Faculty of Veterinary Medicine

Master in Food Safety

ASSESSMENT OF ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI, SALMONELLA* SPP, AND *ENTEROBACTER* SPP IN CHICKEN MEAT SOLD IN INFORMAL MARKETS OF MZIMBA DISTRICT, MALAWI

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MAPUTO

February 22, 2026

UNIVERSIDADE EDUARDO MONDLANE

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DECLARATION

I, Abel Compbel Chipembo, hereby declare that this thesis entitled "Assessment of Antimicrobial Resistance of *E. coli, Salmonella* spp. *and Enterobacter* spp. in Informal Markets in Mzimba, Malawi" is entirely original with no submissions to other institutions for comparable reasons. It has not been submitted to any college or university for a degree or exam".

This dissertation is submitted in partial fulfillment of the requirements for the degree of Master in food safety at the Faculty of Veterinary Medicine of Eduardo.

Date:……. /………/……

Signature

(Abel Compbel Chipembo)

ACKNOWLEDGEMENTS

I thank God the Almighty for giving me the courage, wisdom, and protection this far with gratitude. The future endeavor is so blessed and opens great opportunities to all works that I put on hands to and critical thinking to prosper in life.

I would also take this opportunity to acknowledge my parents, Mr. Paul Chipembo, and Oline Nyasulu their effort, care, grooming, and educating me with all the necessary needs. I am humbled to witness this academic journey.

I am grateful to the Center of Excellence in Agriculture and Nutrition in collaboration with World Bank for choosing me as one of the MSc scholarship beneficiaries and for funding this research at the Faculty of Veterinary Medicine (FAVET).

I thank my research supervisors, Dr Niura Bila, Prof Belisario Moane, and Prof Janelisa Musaya, for the academic guidance, including corrections, strengthening data management, and reviewing, which has significantly improved this research. They committed to supervise me at the times I needed them most in my academic journey.

I also humbly thank Professor Celsatina Tchamo, Prof Laurinda Augusto, Prof Podja for valuable initial supervisory support in conceptualization, data management, and correctional guidance, thus making a great contribution to this research work and my academic career.

Special thanks are extended to the Malawian community and the Mozambique Community who have impressively supported me academically, financially, and have shown extraordinary consideration during my dissertation.

Finally, I thank my classmates, all UEM staff members, and all other people who gave technical and academic.

DEDICATION

I dedicate this work to my wife, Julia Mhango; my mother, Oline Nyasulu; my brother, Paul Chipembo; and my entire family, who have supported, and encouraged me throughout my academic and professional lives. Oline Nyasulu, my mother, instills in me the value of perseverance and never giving up, even in the face of success.

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ABBREVIATIONS AND ACRONYMS

AM: Ampicillin

AMR: Antimicrobial Resistance

AMRB: Antimicrobial Resistance Bacteria

AMU: Antimicrobial Usa

AST: Antibiotic susceptibility Test

ATBR: Antimicrobial Bacteria Resistance

CHL: Chloramphenicol

CIP: Ciprofloxacin

CLSI: Clinical Laboratory Standard Institute

CRO: Ceftriaxone

DNA: Deoxynucleic Acid

EU: European Union

FAO: Food and Agriculture Organization

FAS: Food Animal Source

GEN: Gentamycin

IFT: Implications for the Food System

KAP: Knowledge Attitudes and Practices

LMICs: Low- and Middle-Income Countries

MDR: Multi-Drug Resistant

MEM: Meropenem

Spps: Species

TET: Tetracycline

TSB: Trypticase Soy Broth

UEM: Eduardo Mondlane University

WHO: World Health Organization

ABSTRACT

Background: Antimicrobials are widely used in chicken production all over the world. However, the motivating factors towards the use of antimicrobials by poultry farmers is not well known. Furthermore, awareness of antimicrobial resistance and how such factors can lead to AMR in bacterial poultry pathogens, particularly those isolated from chicken meat in Mzimba district, is not well documented. **Aim:** To assess antimicrobial resistance of *E. coli*, *Salmonella* spp., and *Enterobacter* spp in chicken meat for public food safety. **Methodology:** A cross-sectional study was conducted involving 89 chicken farmers in Mzimba to assess their knowledge, attitudes, and practices (KAP) related to AMU and AMR. The study also included 28 veterinary shop owners to examine the drug value chain. AMR prevalence was assessed through 100 samples collected from the informal market. Data analysis was performed using STATA version 15, survey results were analyzed by regression analysis and Pearson correlations for KAP scores, while laboratory results were analyzed using descriptive statistics and one-way ANOVA. **Results:** Chicken farmers who demonstrated good knowledge, attitudes, and practices were 46.1%, 43.8% and 42.7% respectively. There were gaps in coordination between veterinary shop owners and regulatory bodies. The *E. coli* isolates from samples showed high resistance rates, with 91.3%, 82.6% and 71% for ampicillin, meropem, and tetracycline, respectively. Similarly, the *Enterobacter* spp. isolates from samples showed significant resistance, with 83.3%, 75% and 71% against ampicillin, meropem, and tetracycline. **Conclusions and recommendations**: Chicken farmers exhibited low KAP levels on AMU and AMR. *E. coli* and *Enterobacter spp* showed high resistance rates to ampicillin, tetracycline, and meropenem. Farmers and vet shop owners need to be trained on AMU and AMR.

Keywords: *Antimicrobial resistance, antimicrobial use, awareness, chickens, antimicrobials, Malawi*

RESUMO

Contexto: Os antimicrobianos são amplamente utilizados na produção de frango em todo o mundo. No entanto, os fatores que motivam os produtores de aves a utilizarem antimicrobianos não são bem conhecidos. Além disso, a consciencialização sobre resistência aos antimicrobianos e como esses fatores podem contribuir para a RAM em patógenos bacterianos de aves, particularmente aqueles isolados da carne de frango no distrito de Mzimba, não está bem documentada. Objetivo: Avaliar a resistência aos antimicrobianos em E. coli, Salmonella spp. e Enterobacter spp na carne de frango, em termos de segurança alimentar pública. **Metodologia**: Foi realizado um estudo transversal envolvendo 89 produtores de frango em Mzimba para avaliar os seus conhecimentos, atitudes e práticas (KAP) relacionados com o uso de antimicrobianos (AMU) e resistência aos antimicrobianos (AMR). O estudo incluiu também 28 proprietários de lojas veterinárias para examinar a cadeia de valor dos medicamentos. A prevalência de RAM foi avaliada através de 100 amostras coletadas no mercado informal. A análise de dados foi realizada usando o STATA versão 15, e os resultados do inquérito foram analisados por análise de regressão e correlação de Pearsonpara as pontuações KAP, enquanto os resultados laboratoriais foram analisados usando estatística descritiva e ANOVA unidirecional. **Resultados**: Os criadores de galinhas que demonstraram bons conhecimentos, atitudes e práticas foram, respetivamente, 46,1%, 43,8% e 42,7%. Havia lacunas na coordenação entre os proprietários das lojas veterinárias e os organismos reguladores. Os isolados de E. coli de amostras apresentaram elevadas taxas de resistência, com 91,3%, 82,6% e 71% para ampicilina, meropem e tetraciclina, respetivamente. De forma semelhante, os isolados de Enterobacter spp. de amostras mostraram resistência significativa, com 83,3%, 75% e 71% contra ampicilina, meropem e tetraciclina. **Conclusões e recomendações:** Os criadores de galinhas apresentaram baixos níveis de KAP em AMU e AMR. E. coli e Enterobacter spp apresentaram elevadas taxas de resistência à ampicilina, tetraciclina e meropenem. Agricultores e proprietários de lojas veterinárias precisam de ser treinados sobre AMU e AMR.

Palavras-chave: Resistência a antimicrobianos, uso de antimicrobianos, consciencialização, galinhas, antimicrobianos, Malawi

1.0 INTRODUCTION

1.1 Motivation

The poultry industry is increasing rapidly worldwide with significant contributions from countries like the United States, China, and Brazil (Shamsuddoha *et al*., 2013; Uzundumlu, 2025). Globally, chickens account for 91% of the world's total poultry population and contribute to around 89% and 92% of poultry meat and egg production, respectively (Erdaw & Beyene, 2022). The increase in chicken meat production and chicken meat worldwide will improve human nutrition and health (Alparslan & Demirba, 2019; Uzundumlu, 2025). The USA, China and Brazil are the biggest producers, accounting for more than 40% of global production in 2020 (FAOSTAT, 2020).

In Africa, the trend of poultry product consumption per person is steadily increasing. Currently, Africans consume an average of only 3.3 kg of poultry meat per year, compared to the global average of 14 kg (OECD-FAO, 2018). South Africa stands out as the largest producer of poultry, generating 1.9 million metric tons of chicken per year (Rich *et al*., 2022). Other significant poultry producers include Nigeria, Egypt, Uganda, Tanzania, Morocco, Kenya, and Algeria. The consumption of poultry has been rising at an average yearly rate of 5.4 percent in Sub-Saharan Africa. Notably, countries such as South Africa and Ghana have seen rapid increase in chicken imports, growing by 8% and 9% per annum, respectively, since 2005 (BFAP, 2021).

In Malawi poultry industry is increasing, with a lot of exports primarily to markets like Mozambique and Tanzania (DAHLD, 2021). Poultry production in Malawi is regarded as the main source of protein, and its demand has increased since mid-2000s owing to increased levels of economic growth and urbanization (Gondwe *et al.*, 2023).

The report by CASA, 2020, indicated that poultry farming in Malawi, mostly broiler production makes 79% of the animals consumption rate per person. It is predicted that the consumption of poultry will rise by 27,000 tons by 2030, reaching 109.84 thousand tons of chicken meat overall (Nyondo *et al*., 2024).

The rise in chicken production influence farmers to use antibiotics to combat bacterial illnesses, promote growth rate, and increase feed conversion, all of which improve meat production(Balala *et al*., 2019; Mtila *et al*., 2024). However, a selective pressure give rise to bacteria resistant to antibiotics (Ventola, 2015; WHO, 2017, 2022). This leaves scientists worried about the danger to human and animal health (Ramirez, 2018).

In addition to high prevalence of antimicrobial bacteria in food animals, the misuse or overuse of antimicrobials which can be associated with lack of Knowledge, attitudes and practices(KAP) can result in high toxic levels of antimicrobial residues in animal meat, such as chicken. These residues pose significant risks to vulnerable consumers (Abdeta *et al*., 2024). The hazardous effects of these residues can include toxic allergic reactions and disruption of gut microbiota in human body.

Consumption of chicken meat contaminated with resistant bacteria such as *Escherichia coli* (*E. coli*), *Salmonella* spp., and *Enterobacte*r spp, and with antibiotic residues can lead to infections that are difficult to treat and manage. This results in increased morbidity, mortality, and healthcare costs (Almansour *et al.*, 2023). Additionally, the presence of AMR bacteria in chicken can hinder market access for meat products, especially in regions with strict food safety standards.

Combatting AMR need One-Health approach as various bacteria are becoming increasingly resistant and livestock/wildlife may be a reservoir (Scott *et al*., 2019). A study by Muaz *et al. (*2018), reported that there is a need to implement important measures for low medium income countries (LMIC) to reduce bacterial resistance to antibiotics, through maintaining good hygiene (GHP), and good manufacturing practices (GMP), and educating farmers about use of antibiotics in animal production.

This study aimed at assessing the antibiotic resistant in chicken meat in the Mzimba North district of Malawi for public health and food safety. The assessment provided critical insights into the levels of the knowledge, attitudes, and practices regarding the use of antimicrobial and resistance, preverance of *E. coli, salmonella* spp. and E*nterobacter* spp. and their resistance patterns.

1.2 Problem statement

Antimicrobial resistance among pathogenic bacteria presents an urgent challenge to human and animal health (Scott *et al*., 2019). AMR is a significant global challenge, as evidenced by the 4.95 million people who died from drug resistant infections in 2019; of these 1.27 million death were directly attributed to AMR. Sadly, children under the age of five accounted for one in five of these fatalities. 3,600 of the 15,700 deaths in Malawi in 2019 were directly related to antimicrobial resistance (AMR). According to this figure, out of 204 nations, Malawi had the 23rd-highest age-standardized mortality rate per 100,000 population linked to AMR (Institute for Health Metrics and Evaluation, 2019).

Several studies have reported that in Low- and Middle-Income Countries (LMIC) like Malawi and Zimbabwe, most farmers have limited knowledge, attitude and practices (KAP) on antimicrobial use and resistance.(Abraham *et al*., 2025; Chilawa et al., 2023; Mtila *et al.*, 2024; Ndlovu, 2024) . On top of that evidence suggests that antimicrobial use in livestock is frequently shaped by limited access to trained veterinary services, weak regulation and enforcement, and reliance on advice from veterinary drug shop personnel who may be inadequately trained(Kainga *et al*., 2023, 2025; Kimera *et al*., 2020; MacPherson *et al*., 2022). These factors can contribute to inappropriate antimicrobial selection, under-dosing, prolonged use, and routine prophylaxis. At the same time, poultry husbandry and biosecurity practices may be suboptimal, increasing infection pressure and further incentivizing antimicrobial use (Kainga *et al*., 2025; Mtila *et al*., 2024)

There is a notable rise in chicken consumption in Malawi (Nyondo *et al.*, 2024). Chicken is particularly popular because it is cheap and more affordable as compared to other types of meat. The majority of chicken farmers in Malawi lack proper training in poultry husbandry, including biosecurity and the responsible use of antimicrobials (Mtila et al., 2024). Currently, there are no recommendations regarding the prudent use of antibiotics in poultry and food animals. The limited awareness and knowledge of antimicrobial use among the majority of poultry farmers can lead to inappropriate use of antimicrobials (Kimera *et al*., 2020).

Recent studies in Malawi have reported antimicrobial-resistant bacteria in food animals and food products, raising concerns for food safety and public health (Chisembe *et al*., 2024; Kalumbi *et al*., 2022). For example a study by Chisembe *et al.* (2024), found high resistance rates of 90% and 70% for *E.coli* against tetracycline and co-trimoxazole, respectively, which are commonly used in broiler chickens. Consumption or handling of poultry contaminated with resistant bacteria such as *Escherichia coli* and *Salmonella* may lead to infections that are harder to treat. Inadequate observance of withdrawal periods may also result in antimicrobial residues in edible tissues, posing additional risks to consumers(Kainga *et al.*, 2025). Despite these concerns, the local drivers of antimicrobial use and the burden of AMR in Malawi remain insufficiently characterized, particularly among small-scale poultry farmers in rural districts.

