2909388	6.518183673	3.259091837				

Appendix 21: The concentration of the ciprofloxacin positive in a kidney tissue

Farm management system	**Sample**	**Retention time**	**Peak area**	**conc 1** µg/mL	**conc 2** µg/mL	**conc in 2g(**µg/g)
Medium	Liver	3.36	1408444.333	30.61206608	1.913254	0.956627