Mzimba district is found in the northern region of Malawi. The district is predominantly rural, characterized by small-scale poultry production and limited veterinary service provision. In such contexts, farmers' knowledge, attitudes, and practices (KAP) regarding antimicrobial use and resistance are likely to influence antimicrobial-use behaviours which can lead to antibiotic resistance bacteria and the effectiveness of any stewardship interventions. In addition, anecdotal reports indicate that some farmers may use herbal remedies to treat poultry illnesses, but the patterns of use and perceived effectiveness are not well described.

Therefore, this study aimed to assess the knowledge, attitudes, and practices of poultry farmers in Mzimba District regarding antimicrobial use and antimicrobial resistance, and also determine resistance patterns of *E. coli, Salmonella* spp and *Enterobacter* spp in chicken meat. Understanding these factors is essential for designing targeted training, strengthening antimicrobial stewardship, informing policy and guidelines for prudent antimicrobial use in food animals, and ultimately reducing the risk of AMR at the human–animal interface.

1.3 General objective

This study aimed to investigate the influence of farmers' Knowledge, Attitude, and Practices (KAP) on antibiotic use and associated risk factors on preverance of AMR selected bacteria in chicken meat in Mzimba of Malawi.

1.3.1 Specific objectives

1. To evaluate chicken farmers' knowledge, attitudes and practices regarding antimicrobial use and antimicrobial resistance in chicken farming.
2. To identify key risk factors associated with AMR prevalence in chicken meat, including AMU practices and the drug supply chain.
3. To determine the preverance of *Escherichia coli*, *Salmonella* spp., and *Enterobacter* spp in fresh chicken meat.
4. To determine the preverance of antimicrobial resistance(AMR) in *E. coli*, *Salmonella* spp and *Enterobacter* spp isolated from fresh chicken meat.

1.3.2 Research Questions

1. What was the level of knowledge, attitudes and practices regarding AMU and AMR?

2. What risk factors contribute to the AMR?

3. What was the prevalence of *E. coli*, *Salmonella* spp., and *Enterobacter* spp. in fresh chicken meat in the study area?

4. Which pathogen *E. coli*, *Salmonella spp.*, and *Enterobacter* spp. was most resistant to the class of antibiotics tested?

1.4 Contributions of the study

The results found will:

a. Raise awareness to the public about AMR in chicken meat.
b. Help policymakers such as the government through the Department of Public Health, and Department of Animal Health to make new policies on how antimicrobials should be used and sold in poultry farming.
c. Help in the formulation of food safety guidelines to prevent and control of food-borne illness
d. Open ways for scientists to study more on AMR levels in meat and formulate new antimicrobials that can be effective to both humans and animals

2.0 LITERATURE REVIEW

2.1 Poultry sector in Malawi and its importance

The process of chicken production starts with breeding to produce offspring, which are then raised to maturity. Once the offspring reach the appropriate age and size, they are sent to processing facilities, where they are butchered and prepared for distribution to grocery stores, restaurants, and consumers. In Malawi, poultry farming is dominated by smallholder farmers, with the majority engaged in broiler production, which accounts for 79% per capita livestock consumption (CASA, 2020).

In Malawi approximately 1.3 million households are involved in chicken farming, and as of 2019, the total chicken population was 160 million which includes 67 millions indigenous chickens,83 million broilers, 8 million layers, and 2 million Black Australorps.

Production estimates indicate that the chicken population increased from 84.5 million in 2015 to 160 million in 2019, while egg production rose significantly from 4,700 tonnes in 2015 to 10,300 tonnes in 2019 (CASA, 2020). The three commonly raised breeds in Malawi are local chicken, mikolongwe, and hybrids (such as broilers and layers). While many people prefer rearing local chicken due to their taste, there is a high demand for broiler chickens because they are cheap and more affordable.

Most farmers in Malawi primarily engage in intensive systems, while only a small number adopt backyard or commercial systems. These farming systems are influenced by climate change, which has led to arise in the prevalence of diseases and external parasites, increased resistance of some diseases to treatment, and a decline in overall production and productivity. Despite these challenges, the demand for poultry and poultry products is growing due to human population growth and changes in consumer preferences (DAHLD, 2021).

2.2 Epidemiology of *E. coli, Salmonella* spp. and *Enterobacter* spp.

E. coli is a gram-negative bacillus that is non-spore-forming and is considered part of the normal intestinal flora. However, it can cause both intestinal and extraintestinal illnesses in animals and humans (Ema *et al*., 2022; Skurnik *et al*., 2016). There are five main subtypes of *E. coli* is known to cause illness: Enter0toxigenic *Escherichia coli* (ETEC), Enterohemorrhagic *Escherichia coli* (EHEC), Enteroinvasive *Escherichia coli* (EIEC), Enteropathogenic Escherichia coli (EPEC), Shiga toxin *E.coli* (STEC) and Enteroaggregative *Escherichia coli* (EAEC) (Makvana & Krilov, 2015). These subtypes possess specific O and H type antigens that are associated with *E. coli* resistance (Skurnet *et al*. 2016).

Salmonella spp are facultative intracellular gram-negative bacteria that cause high morbidity and mortality across a wide range of hosts, including humans, birds, mammals, and insects. The pathogenic strains include *S. enterotica S. enteritidis S. typhimurium* and *S. dublin* (Bäumler *et al*., 1998; Darshana *et al*., 2021; Dougnon *et al.*, 2017). These bacteria are among the most problematic foodborne and zoonotic pathogens, posing significant health threats and challenges to human well-being (Balasubramanian *et al*., 2019). *Salmonella* spp. typically reside in the gastrointestinal tract of warm-blooded animals, and in humans, they cause salmonellosis, a disease primarily characterized by mild diarrhea, commonly referred to as food poisoning (Abrahan *et al*., 2012; Rahman & Othman, 2017).

Enterobacter spp are gram-negative, rod-shaped bacteria that are facultatively anaerobic, meaning they can grow in both the presence and absence of oxygen (Ramatla *et al*., 2022). These bacteria are commonly found in the intestinal tracts of humans and animals, as well as in soil, water, and plant surfaces. Notably, two species, *Enterobacter cloacae* and *Enterobacter aerogenes*, can act as opportunistic pathogens. They are associated with infections in immunocompromised individuals, leading to conditions such as urinary tract infections, bloodstream infections, and respiratory infections. Additionally, *Enterobacter* spp can exhibit resistance to multiple antibiotics, which poses significant challenges for treatment (Ansarifar *et al*., 2023). This resistance can be due to the production of beta-lactamases and other mechanisms. In clinical settings, these factors complicate effective management of infections caused by *Enterobacter* spp.

These pathogens can contaminate meat through various ways. One of the occurrences is natural contamination, which can be due to lack of biosecurity measures during chicken rearing. When biosecurity is lacking, chicken may harbor these pathogens in their intestines, feathers, and skin, leading to contamination during slaughter (Lichtner *et al.*, 2024; WHO, 2023). Another method of contamination is cross-contamination, which occurs when equipment contaminated with bacteria is used on different chickens during the slaughtering process (Lichtner *et al*., 2024; Mazengia *et al*., 2015; Munther *et al.*, 2016). Additionally, poor hygiene practices by workers or meat sellers can introduce bacteria to the meat through contamination (Ogutu *et al*., 2024; Siluma *et al*., 2023). Finally, environmental exposure can also lead to contamination, as chicken meat may become tainted by feed or water that contains bacteria (Sajid *et al*., 2023; Sinhamahapatra, 2022; WHO, 2023).

2.3 Antimicrobial use

Antimicrobials are substances or compounds, either natural, synthetic, or semi-synthetic, that prevent the growth or kill microorganisms (Chi, 2024). The following are types of antimicrobial substances: antiprozoa, antibiotics, antifungal, antiparasites and antiviral (WHO, 2015). Among all antimicrobials, antibiotics are frequently used. This is because when human or animals are infected with other pathogens, the resulting conditions can lead to secondary infections that require antibiotic treatment. Globally, antimicrobial usage is estimated at 99,502 tons (95% CI 68,535–198,052) in 2020 and is projected, based on current trends, to increase by 8.0% to 107,472 tons (95% CI: 75,927–202,661) by 2030 (Mulchandani *et al*., 2023).

2.4 Antibiotic use in poultry

According to the World Organization for Animal Health, there are a lot of classes of antibiotics that are used in chicken production according. These antibiotics serve various purposes: they are employed therapeutically to alleviate bacterial illnesses, prophylactically for disease protection in animals considered at risk of infection, and for growth to enhance in growth rates or feed efficiency (Mikecz *et al*., 2020; Neill, 2016; WHO, 2017).

A study in low- and middle-income countries in Africa reported high levels of antimicrobial usage in animal production with nearly 80% of farms using antimicrobial agents(Van *et al*., 2020). The most commonly used antibiotics are tetracycline, aminoglycosides, and penicillin (Van *et al*., 2020). According to Kimera *et al.* (2020), the use of antimicrobials in intensive animal and food production is essential for maintaining animal health and optimum production. This usage has not only reduced the costs associated with food animal production but has also facilitated the rearing of food animals (FAs) in small and confined spaces, thereby increasing profit margin (Mankhomwa *et al.*, 2022).

Ayukekbong *et al*. (2017), reported that several factors contribute to antimicrobial resistance in developing countries. These include the high cost and inaccessibility of proper veterinary services, which leads farmers to seek advice from peers, family, and friends instead. Additionally, antimicrobial drugs are often available and easily accessible without restrictions, further exacerbating the issue. Moreover, a low level of illiteracy among livestock farmers also plays a significant role in this growing problem.

A study conducted among intensive small-scale farmers in Blantyre, Malawi, the primary factors influencing the district's use of antibiotics in food animals were the expense of veterinary care, the necessity to compete profitably with large-scale farmers, and the cost of putting in place and maintaining appropriate biosecurity measures. This situation could contribute to AMR (Mankhomwa *et al*., 2022). Additionally, Mtila *et al.*, (2024), reported that inadequate biosecurity practices influence antibiotic the use among poultry farmers in Lilongwe.

Kiambi *et al*. (2021), reported that tetracycline is widely used for treating animals across southern Africa. For example, in Blantyre, Malawi, tetracycline was the most commonly used antibiotic poultry treatment, followed by streptomycin, colistin and erythromycin following (Mankhomwa *et al*., 2022). Additionally, a study done by Mtila *et al*., (2024) in Lilongwe found out that farmers frequently used oxtetracycline, erythromycin, enrofloxacin, trimethoprim, sulfonamides, and colistin.

The overuse or misuse of antibiotics in farm animals contributes to AMR and leads to the accumulation of antimicrobial residues. This accumulation exerts selective pressure on microbial populations, enhancing the selection of resistant bacteria. Many genes responsible for antibiotic resistance are located on mobile genetic elements, which facilitate the spread of resistant bacteria (Cheng *et al.*, 2020).

2.5 Mechanism of antimicrobial-resistant bacteria

Antibiotic resistance can occur through natural and acquired(Ahmed *et al*., 2024). Bacteria can develop resistance naturally due to genes that are only expressed at resistance levels after antibacterial exposure(Munita *et al*., 2016). The organisms that are intrinsically resistant typically encode efflux pumps which help to transport the drug out of the cell, or have reduced membrane permeability which prevents the drug from entering the cell(Xu *et al*., 2019).

Acquired antibiotic resistance can occur through mutations and the acquisition of resistance genes. Mutations cause changes in the structure of chromosomes elements such as plasmids and transposons (Belay *et al.*, 2024). Mutations can occur due to physical factors such as ultraviolet radiation and chemical factors such as deamination agents, alkylating agents and intercalating agents, which induce structural changes in bacterial cells. This can result in reduced permeability to drugs or alterations in drug targets within the cell(Abbas *et al*., 2024).

Acquisition of genes is done through gene transfer. These genes are often exchanged between bacteria through horizontal gene transfer (HGT), a process that enables the sharing of genetic material across species, strains, and even genera within the same ecological niche. Alarmingly, HGT can transfer resistance genes non-pathogenic to pathogenic bacteria, amplifying the spread of resistance (Sun, 2018). Factors such as the availability of a large pool of resistance genes and favourable ecological conditions increase the likelihood of HGT, fuelling the evolution of antibiotic-resistant pathogens (Wintersdorff *et al.*, 2016).

Both acquired and mutation are associated with the use of antibiotics. Bacteria develop resistance due to inappropriate use of antibiotics (Adebowale *et al*., 2020; Al Amin *et al*., 2020; Dyar *et al*., 2020; Geta & Kibret, 2021; Mankhomwa *et al*., 2022). The overuse or misuse of antibiotics during chicken production causes the accumulation of antimicrobial residues which put selective pressure on microbial populations, enhancing the selection of resistant bacteria, since several genes coding for antibiotic resistance are located on mobile genetic elements (Cheng *et al.,* 2020).

Bacteria cause antibiotic resistance through altering structure of drug thereby preventing from binding to the target site on the pathogens cell. They can also actively transport the drug out of the cell thereby preventing from reaching target, bacteria also release enzymes which modify antibiotics making it ineffective e.g production of beta lactamase enzyme which make most of antibiotics ineffective(CDC, 2019). Bacteria also form biofilm formation thus a shielding matrix which prevent the antibiotics to kill the bacteria(Zhao *et al.*, 2023).

There is interaction between use of antimicrobial in chicken and development of AMR bacteria. Xu *et al*., 2022 reported that the use of antibiotics could lead to antibiotic residues which can lead AMR bacteria in meat which can spread to human through food chain (

Figure 1).

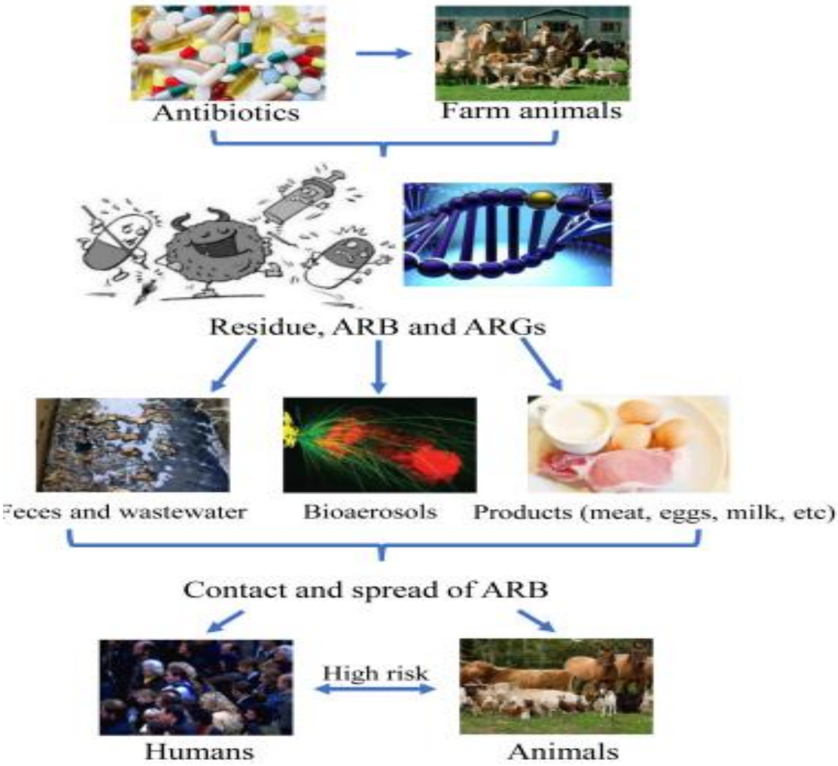


Figure 1:Pathway of AMU and AMR bacteria

Showing how the use of antibiotic in animals like chicken could lead to antibiotic residues and AMR bacteria through development of resistant genes. The residues and AMR bacteria can contaminate meat, hence spread to human, at same times human can get contact from faeces or waste water contaminated by bacteria hence spread of AMR bacteria (Source: Xu 2022

2.6 Burden of AMR globally

Antimicrobial resistance is a growing threat to human and animal health on a global scale (WHO, 2022a). This is because AMR reduces the effectiveness of antibiotic treatment and thus leads to increased morbidity, mortality, and healthcare expenditures (Hlashwayo *et al*., 2023). The AMR can lead to a global death toll to 700,000 at present and this can soar to 10 million by 2050 and loss of global production between 2016 and 2050 can top up to US$100 trillion (Neill, 2016).

The direct negative impact of AMR in the animal sector is production losses which ultimately result in reduced food security, and developing countries are more vulnerable to AMR (Hagos *et al*., 2021). AMR affects greatly humans and animals, and the main drivers of the development and emergence of AMR in low-income countries such as Malawi include 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 and affordable medicines, lack of knowledge and awareness about AMR; and lack of enforcement of legislation (OIE, 2020).

In Malawi, as in many low-income countries, there is a shortage of technical experts, inadequate microbiology services, limited funding, and insufficient epidemiological data to support improved preventive and treatment strategies against AMR (Kumwenda *et al*., 2021). As a result, AMR is evolving at an alarming rate, outpacing the development of new countermeasures from high-income countries. This situation poses significant threats to infection control in both humans and animals, threatening patient care, economic growth, public health, agriculture, economic security, and national security (WHO, 2020).

2.7 Food safety, quality, and contamination

Food safety, quality, and management are important in preventing foodborne illness. Agarwala & Kassie (2022) and Alex *et al*. (2017), reported several factors contributing to food-borne diseases. These include changing patterns of food consumption, decreased use of preservatives, and less processing by companies, all which can cause development of pathogens. Additionally, changes in farming practices by livestock farmers, government shortcomings in implementing effective food safety policies, and a lack of a multidisciplinary research approach in food safety further exacerbate the issue. The importation and exportation of meat also pose risks of bacterial contamination. In sub-Saharan Africa, imported meat can be contaminated with *E. coli* in up to 54% of cases (Olaru *et al.*, 2023).

Foodborne diseases and microbial food safety are emerging as significant global public health issues. Most foodborne diseases are generally caused by the consumption of contaminated food and beverages, such as raw milk, beef, and chicken (Boudjerda & Lahouel, 2022). These illnesses result from inadequate knowledge of food hygiene and frequent practice of unsafe food handling methods.

An estimated 600 million people-nearly one in ten worldwide-fall ill from eating contaminated food, resulting in a global annual burden of 33 million disability-adjusted life years (DALY) and 420,000 premature deaths (WHO, 2022). The impact of foodborne diseases is substantial, affecting individuals of all ages, particularly children under five and persons living in low-income regions.

Street food, especially meat sold in markets, poses significant health risks due to unhygienic practices that can lead to contamination by foodborne pathogens (Iwuagwu *et al*., 2023). Microbiologically contaminated street food is recognized as a global problem that contributes to the spread of food-borne diseases (Odwar *et al*., 2014; Salamandane *et al*., 2023).

Foodborne diseases cause high morbidity, mainly in developing countries, due to poor hygiene during food preparation and sales, as well as a lack of awareness about food safety (Eromo *et al*., 2016). According to Lukman *et al.* (2020), the food production chain plays a crucial role in the transmission of AMR pathogens. Throughout the production of food and packaging processes, many foods may not be sterile and can harbor inherent microorganisms from their sources or become contaminated through recontamination or cross-contamination along the processing line.

The absence of tap water in the markets, combined with improper waste disposal, creates a breeding ground for flies and rodents, which can contaminate meat and contribute to foodborne illness. Pathogens such as *E.coli, Salmonella spp, Clostridium spp, Bacillus spp, Shigella* spp, *Enterobacter* spp., *Campylobacter spp.,* and *S. aureus* can thrive in these conditions. Additionally, contamination can occur during food handling when products are sold to customers (Eromo *et al,* 2016).

2.8 Transmission of AMR bacteria between humans and livestock meat

Global human health is at risk due to the antibiotic resistance (AMR) pandemic. Although the primary means of acquiring and dispersing AMR is through human-to-human transmission of antibiotic-resistant bacteria, its genesis and spread are also influenced by other environments. Among them, livestock such as chicken is considered to play an important role (Hernando-amado *et al*., 2019). The ecology between livestock and human populations can create diverse interfaces, which present opportunities for either population to act as a reservoir from which antimicrobial-resistant bacteria (ARB) or their antimicrobial resistance genes (ARGs) could be transmitted in either direction (Hassell *et al*., 2017).

There are two primary conceptual models for the transmission of AMR between humans and livestock, such as chickens: clonal transfer of ARB and horizontal transmission of ARGs (Chang *et al*., 2015). AMR can be transmitted from animals to humans in various ways, including both direct and indirect contact. Direct transmission occurs through interactions such as farmers consuming meat contaminated with pathogens. Indirect transmission can happen via contaminated materials and environments, such as livestock excretions that contain unmetabolized antimicrobials, as well as through water, food, and air contaminated with microbes (Wee *et al*., 2020).

There are two primary conceptual models for the transmission of antimicrobial resistance (AMR) between humans and livestock, such as chickens: the clonal transfer of antimicrobial-resistant bacteria (ARB) and the horizontal transmission of resistance genes (ARGs) (Chang *et al.*, 2015). AMR can be transmitted from animals to humans in several ways, including both direct and indirect contact. Direct transmission occurs through interactions, such as when farmers consume meat contaminated with pathogens. In contrast, indirect transmission can happen via contaminated materials and environments. For example, livestock excretions may contain unmetabolized antimicrobials, while water, food, and air can also become contaminated with microbes(Wee *et al*., 2020).

Animal-derived food products play an important role in the transmission of AMR bacteria (Wee *et al*., 2020). However, the role of livestock in the dissemination of AMR and its resistance determinants to humans remains poorly understood (Muloi *et al*., 2019; Wee *et al*., 2020). The overuse of antimicrobials in livestock can lead to the emergence of antibiotic resistance, resulting in the presence of antimicrobial residues in meat and eggs, which poses risks to human health (Agyare *et al*., 2019). A study by Economou & Gousia (2015), reported that high levels of bacterial bloodstream infections (BSIs) and mortality in Sub-Saharan Africa may be linked to AMR bacteria originating from meat consumption in the region.

2.9 Conceptual framework

The research adopted a conceptual framework that links chicken demand, disease management and antibiotic use, while outlining potential outcomes. It incorporates key elements such as farmer's knowledge, attitudes and practices related to AMU and AMR, along with their education levels training in AMR and antimicrobial management. The framework also accounted consumption of chicken meat contaminated with resistant bacteria which can lead to AMR resistant of the *E.coli*, *Salmonella* spp and *Enterobacter spp* (**Figure 2**).

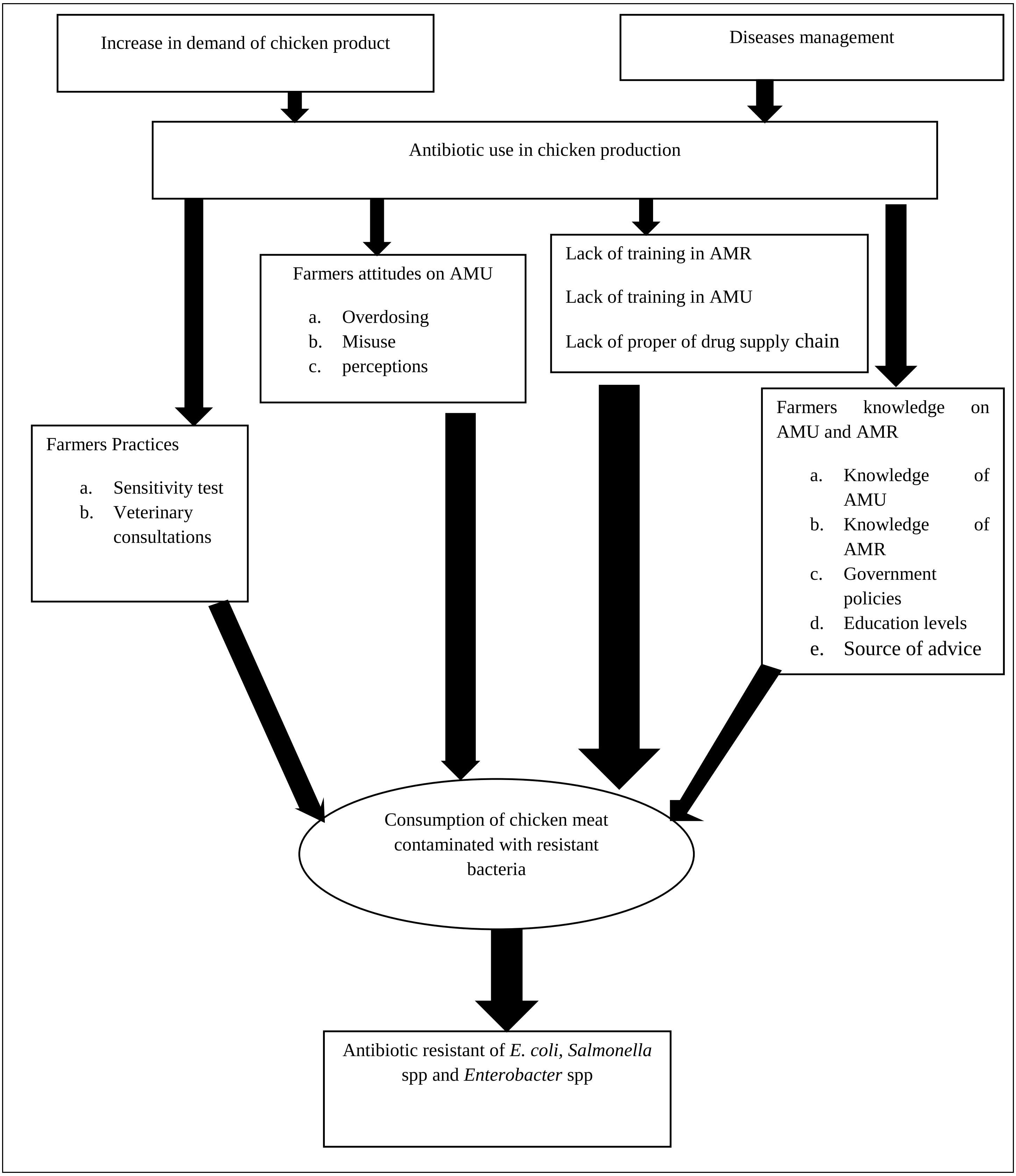


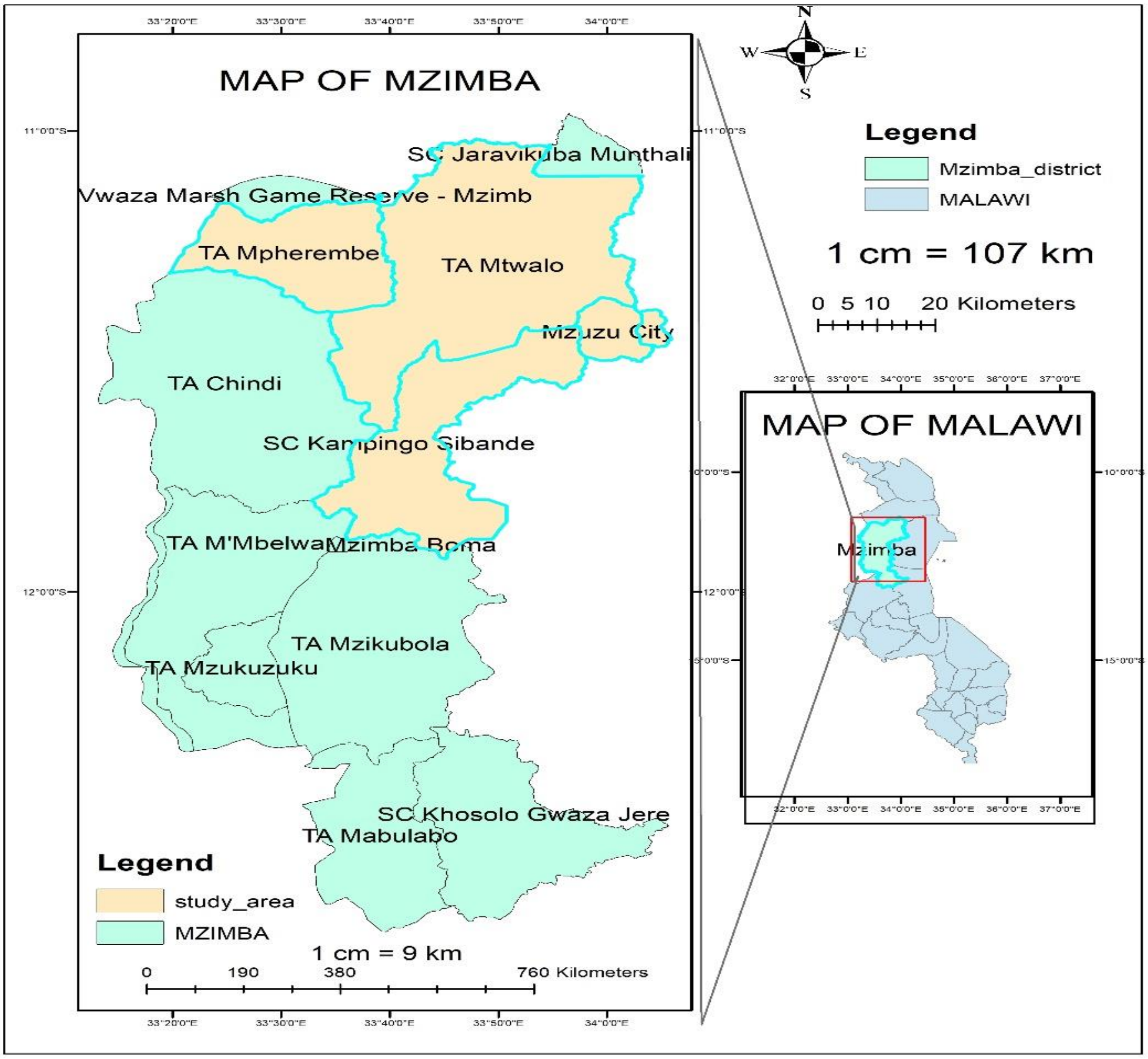
Figure 2: Conceptual framework of AMU and AMR

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in the Northern region of Malawi, Mzimba district targeting households of farmers keeping broiler chickens from February to May 2025. The area is located at latitude: 11 ° 54’ 0" S and longitudes 33 ° 36’ 0" E. Mzimba North . The study targets Mzuzu, Ezondweni, Vwaza Wildlife Reserve, Ekwendeni, Mpherembe, and Mtwalo and Kampingo Sibande which are notable for having a large population of people keeping chickens for both food and income (

Figure *3*).



Map of Malawi showing the location of Mzimba district (highlighted in green rectangle on the right, where yellow colour areas represents study area. The map was drawn using ArcGIS Vision 15 Software ,(Esris California, USA)

Figure 3: Study area

3.2 Study design, study participants, sampling method, and Sample size

A cross-sectional design was used to survey broiler farmers, and to collect meat samples in Mzimba. A comprehensive list of poultry farms was obtained from the district Animal Health Office (n = 197 farms). However, most farmers are seasonal and due to the high cost of feed, most farmers were not doing chicken farming during the time of data collection, and only 114 farmers were reported as active farmers during the study period. The targeted respondents were farm owners or managers.

For vet shop owners, 28 shop owner were considered in the study out of 30 estimated populations. Additionally, there were 124 vendors involved in selling or slaughtering chickens, and the sample size collected from this group was 100 instead of 97 as initiary planned, achieved at 95% confidence interval.

The sample size was calculated using OpenEpi software version 3, hypothesized at a 95% confidence level. The formula below was employed: n = [DEFF*Np (1-p)]/ [($d^2/Z^2_{1-\alpha/2}$*(N-1) +p*(1-p)], where: DEFF is Design effect: 1.0, N is population size: (the total number of household farmers keeping broilers in Mzimba North), d: confidence limits as % of 100 (absolute +/-%: 5%), p: hypothesized % frequency outcome in apopulation:50%+/-5, and α=0.05, Z= 1.96. The assumed prevalence of farmers using antibiotics use was 50% of all farms. The total sample size calculated was 89 farms. A purposive sampling was used to target the calculated sample size that were active in chicken farming to evaluate antibiotic use and the farmer's awareness on antimicrobial resistance. Potential participants were identified with the help of veterinary assistants, and a purposive and stratified sampling method was used to enroll chicken farmers based on the age group differences, education level, gender, and marital status in the study and farmers been trained on AMU and AMR.

For vet shops survey, purpose sampling was used potential participants were also identified with help of veterinarians. For the collection of chicken meat samples, convenience and stratified sampling methods were utilized.

OpenEpi software, version 3 (www.openEPI.com) was used for calculating sample size in this study. This tool provides a user-friendly interface for conducting various epidemiological statistical analyses, including sample size determination.

3.3 Inclusion and exclusion criteria

3.3.1 Inclusion criteria

The study enrolled farmers residing in the aforementioned area, who were actively rearing chickens for meat during the time of the study and had provided consent. Those farmers with multiple animal types were also considered in this study, as long as they reared broiler chickens for commercial purposes. For laboratory data, the study included those farmers involved in selling live or slaughtering chicken meat. The meat samples were collected from fresh chicken meat.

3.3.2 Exclusion criteria

The study excluded all farmers who kept other animals, those farmers under 18 years of age, farmers who declined to participate, and those who did not provide consent. For meat samples, all chicken meat sold in shops or at retail were not considered.

3.4 KAP survey development

The questionnaires were designed to cover a range of topics, including demographic information, an assessment of AMU conditions, and an evaluation of KAP connected with AMU and AMR among farmers. Most questions in the questionnaire were multiple-choice. Demographic information included age, gender, educational level, training in AMR, training in AMU, and years of experience in chicken farming. AMU-related questions addressed various aspects of poultry management, such as the types of antimicrobials used, their sources, storage practices, and overall farm management, including flock size, bird health, the specific antimicrobial used by farmers, and the recurrence of illness. Knowledge-related questions centered on AMU, antibiotic withdrawal periods, AMR transmission, and government regulations concerning AMU. Regarding perceptions of AMU and AMR, the attitude section investigated factors such as the use of prescribed antimicrobials and instances of overdosing. Finally, the practice-related questions aimed to gather information on sensitivity testing, adherence to full courses of antimicrobials, and whether farmers consult veterinarians.

For vet shop questionnaire, it covered the following: demographic information, source of antibiotics, the advice which they were giving farmers and also interaction between vetshop owner and regulatory bodies.

3.5 Sample collection

3.5.1 Semi-structured interviews (SSI)

Data collection was done using a self-administered structured questionnaire facilitated by the Kobo Collect tool. The researcher administered pretesting of the questionnaires, where 9 farmers were given the questionnaires to respond to and to see if the questions were relevant, clearly understood, and made sense before going for data collection of the study. The questionnaires were checked and validated by experts to ensure the objectives of the study were answered. The reliability was checked by factor analysis of all knowledge, attitudes, and practices, and the results were above 0.7.

3.6.2 Meat sampling

Sample collection was conducted in accordance with ISO standard 17604:2015 (ISO, 2015) (Terentjeva *et al.*, 2017). Aseptic techniques were used to collect two grams of chicken meat using forceps and a sterile surgical knife, ensuring that the blade will be replaced between samples to prevent contamination. Additionally, a sterile swab was used to collect samples by swabbing all internal parts and neck regions of the carcass during slaughter.

After collection, the samples were transported to the Mzuzu veterinary laboratory in an isothermal box with ice packs, maintaining a temperature of less than 7 degrees. In the laboratory, the samples were kept under refrigeration (4 °C) and were processed within 48 hours (Saha, 2018).

3.7 Sample processing, bacterial isolation, and identification

3.7.1 Quality control

All of the culture media used in the study underwent quality control (QC) during the media preparation phase. This involved recording the name, brand, color, texture, gross appearance, and quantity of weighted powdered culture. After that, the right amount of water was measured, and the broth's pH was evaluated. Quality control (QC) was performed after media preparation to verify the media's physical appearance, check for issues such wrinkles or precipitates, and ensure the media was sterile. The strain E. coli ATCC 25922 was utilized as a QC reference for E. coli quality assurance. Every internal quality control method was carried out in compliance with the CLSI 2022 requirements. (CLSI, 2022).

3.7.2 Sample Preparation

Each meat sample plus carcass swab was placed in 30 ml peptone water (BPW) in a Falcon tube and incubated overnight at a temperature of 37°C.

3.7.3 Isolation of E. coli

Isolation and identification were performed according to ISO 7251:2005 (ISO, 2005). Aseptic techniques were followed while isolating *E. coli*. A small drop of the incubated sample in peptone water was collected using a sterilized inoculating loop, and the inoculated plate was then incubated at 37°C for 24 hours.

After being sub cultured onto MacConkey agar, positive colonies, which were identified by their pink and yellow coloring, were incubated for a further twenty-four hours at the same temperature. Colonies that showed a purplish-red hue or a metallic green sheen on MacConkey agar after 24 hours were identified by microscopic characterization using Gram's stain (Smith & Hussey, 2016). The Triple Sugar Iron (TSI) test, catalase, indole, oxidase, lysine, growth in urea, Simon citrate test, and further catalase tests were the eight biochemical tests that came next.

3.7.4 **Isolation of *Salmonella* spp.**

The preparation and isolation of meat samples were conducted using ISO 6579-1:2017 (ISO, 2017). Aseptic techniques were followed during the isolation of *Salmonella* spp. A small drop of the incubated sample in peptone water was collected using a sterilized inoculating loop and inoculated on Xylose lysine tergitol 4 (XLT4) agar. The inoculated plate was then incubated at 37°C for 24 hours.

After incubation, presumptive colonies that appeared blue-green to blue with black centers, or pink with black centers, were considered positive. Following this, microscopic characterization was performed using Gram's stain (Smith & Hussey, 2016), with observations made using a Ceica DM microscope (UK). After the microscopic examination, a series of biochemical tests were performed, including Triple Sugar Iron(TSI), motility, indole, oxidase, lysine, growth in urea, Simon citrate test, and catalase tests.

3.7.5 Isolation of Enterobacter spp.

All samples for *Enterobacter* spp. were handled in accordance with ISO 16654. Aseptic techniques were followed during the isolation process. A small drop of the incubated sample in peptone water was collected using a sterilized inoculating loop and then inoculated onto MacConkey agar and XLT-4. The inoculated plates were then incubated at 37°C for 24 hours.

Presumptive colonies that appeared pink-colour and yellow on MacConkey agar and (XLT4) agar respectively, were subculture onto MacConkey agar. These subcultures were incubated aerobically for 24 hours at 37°C to obtain pure colonies. For identification, colonies exhibiting round pink on MacConkey agar and round yellow colonies on XLT4 underwent microscopic characterization using Gram's stain (Smith & Hussey, 2016). This was followed by biochemical tests, which include the Triple Sugar Iron (TSI) test, urea test, catalase, indole, oxidase, lysine, growth in urea, Simon citrate test, and catalase tests.

3.7.6 Antibiotic Susceptibility Testing (AST)

Sowunmi *et al*. (2022), stated that the Bauer, Kirby, Sherri's and Turk 1966 disc diffusion approach is advised for antibacterial testing. This method was used for the antimicrobial susceptibility testing of *E. coli*, *salmonella* spp., and *Enterobacter* spp. The AST was conducted on Mueller-Hinton agar according to the guidelines of Clinical and Laboratory Standards 20222 (CLSI, 2022).

All isolates were evaluated for antimicrobial susceptibility using the following antibiotics: Ampicillin (10 µg), Ceftriaxone (30 µg), Chloramphenicol (10 µg), Cefepime (30 µg), Meropenem (10 µg), Gentamycin (10 µg), Tetracycline (5µg and 10 µg). The selection of these antibiotics was based on their priority classification, considering their important for humans and animals (WHO, 2024), as well as their significance in animal production (WOAH, 2021). Additionally, the availability of these antibiotic types in the testing laboratory of Mzuzu was taken into account.

From pure cultures of *Salmonella* spp., *Enterobacter* spp., and *E. coli* agar plates, one colony was selected and suspended in 0.9 percent saline water, with the solution adjusted to meet a 0.5 McFarland turbidity criterion. On Mueller-Hinton agar plates, 0.1 mL of the 0.5 McFarland suspension was then uniformly swabbed in at least three directions. Antimicrobial discs were positioned at a particular spot on the agar plates for additional testing after each plate had time to dry.

To guarantee even contact, the discs were positioned 24 mm apart using a disc dispenser and gently pressed down onto the agar surface. Following inoculation, the plates were incubated for 18 to 20 hours at 37 ^{o}C. Following incubation, each plate was inspected, and using a measuring clipper ruler on a nonreflecting backdrop, the diameter of the zone of inhibition was measured to the closest full millimeter. The CLSI interpretation table and FAO-WHO-NET recommendations were used to evaluate the results and classify them as Sensitive (S), Intermediate (I), and Resistant (R)(**Table 1**).

Table 1: AST breakpoints of *E.coli*, *Salmonella* spp and *Enterobacte*r spp

Disk diffusion test of antibiotic breakpoints in E. *coli*				
Antibiotic agent	Disc contents	Susceptible(**≥mm)**	Intermediate(**mm)**	Resistance(**≤mm)**
Ampicillin	10 µg	18	15-17	14
Tetracycline	30 µg	18	15-17	14
Chloramphenicol	10 µg	18	15-17	14
Ciprofloxacin	5 µg	24	22-23	20
Ceftriaxone	30 µg	21	18-20	17
Gentamycin	10 µg	15	12-14	11
Meropenem	10 µg	23	20-22	19
Cefepime	30 µg	24	18-23	17
Disk diffusion test of antibiotic breakpoint in *Enterobacter* spp and *Salmonella* spp				
Antibiotic agent	Disc contents	Susceptible(≥mm)	Intermediate(mm)	Resistance(≤mm)
Ampicillin	10 µg	17	13-16	12
Tetracycline	30 µg	18	15-17	14
Chloramphenicol	10 µg	18	15-17	14
Ciprofloxacin	5 µg	30	24-29	23
Ceftriaxone	30 µg	23	19-22	18
Gentamycin	10 µg	15	13-14	12
Meropenem	10 µg	20	17-19	16
Cefepime	30 µg	26	22-25	21

3.8 Data management and analysis

3.8.1 KAP survey data

For KAP survey, questionnaire was stored online on kobo Toolbox. The data were then exported into Microsoft Excel worksheet-2016 for cleaning and management, and thereafter posted into STATA version 15 software, where all statistical analyses were done. Descriptive statistical analysis of the responses captured using questionnaires was performed using Microsoft Excel worksheet 2016 and STATA. STATA version 15 was used to compute correlation coefficients of the independent variables, such as age, sex, marital status, education level, years in practice, formal training in AMU, and formal training in AMR, against their dependent variables, such as knowledge, attitude, and practices scores. After computing mean scores, linear regression was used to examine associations between knowledge, attitudes, and practice scores and. The assessment of knowledge, attitude, and practice was performed with a scoring system. The KAP scores of the participants were calculated as the sum of correct responses to each question. The correct response was scored 1 and the wrong response 0. Data coding was done for the variables, for instance, variables like knowledge, attitudes, and practice, each correct response was given a score of 1, while a wrong or doubtful response was scored as 0. Each respondent could obtain a score of 0–11 for knowledge, 0–13 for attitude, and 0– 9 for Practices.

The study used the mean scores with its standard error(SE) as a cut-off point because there was no cut-off point to assess poor and better /good knowledge, attitudes, and practices. Scores above and equal to the mean were regarded as better knowledge, practice, and a positive attitude, while scores below the mean were considered as low knowledge, practice, and a positive attitude. After performing mean scores, linear regression was used to compare the knowledge, attitudes and practice score and demographic variables. The linear regression was validated by checking Variance Inflation Factor (VIF). The VIF for all demographic variables vs Knowledge, attitudes and Practices scores were between 1.02 to 1.3. All had no problem of multicollinearity, hence the analysis was suitable for the test. Pearson's correlation test was further used to compare wheather there was a relationship among knowledge, attitudes, and practices. As per the criteria by(Said et al., 2020), the correlations were classified as 0 - 0.25 = weak correlation, 0.25 - 0.5 = fair correlation, above 0.5 = Good correlation.

3.8.2 Vet shop owners survey

For the KAP survey, questionnaire was stored online on kobo Toolbox. The data were then exported into Microsoft Excel worksheet-2016 for cleaning and management, and thereafter posted into STATA version 15 software, where all statistical analyses were done. Descriptive statistical analysis of the responses captured using questionnaires was performed using STATA.

3.8.3 Laboratory data

Data from the laboratory was entered into FAOWHONET 2020, then data from FAOWHONET was extracted as excel then cleaned and analyzed using Stata version 15.0. Descriptive statistics was used to summarize the data and expressed in terms of frequency and percentage to determine the occurrence of preverance and resistant patterns of microbes (*E.*

coli, salmonella spp, and *Enterobacter* spp). One-way NOVA was used to compare MAR levels of bacteria in different locations.

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula MAR = a/b, where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility(Sandhu *et al*., 2016).

In all the analyses, alpha was set at $P \leq 0.05$. Sensitivity analysis were used in the data management to deal with confounding variables.

3.9 Ethical approval

Ethical approval was obtained from the Department of Animal Health and Livestock (DAHLD) in Malawi (Ref: NO DAHLD/AHS/01/2025/02). Informed oral consent was obtained from every chicken farmer and chicken vendor before the interview and sample collection respectively. Before the interviews, the study objectives, interview process, and the usage of the data were fully understood by the participants. All participants were allowed to withdraw the interview at any time they want, the data obtained from participants were secured and all identified information obtained was not disclosed to any party, it was solely used for research. All participants were informed of how their data will be used, stored, and processed.

4.0 RESULTS

4.1 KAP survey results

4.1.1 Demographic information

The interview results showed that the majority of farmers were male (51%) with ages ranging from 31 to 40 years (30.3%). In addition, 70% (n = 62) of the farmers had completed high school, at least 74% had never received formal training specifically in AMR or antibiotic use, and 32% (n = 32) of the farmers had less than 5 years of experience raising broilers (**Table 2**).

Table 2: Demographic characteristics

Variable	Categories	Frequency	Proportion(%)
Gender	Male	51	57%
	Female	38	43%
Age group	20-30	13	14.6%
	31-40	27	30.3%
	41-50	21	23.6%
	Above 50	28	31.5%
Marital status	Single	15	17%
	Married	74	83%
Education level	No formal education	2	2%
	Primary	11	12%
	Secondary	62	70%
	Tertiary	14	16%
Number of years in poultry practice	0-1	10	11%
	1-4	29	32%
	4-10	21	24%
	More than 10	29	32%
Formal training of AMR	No	86	97%
	Yes	3	3%
Formal training of AMU	No	69	78%
	Yes	20	22%

4.1.2. AMU Situation Analysis Among Chicken Farmers

Most farmers relied on veterinary shop owners for advice on antimicrobial use, for 58% of respondents. In contrast, 28% were relying on their own knowledge, only 11% were seeking advice from veterinarians. When it comes to sourcing antimicrobials, 89% were purchasing from agrovets unlike veterinarians and the chief animal development officer(**Figure 4**).

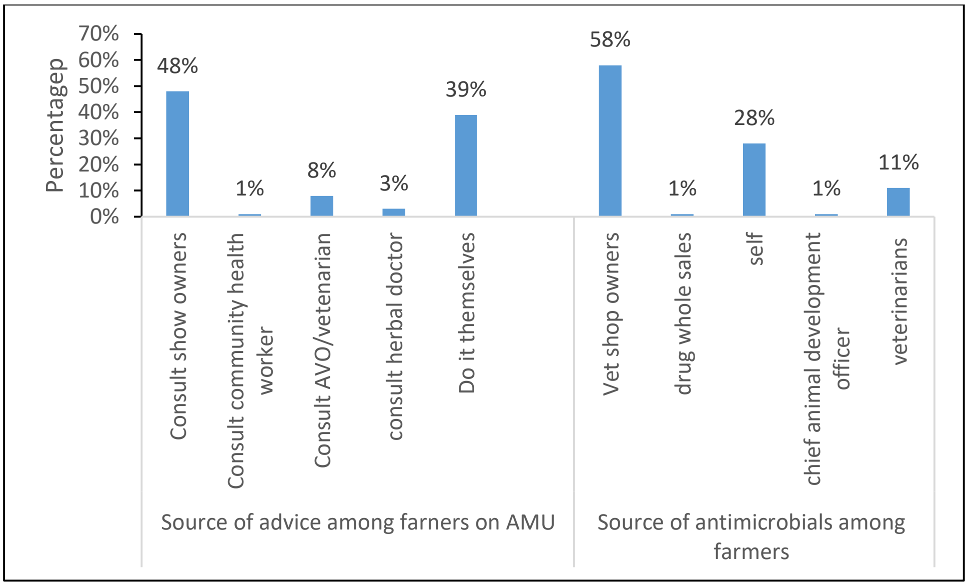


Figure 4: Information on the source of antimicrobials and advice on AMU among farmers

When it comes to selecting antimicrobials, the majority of the farmers (49%) indicated that they consulted shop owners, whereas a small proportion sought guidance from veterinarians and community health workers. For determining dosages, 52% (n=46) relied on their own judgment, 38% (n=34) consulted shop owners, and 7%(n=6) sought advice from Animal Vaccinators (AVOs) (**Figure 5).**

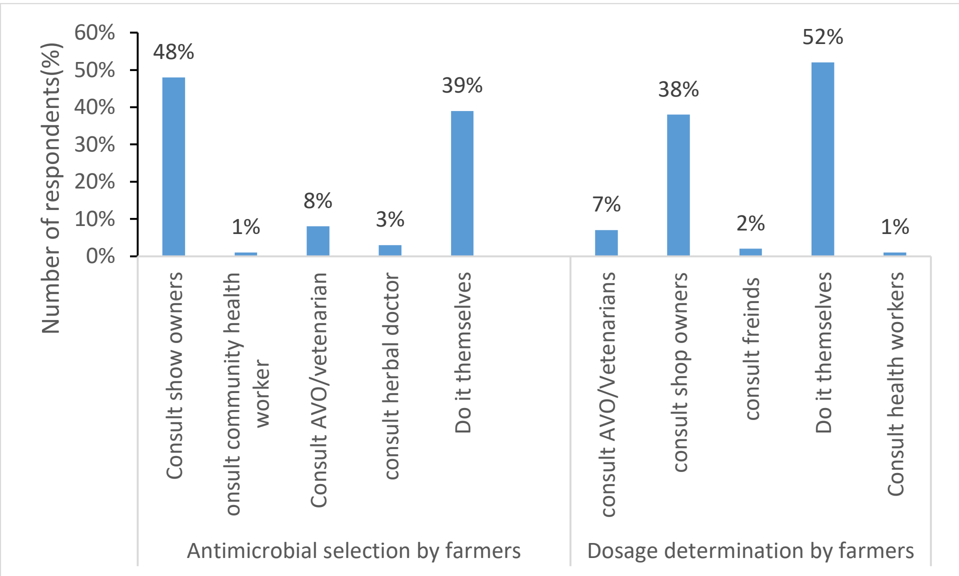


Figure 5: Information on selection and usage of antimicrobials among farmers.

4.1.3. Information of antimicrobial prescription and antimicrobial use

Seventy-six percent of farmers were aware that veterinarians have the authority to write prescriptions. However, 90% of respondents reported that veterinarians do not actually write prescriptions. Additionally, 97% of respondents indicated that veterinary shops do not require prescriptions (**Table 3***)*. In terms of accessing antibiotics, 92% (n=82) obtain them from shops without prescriptions. Meanwhile, 6% (n=5) indicated that they use prescriptions, 2% (n=2) stated they source antibiotics from other shops, and 5% reported accessing them from shops that do require prescriptions.

Table 3: Prescription of antimicrobials information

Variable	Category	Frequency	Proportion(%)
Who has the authority to write a prescription	Registered veterinarian	68	76%
	Not sure	9	10%
	Vet shop owners	6	7%
	Anyone	6	7%
Are prescriptions written	No	80	90%
	Yes	9	10%
Do the Veterinary medicine stores request for prescriptions for some antimicrobials like antibiotics	No	87	97%
	Yes	2	2%

To evaluate how antimicrobial use (AMU) is monitored in chicken production, farmers were asked to identify the responsible individuals overseeing AMU on their farms. According to the responses, 93.3% indicated that the Department of Animal Health and Livestock Development (DAHLD) is responsible for monitoring AMU among farmers. Additionally, 31% reported that agrovets shops play a role in checking AMU on farms, while 20% indicated the Malawi Pharmacy and Medicines Regulatory Authority as responsible. Furthermore, 11% mentioned the Malawi Bureau of Standards(MBS), and 6.7% noted that the Poison and Medicine Board is accountable for this oversight.

4.1.4 Challenges faced by farmers in reducing using antimicrobials in chicken farming

A high prevalence of chicken diseases was reported by 84.2% (n=75) of respondents as the primary challenge preventing them from using antimicrobials. Additionally, 2.25% cited the limited availability of vaccines as a reason for antimicrobial use. Furthermore, 12.36% (n=11) indicated that they use antimicrobials to maintain productivity and profitability, while 1% reported using antibiotics due to difficulties in accessing veterinary professionals. Some of the diseases reported infecting chickens include viral diseases e.g gumboro disease, bacterial infections, worm infestations, and other unidentified diseases (**Figure 6**).

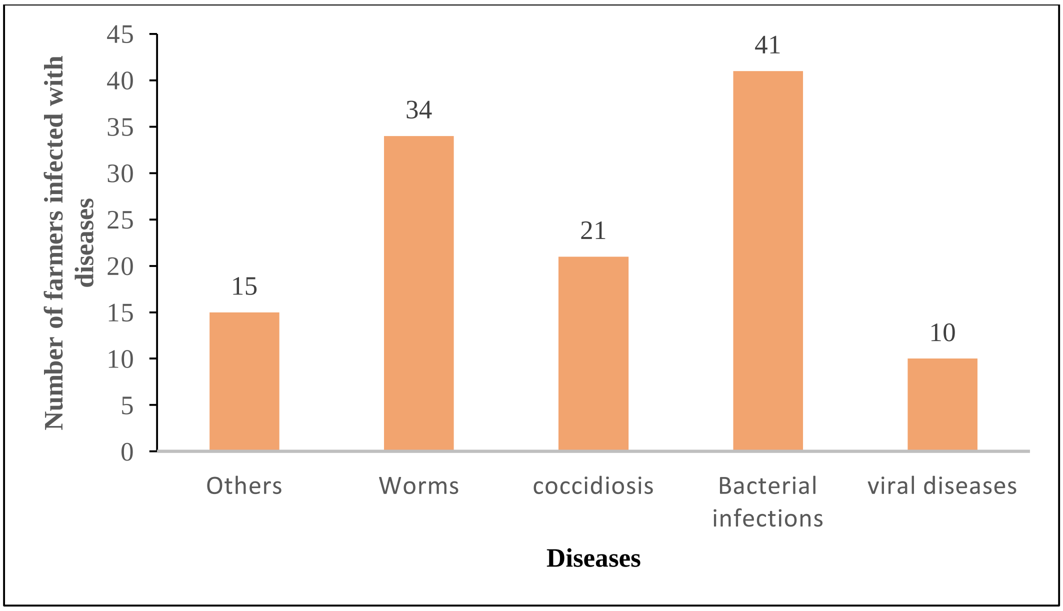


Figure 6: Diseases infecting chickens mentioned by farmers

In terms of antibiotics commonly used by farmers, most farmers were using tetracycline (63%), sulfonamides (22.5%) and quinolone (16.8%) (**Table 4**).

Apart from commercial antibiotics, 11.2% farmers (n=10) were using herbal medicine. The following were some of the herbal antibiotics used by farmers: Guava extracts, Moringa, majick powder, alouvela, avocado (Persea Americana), eucalyptus, duhat, tamarind (tamarind indica), and natural antibiotics. Apart from herbal medicine, 6.7% farmers (n=6) were using diaglow (a pesticide) as an antibiotic.

Table 4: Class of antibiotics used by farmers in the study area

Class of antibiotics	Antibiotics used by farmers in poultry farming	n (%) farmers
Tetracycline	Alamycine, Trimovate, oxyfarm, Trimo farm, egocin, oxysol, tetracycline, limoxin,	56 (63)
Quinolone	Arysel, interflox, livita, enflolaxin, limox.	15 (16.8)
Trimethoprim/sulphamethzole	Intertrim, Vitamed, Trisulmycine, Bactrim, co-trimozaxole	20 (22.5)
Penicillin	Contrivet(amoxicillin),democycline,piperacillin,	5 (5.6)
Aminoglycosides	Neomycin, biosole	3 (3.4)
Cephalosporin's	Vetox	1 (1)
Tylosin	Dawa tylodoxy, Batylocin	2 (2.2)

4.1.5 Knowledge about national policies related to use of antimicrobials and resistance

To assess knowledge of farmers about national policies related to antimicrobial use in chicken farming, 93.2% (n= 83) farmers were not aware of national polices, while 6.8% (n=6) farmers were aware of national policies. Those farmer's aware national policies reported that these policies are not followed by farmers, and they provided the following recommendations tp be done: providing increased access to antimicrobials, and increasing farmers training and awareness.

4.1.6 Knowledge of chicken farmers about AMU and AMR

A total of 11 questions were designed to assess the knowledge of respondents toward AMU and AMR (**Appendix 5).** The total score was 9.4±0.49. using the mean score as a cut point, only 46.1% (of respondents demonstrated proficiency by providing correct answers to over mean of the questions, while the remaining 53.9% yielded responses falling below mean.

Of the 89 farmers, 25% (n=22 respondents) recognized AMR as a serious public health problem, while 75% (n=67 respondents) did not. 95% of respondents had heard of AMU, and 98% had heard of AMR. Regarding knowledge of antimicrobial transfer via chicken products, 96% (n = 85 respondents) knew that antimicrobials can be transferred to humans through chicken products, and 91% of them understood that the inappropriate use of antimicrobials can lead to the development of AMR. Among 89 farmers, 97% reported that it is wrong to sell chicken meat before the withdrawal period, and 94% reported that antimicrobial residues could be serious to public health; however, 27% were aware that the use of antimicrobials in feed formulation is inappropriate. 63% were aware of institutions that control sales of antimicrobials, and 70% were aware that the lack of control of sales of antimicrobials can lead to AMR **(Appendix 5**).

Coefficient analysis between the independent variables of age, gender, marital status, years in practice, and education level of the poultry farmers was tested against the dependent variable of knowledge using STATA, with the correlation factor of p-value ≥ 0.05. The findings revealed that gender, education level, training in AMR and training in AMU were significant, meaning that the gender and education level of the poultry farmers affected their responses on the knowledge and awareness on AMU and AMR, as shown in (**Table 5**).

Table 5: Correlation of independent variables with knowledge scores

Variable	T-test	Significance
Age range group	-0.04	0.969
Gender	2.65	0.010
Marital status	0.42	0.678
Years in poultry farming	0.03	0.978
Education level	54.64	0.000
AMU training	2.59	0.011
AMR training	3.27	0.002

4.1.7 Attitudes of chicken farmers on AMU and AMR

A total of 14 questions were designed to assess the attitudes of respondents toward AMU and AMR. The mean attitude score was 30.47±49. Using this as a cut point, only 43.8% of respondents demonstrated proficiency by providing correct answers to over 30%% of the questions, while the remaining 56.2% yielded responses falling below. 98% (n=87) respondents strongly agree that antimicrobials are safe for humans and animals, and 91% (n=81 respondents) strongly agree that antimicrobials are needed for any animal illness. 93.26% (n=83 respondents) strongly agree that broad-spectrum antimicrobials cured for any infection,

Out of 89 farmers, 82% (n=73 respondents) strongly agreed that antimicrobials are needed to prevent serious illnesses but only 35% (n=34) strongly disagreed of adding antimicrobials in feed. 94% of respondents strongly agree that non-prescribed antimicrobial sales should be prohibited,57% respondents strongly agree that antimicrobials are needed during weather/season changes, and 63% strongly agreed that they are needed for fever/cold.

In regards to diseases symptoms, 79% (n=70 respondents) strongly agreed that stopping antimicrobials after symptoms disappear is safe to preserve them for future, and 96% (n=85 respondents) strongly agreed that missing doses contribute to AMR.

In terms of overdosing of antimicrobials, only 33% (n=29 respondents) strongly agreed that overdosing could lead to AMR, with 39.32% (n=35 respondents) strongly disagreeing, 62% (n=55 respondents) strongly agreed that vaccination can reduce antimicrobial use. Seventy-nine percent (n=70 respondents) strongly disagreed that it is not good to use expired drugs in treating chicken diseases and 78% (n=69) of respondents strongly disagreed that it is safe to eat animals that die during course of treatment (

Appendix 6).

Coefficients analysis between the independent variables of age, gender, marital status, years in practice, and education level were tested against the dependent variable of knowledge using Stata software, with the correlation factor of p-value ≥ 0.05. The findings revealed that all variable had no significant difference to their attitudes on Antimicrobial Use and antimicrobial resistance (**Table 6**).

Table 6: Correlation of independent variables with attitudes scores

Variable	T-test	Significance
Age range group	1.14	0.257
Gender	-0.55	0.585
Marital status	-0.67	0.508
Years in practice	-0.95	0.345
Education level	-0.65	0.519
Training AMU	1.00	0.319
Training AMR	0.95	0.346

4.1.8 Practices of chicken farmers about the use of antimicrobials

A total of 9 questions were administered to poultry farmers regarding their practices in AMU. The total mean score was 4.4±0.18. Among the respondents, 47.5% demonstrated proficiency by correctly answering >4.4 of the questions, while the remaining 59.3% exhibited responses fell below mean score.

Notably, 94% (n=84) reported treating animals without conducting sensitivity tests, while 6% use lab tests. It was also found that 51% reported that do not consult anyone when chickens are sick. Apart from the consultation, 63% (n=56 respondents) select antibiotics themselves, and 68% (n=61) decide dosage themselves.

Despite of the farmers' administering drugs themselves, 91% (n=81) reported that they allow their chickens to complete the full course of antimicrobial treatment when treated by veterinarians. Again 84% (n=75) reported that they check expiry dates, It was also found that 92% (n=82) stop administering antibiotics when symptoms disappear. In terms of disposing drugs, 92% (n=82) responded that they burn or bury expired drugs, 64% (n=57) reported that they throw them away, 6% (n=5) put in animal feed, 2% reported that they return to the veterinary laboratory. Most farmers dispose of expired drugs appropriately, but a small minority misuse them. It was also found that 80% of farmer's increase dose/frequency when clinical symptoms persist (Error! Reference source not found.).

Coefficients analysis between the independent variables of age, gender, marital status, years in practice, and education level were tested against the dependent variable of practices using Stata software, with the correlation factor of p-value ≥ 0.05. The findings revealed that only those farmers trained in AMR their P-value was a significant at 0.024, meaning that the farmers trained in AMR affected their responses on the knowledge and awareness of antimicrobial use and AMR, as shown in (**Table 7**).

Table 7: Correlation of independent variables with Practices scores

Variable	T-test	Significance
Age range group	-0.44	0.659
Gender	0.87	0.388
Marital status	-0.54	0.591
Years in practice	-0.53	0.596
Education level	0.24	0.809
AMU training	1.32	0.191
AMR training	2.29	0.024

A correlation test between knowledge against attitude, and practices was further conducted by using Pearson's correlation test. As per the criteria by Said *et al.(*2020), the correlations were ranked as 0 - 0.25 = weak correlation, 0.25 - 0.5 = fair correlation, above 0.5 = Good correlation. Therefore, the correlation analysis between knowledge and attitude score indicated there is weak positive relationship between knowledge and attitude among participants in the study (0.22), this means that the attitude of the poultry farmers towards AMU and AMR does increase with their knowledge. At same times knowledge about AMU and AMR does influence attitudes (**Table 8**).

The correlation analysis between knowledge and practice score indicated that there is no linear relationship between knowledge and practice among participants in the study (0.07), this means that the practices of the poultry farmers towards AMU and AMR do not increase or decrease with their knowledge (**Table 8**). In contrast, the correlation analysis between practices and attitude score indicated there is a negative relationship between practice and attitude among participants (-0.0539). This means that the attitude of the poultry farmers towards AMU and AMR does increase with their knowledge. At the same times, attitudes about AMU and AMR does influence practices (**Table 8**).

Table 8: Correlation of knowledge, attitudes and practices on AMU and AMR

Variables	**Coefficient**	**P-value**
Knowledge and attitudes	0.2097	0.0486
Knowledge and practices	0.0732	0.4957
Attitudes and practices	-0.0539	0.6162

4.2 Drug value chain

4.2.1 Demographic information of vet shop owners

The sociodemographic characteristics of vet shop owners are presented in **Table 9**. Of the 28 respondents, 50% were male and 50% were females. 43% were from age group between 31-40 years. 64% were married, 32% of farmers had diploma qualification and majority of vet shop owners had long years of experience in selling drugs and only 11% had experience between 0-1year.

Table 9: Demographic characteristics of vet shop owners

Variable	Category	Frequency(%)
Gender	Male	14 (50%)
	Female	14 (50%)
Age group	20-30	11 (39.3%)
	31-40	12 (42.9%)
	41-50	3 (11%)
	Above 50	2 (7%)
marital status	Single	10 (36%)
	Married	18 (64%)
Education level	Primary	1 (4%%)
	High school	5 (18%)
	Certificate	3 (11%)
	Diploma	9 (32%)
	Diploma in animal health	5 (18))
	Degree	4 (14%)
	Bachelor's degree in animal health	1 (4%)
	Tertiary	14 (16%)
Number of years in poultry practice	0-1	3 (11%)
	1-4	9 (32%)
	4-10	8 (26%)
	More than 10	8 (26%)

Malawi's veterinary antibiotic supply chain includes both public and private sector participants (Figure 7). The Pharmacy Medicines Regulatory Authority (PMRA), the Department of Animal Health and Livestock Development (DAHLD), and the Department of the Registrar General are important public sector players which control antimicrobial importation and use. Pharmaceutical businesses that operate outside of Malawi make up the majority of the private sector because the nation depends solely on imports of veterinary antibiotics. The importation of antibiotics is restricted to recognized importers that possess appropriate pharmaceutical wholesale permits. Prescribing wholesalers who supply large-scale farms with veterinary antibiotics, non-prescribing wholesalers who exclusively offer veterinary medicines in bulk, and regional pharmaceutical enterprises that produce veterinary antibiotics are among them. (**Figure 7**).

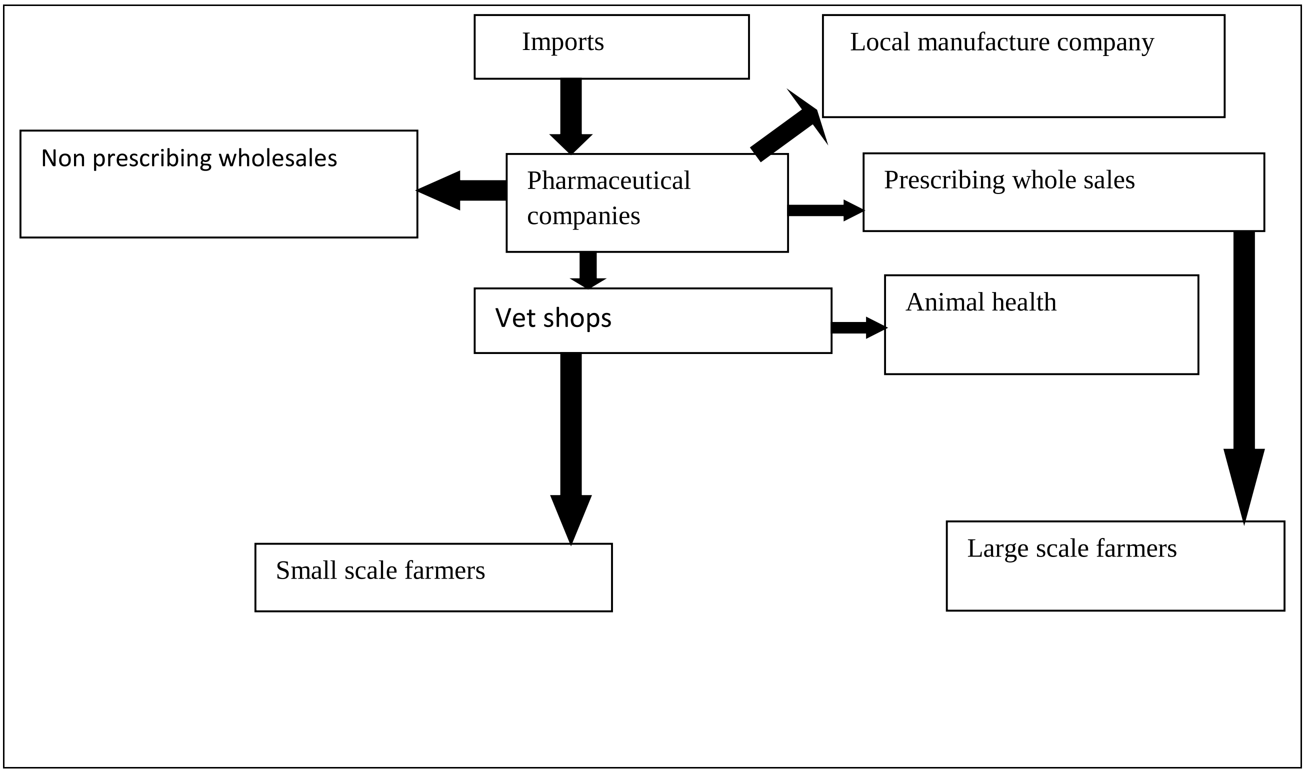


Figure 7: The conceptual map of Malawi's veterinary antibiotic flow that shows the different distribution levels .

The majority of veterinary shop owners sourced antimicrobials from distributing companies (71%), followed by manufacturers and retailers (21%) each. Other sources like agrovets, and veterinarians, were less reported by chicken farmers (**Figure 8**). Only 7% involve veterinarians or animal health practitioners directly in sourcing, suggesting limited professional oversight in the supply chain. Demand was the most dominant factor influencing drug stocking decisions by vet shop owners with 46%, followed by condition (15%) and storage method was least factor influencing the stocking of antimicrobials (**Figure 8**).

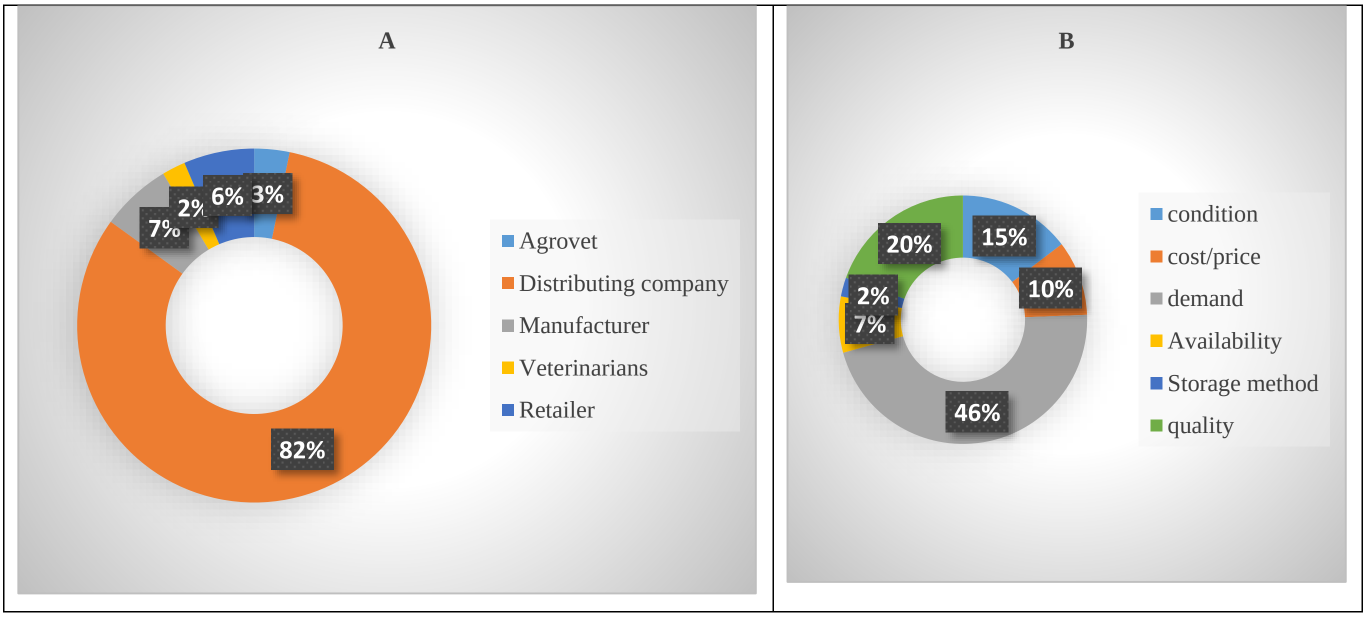


Figure 8: Antimicrobial source(A) and factors influencing the stock by vet shop owners(B)

4.2.2 Information on drug supply chain

In terms of recommending antimicrobials to farmers, history and clinical signs were most reported as the primary basis for recommending drugs (96%), with 21% combining this with prescriptions. Only 32% rely solely on prescriptions, and 7% depend on laboratory reports.

Almost 82% (n=23) vet shop owners sell drugs both with and without prescriptions, however 71% (n=20) of vet shop owners reported that they decide themselves if there is no prescription, 11% (n=3) reported that they decide based on what that client asked them, 14% (n=4) reported that they ask their fellow vet shop owners and 4% (n=1) reported that him or her asked the veterinarian for guidance.

Every owner of shop stated that they advise farmers on formulation, dose, duration, withdrawal period, and storage conditions. As indicated by 71 percent (n=20), the most frequently given advise involves dosage and duration. Of the responses, 42 percent (n=12) noted the withdrawal period, which is the amount of time before animal products can be taken. Less frequently, 36 percent (n=10), were included in storage and formulation. Advice to farmers was given "all the time" as reported by 54% (n=18) of respondents, while 29 % (n=8) provided it only upon request and 7% (n=2) rarely gave the advice.

In terms of dispersing drugs, 75% (n=25) of farmers reported that they disperse drugs as whole package for the whole treatment, and 25% (n=7) reported that they disperse drugs depending on the proportion/measurement, depending on the client's capacity to purchase.

A majority 75% (n=21) prescribed drugs to healthy animals for prophylaxis, with 25% (n=7) not doing. When farmers complain about ineffective drugs, 61% (n=17) recommend changing the drug, 21% (n=6) refer to a veterinarian, and 18% (n=5) do not recommend anything.

In regards to the clients who buy antibiotics from vet shops, 60.7% of vet shop owners reported that poultry farmers were major clients and few vet shop owners reported that farmers of other animals were buying drugs (**Figure 9**).

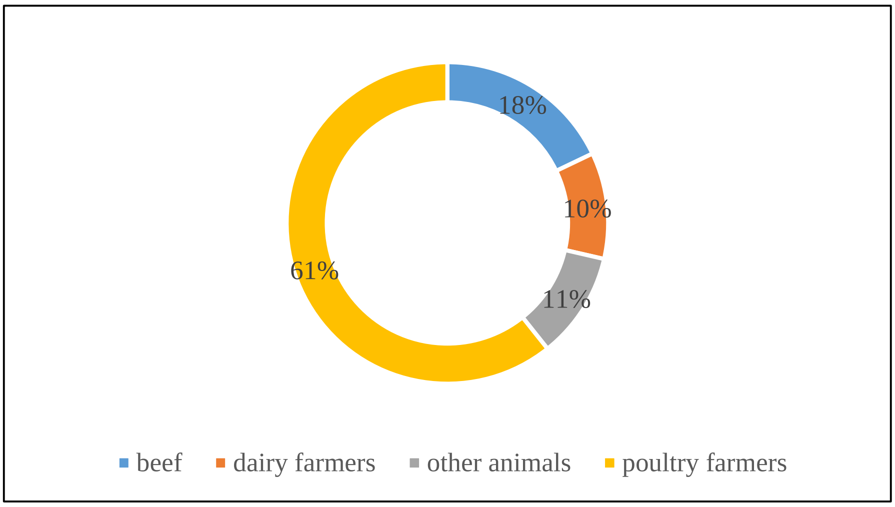


Figure 9 : Clients of vet shops

Showing farmers who buy drugs from vet shops.

The most common storage method was open shelves with refrigeration as reported by 43% vet shop owners, followed by combinations involving open shelves, cool boxes, and cabinets. Only 11% use refrigeration with open shelves exclusively. A small percentage (4%) use other unspecified methods (**Table 10**)

Table 10: Storage method

Storage method	Frequency(%)
Open shelves	4 (14%)
Open shelves and cabinet refrigeration	1 (4%)
Open shelves, cool boxes and refrigeration	1 (4%)
Open shelves and refrigeration	12 (43%)
Open shelves, refrigeration and cabinet	2 (7%)
Open shelves, refrigeration and cool boxes	3 (11%)
Open shelves, refrigeration, cool boxes and cabinet	1 (4%)
Refrigeration and open shelves	3 (11)
Refrigeration, open shelves, cool boxes and cabinet	1 (14%

In terms of disposition of drugs, 64.3% (n=18) reported that they disregard drugs, 3.6% (n=1) return to the distributor, 25% (n=7) burn or bury. Only 7% (n=2) reported encountering counterfeit drugs. Of the two respondents encountering counterfeits, one keeps them in store, and one returns them to the distributor.

When farmers complain about ineffective drugs, 61% recommended changing the drugs, 21.4% refer to a veterinarian, and 18% use other unspecified methods. All vet shop owners reported that they keep records of dispersed drugs.

Regarding the awareness of institutions governing drug use, 93% (n=26) of respondents were aware, with 7% (n=2) being unaware. The Pharmacy and Poisons Board was frequently mentioned (cumulatively ~30%). Policies mentioned include storage practices (e.g., cold chain, avoiding sunlight), checking expiry dates, and restricting unregistered drugs.

Monitoring of vet shop by certified institutions was most often reported during routine inspections as reported by 50% (n=14), with 25% (n=7) reported didn't know, 3.6 reported that frequent monitoring was not being done and 21% (n=6) reported that inspection was done often.

In regarding to drug policies, 64% (n=64) were aware of policies governing drug use, storage, and handling, while 35% were not. The policies which were mentioned by vet shop owners include best storage practice, keeping drugs in a safe place, don't sell drugs that are not registered by governing bodies, handling of vaccines, purchase of drugs, knowing shelf life, prescription, and factors contributing to AMR, cold chain has to be maintained, meat policies, registration of drugs and vaccines. Only 32% (n=9) believed that existing policies were effective, with 67% (n=19) disagreeing.

Respondents suggested improving enforcement (25%) and increasing sensitization (36%) and training and punishment (39%) as ways to improve policies related to drug use and selling drugs. Only 50% attended the stakeholders meeting.

Those who were not attending indicated that owing to the lack of awareness about the meeting contributed to lack of participation of the conference. Only 34% (n=10) reported that there is existing channels for information sharing with regulators, with 64% (n=18) reporting none. Channels mentioned include PMRA contacts, social media, and monitoring visits. On the other hand, 61% (n=17) stated that there is sufficient interaction with regulators, while 39% (n=11) disagreed.

4.3 Prevalence of *E. coli, Salmonella* spp, and *Enterobacter* spp

To assess the preverance of *E. coli*, Salmonella, and *Enterobacter* spp, the positive isolates were divided by total number of samples collected. Out of 100 samples, 69 samples were positive for *E. coli*, 12 samples were positive for *Enterobacter* spp and 2 samples were positive for *Salmonella* spp. The E. coli prevalence was 69% higher than Salmonella spp and Enterobacter spp significant at 0.001 (**Figure 10**).

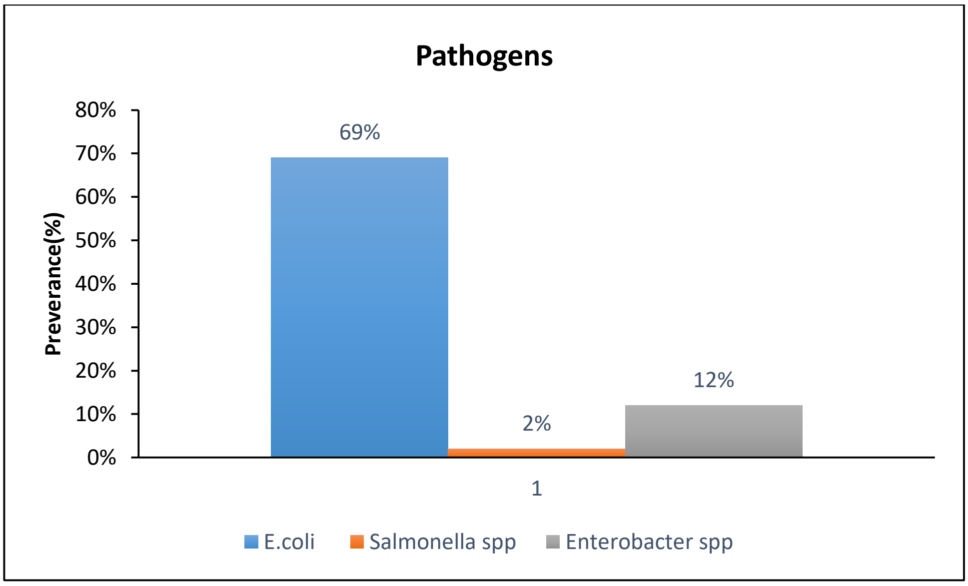


Figure 10: Prevalence of *E.coli, Enterobacter* pp and *Salmonella* spp in chicken meat

The samples from all locations were positive for *E.coli* , only 2 samples from 2 locations were positive for *Salmonella* spp and 12 samples from 7 locations were positive for *Enterobacter* spp (**Table 11**).

Table 11: Positive and negative samples for study bacteria per location

Location	***E.coli***		***salmonella***		***Enterobacter***	
	Positive	**Negative**	**Positive**	**negative**	**Positive**	**negative**
Mzuzu town	11	5	0	16	1	15
Chibavi	5	2	0	7	1	6
Mchegautuwa	3	5	0	8	2	6
Zolozolo	6	2	0	8	0	8
Luwinga	8	4	0	12	0	12
Area 1 B	9	3	1	11	0	12
kholongo	4	0	0	4	0	4
Kavibale	5	1	0	6	2	4
Ekwendeni	9	1	0	10	1	9
Enukweni	3	2	0	5	2	3
Kafukule	6	6	1	11	3	9
Total	**69**	**31**	**2**	**98**	**12**	**88**

4.4 Prevalence of *E. coli, Salmonella spp,* and *Enterobacter* spp resistance

To assess the degree of resistance of the isolated microorganisms, susceptibility assay was performed on various antibiotics against each microorganism.

4.4.1 Prevalence of resistance of E. coli

The *E. coli* isolates from the samples showed high resistance, of more than 70%, to ampicillin (91.3%), meropenem (82.6%), and tetracycline (71%). On the other hand, isolates were more susceptible to ceftriaxone, gentamicin, and ciprofloxacin, with percentages of 94.2%, 89%, and 85.5%, respectively (**Table 12**).

Table 12: Prevalence of resistance of *E.coli* isolates

Antibiotics	Susceptible(%)	Intermediate(%)	Resistance(%)
Ampicillin	5 (7.2%)	1 (1.4%)	63 (91.3%)
Ciprofloxacin	59 (85.5)	3 (4.3%)	7 (10.1%)
Meropenem	10 (14.5)	2 (3%)	57 (82.6%)
Chloramphenicol	47 (68.1%)	0 (0%)	22 (31.9%)
Tetracycline	18 (26%)	2 (3%)	49 (71%)
Gentamycin	62 (89.8%)	0	7 (10.1%)
Cefepime	31 (45)%)	21 (30%)	17 (24.6%)
Ceftriaxone	65 (94.2%)	1 (1.4%)	3 (4.3%)

4.4.2 Prevalence of resistance of Enterobacter spp

The *Enterobacter* spp isolates from samples showed high resistance, of more than 70% to ampicillin (83.3%), tetracycline (83.3%), meropenem (75%), Conversely, isolates were more susceptible to ciprofloxacin, gentamicin, and ceftriaxone, with percentages of 100%, 91.7%, and 91.7%, respectively. (**Table 13**).

Table 13 : Prevalence of *Enterobacter* spp isolates resistance

Antibiotics	Susceptible	Intermediate	Resistance
Ampicillin	0 (0%)	2 (16.7%)	10 (83.3%)
Ciprofloxacin	11 (91.7%)	0 (0%)	1 (8.3%)
Meropenem	3 (25%)	0 (0%)	9 (75%)
Chloramphenicol	9 (75%)	1 (8.3%)	2 (16.7%)
Tetracycline	2 (16.7%)	0 (0%)	10 (83.3%)
Gentamycin	11 (91.7%)	0 (0%)	1 (8.3%)
Cefepime	6 (50%)	3 (25%)	3 (25%)
Ceftriaxone	12(100%)	0 (0%)	0 (0%)

4.4.3 Resistance Rate of isolates by location

The majority of the isolates from every location were responsive to ceftriaxone, ciprofloxacin, gentamycin, chloramphenicol, and cefepime, while the majority were resistant to ampicillin, meropenem, and tetracycline. (**Table 14**).

Table 14: *E.coli, Salmonella* spp, and *Enterobacter* spp isolates' resistance rates to antibiotics by location

Location	ORGANISM	CIP	AM	MEM	TCY	FEP	CRO	GEN	CHL
Area 1B	*E.coli*	33	100	100	100	0	11	55	33
	Salmonella spp	0	0	100	0	0	0	100	0
Chibavi	*Enterobacter spp*	0	100	100	100	100	0	0	0
	E.coli	0	100	100	80	60	0	0	20
Ekwendeni	*E.coli*	0	0	100	100	100	0	0	0
	E.coli	11	89	89	66.7	89	0	0	44
Enukweni	*Enterobacter* spp	0	100	100	100	0	0	0	0
	E.coli	0	100	33	66.7	0	33	0	0
Kafukule	*Enterobacter* spp	0	100	0	66.7	0	0	0	33
	E.coli	0	100	0	66.7	0	0	0	16.7
	Salmonella	0	100	0	100	0	0	0	0
Kaviwale	*Enterobacter* spp	0	100	100	100	0	0	0	0
	E.coli	20	100	80	100	20	0	20	20
Luwinga	*E.coli*	0	87.5	87.5	63	13	13	0	50
Mchengautuwa	*Enterobacter* spp	0	50	100	50	0	0	0	50
	E.coli	0	100	100	0	0	0	0	33
Mzuzu town	*Enterobacter* spp	100	100	100	100	100	0	100	0
	E.coli	9	73	100	81	36	0	9	45
Nkholongo	*E.coli*	0	100	100	75	0	0	0	0
Zolozolo	*E.coli*	16.7	83	83	33	0	0	0	33

4.4.4 Multiple antibiotic resistance index of E. coli and Enterobacter spp

To determine MAR index, the antibiotics resistant by isolates were divided by total antibiotics tested (Sandhu *et al.*, 2016).

The MAR index shows that 27 samples had a MAR index of 0.4, seconded by 18 samples with MAR index of 0.5, then 6 samples with MAR index of 0.63 then 2 samples with MAR index of 0.75 and 1 sample with 0.1 MAR index (**Figure 11**). The MAR index was significant at 0.001 among all locations (**Appendix 8).**

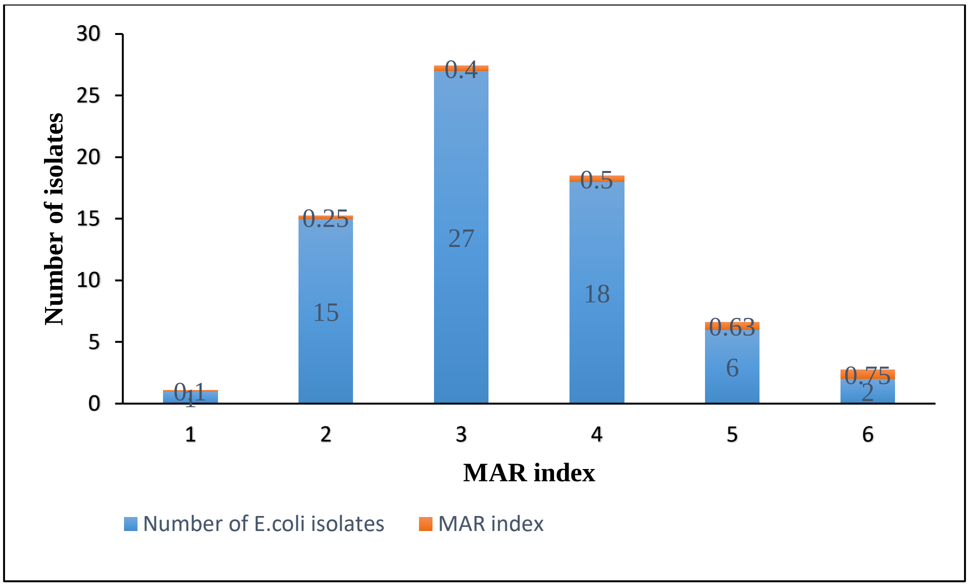


Figure 11: MAR index of *E.coli* Isolates

The turkey test was used to compare MAR index levels of *E. coli* differences among all locations, only 4 areas comparison were observed that the areas were significant, KAFUKULE vs AREA 1B, ZOLOZOLO vs AREA 1B, KAFUKULE vs EKWENDEN, MZUZU TOWN vs KAFUKULE were significant with p value of 0.000, 0.015, 0.004, and 0.021 respectively. The rest of areas comparison were not significant to each other (**Appendix 8**).

The MAR index of *Enterobacter* spp showed that 7 isolates have MAR index of 0.375, 2 isolates with MAR index of 0.25 and 1 isolate with a MAR index of 0.75, and one with MAR index of 0.5(**Figure 12**).

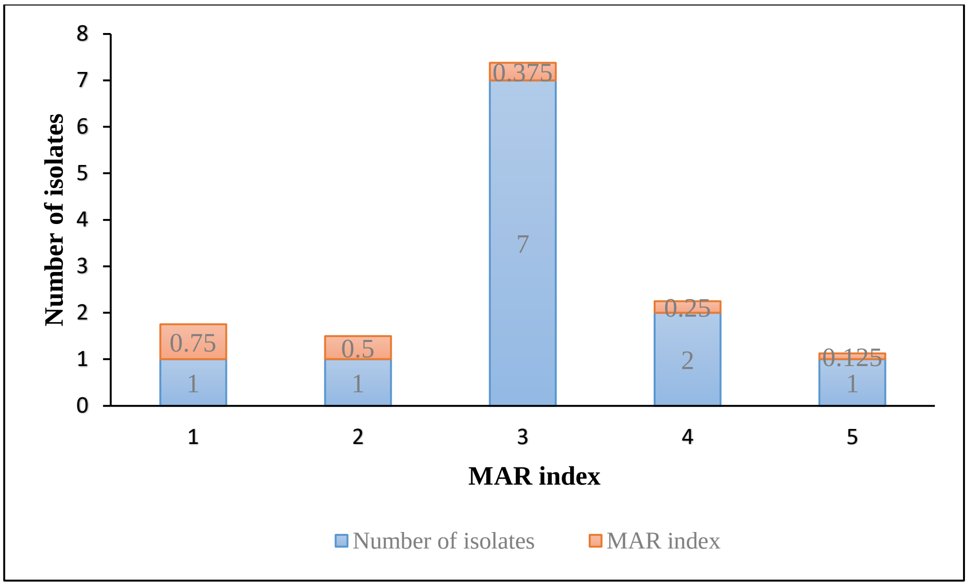


Figure 12: MAR index of Enterobacter spp

Showing multiple antibiotic resistant tests of isolated Enterobacter spp.

4.4.5 Prevalence of multidrug-resistant E. coli and Enterobacter spp

To determine MDR, all isolates resistant to 3 or more antibiotics were regarded as MDR. The MDR of *E.coli* was 75.4% (n=52) and the MDR for Enterobacter spp was 75% (n=9)

5.0 DISCUSSION

5.1. AMU Situation Analysis

This study revealed that the majority of the poultry farmers in Mzimba, Malawi obtain antimicrobials from agrovets and rely on veterinary shop owners for advice related to antimicrobial sources and selection of antimicrobials that are used in broiler chicken production. This is a similar scenario in other settings, such as in Bangladesh, Nigeria, and Fiji, where the majority of the poultry farmers obtain antimicrobials from feed sellers, and they also do not consult veterinarians for proper prescriptions (Al-Mustapha *et al.*, 2020; Hassan *et al.*, 2021; X. Khan *et al.*, 2022; Tasmim *et al*., 2023) . This is largely due to economic reasons and dissatisfaction with the previous services offered by the government's veterinary officers, which exacerbates AMR (Al-Mustapha *et al.*, 2020; X. Khan *et al*., 2022). The results reveal high antimicrobial misuse, self-prescription, and reliance on agrovets shops, indicate that policies related to antimicrobial use are failures and lack of regulation related to accessibility to antimicrobials in chicken production.

The most commonly used antimicrobials from this study were comparable with the studies conducted in Malawi and other global settings(Al-Mustapha *et al*., 2020; Geta & Kibret, 2021; Mankhomwa *et al*., 2022; Mtila *et al.*, 2024; Tasmim *et al*., 2023). -For instance, tetracycline, sulphonamides, Oxytetracycline, and quinolones have been reported previously in Malawi, Bangladesh, Ethiopia, and Nigeria. However, quinolone use was not rampant in the present study compared to other settings. These differences suggest variation in geographical location, disease burden, management systems, economic related matters and distribution of the antibiotics. Interestingly, most of these antibiotics are mostly dispersed by veterinary shop owner as reported by the present study and others(Al-Mustapha *et al.*, 2020; Geta & Kibret, 2021; Mankhomwa *et al.*, 2022; Tasmim *et al.,* 2023). This implies that distribution of these antibiotics is not satisfactory and the regulatory bodies must tighten access to such antibiotics because these are similar antibiotics used in treatment of human infections. The overuse of tetracyclines and other antibiotics in the poultry and other animal production increases the risk of AMR (Jahantigh *et al*., 2020; Mankhomwa *et al.*, 2022; Sangeda *et al*., 2021).

World Organization for Animal Health, indicate that critical important antibiotics should be administered by well qualified person only (OIE, 2007). Tetracyclines are classified as highly important antibiotics used in humans, while aminoglycoside, macrolides, polypeptides, and sulfonamides have been listed as important antibiotics (WHO, 2019). 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.

WHO recommends that medically important antibiotics should not be used in animals which is not the case in Malawi where most of the farmers use Bactrim and amoxicillin to treat infections in chickens (Aidara-kane *et al*., 2018). These antibiotics are supposed to be reserved for the treatment of infections in humans. However, poverty drives the famers to be using such antibiotics in chicken production with the aim to increase productivity of their enterprises. For instance, farmers reported that they are unable to buy recommended antibiotics to treat chickens because of financial problems. Others reported that they use bactrim and amoxicillin because they get them for free from government hospitals, on top of that other farmers were using antiretroviral(ARV) drugs for HIV/AIDS which other farmers reported that they could combine with bactrim and give chicken for growth promotion and as antibiotics, this is serious public health concern. Farmer using expired antibiotics is serious public health concern. AMR is fueled by this type of antibiotic misuse and overuse. When antibiotics are used in broiler chicken treatment and not follow the antibiotic withdrawal period, their residues are consumed together with the meat by humans. This promotes the development of bacterial antibiotic resistance (AMR) due to the inadequate concentration of antibiotics (Moffo *et al*., 2020).

Although World Animal Protection bans routine antibiotic feeding to farm animals, (Protection, 2023). In this study, most farmers reported using Egocine as growth promoter as well as antibiotic. Egocine contains oxy-tetracycline hydrochloride as a main antibiotic and water- and fat-soluble vitamins which is used as routine growth promoters in chickens. This practice is not only unacceptable but also is against action plan implemented by FAO which prohibits the use of antibiotics as feed additives for livestock and poultry growth aimed at combating AMR (FAO, 2021). Some nations have outlawed the use of antibiotics as growth promoters in livestock production, but Malawi is an exception.

This study shows, that farmers' judgment when choosing and determining the dosage of antibiotics can result in unintentional misapplication, including incorrect dosages, inappropriate treatment durations, and unnecessary use, which ultimately contributes to antibiotic resistance and poses risks to public health. (Dyar *et al*., 2020). Farmer’s must follows 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 and food safety implications.

This investigation found that some farmers were using herbal remedies. The majority of farmers who acknowledged using these remedies said they don't use synthetic antibiotics. Although they are not sure of their dosage and withdrawal period, because the pharmacokinetics and pharmacodynamics of the majority of these herbal medications are still unknown. Even so, results revealed that most farmers using herbal remedies to their chickens do not face bacterial infections. Although this is a promising solution, further research is needed to assess the efficacy and safety of the herbal medicines used by poultry farmers so that they can be a substitute for synthetic antibiotics. Some studies have reported that most bacteria are susceptible to natural remedies such as aloe vera and moringa(Bengharbi *et al*., 2024), but more should be done to standardize the dosage and assess the residues of these herbal in meat. In this study, it was also found that some farmers were using diagrow pesticide as an antibiotic; the use of pesticide can accumulate in chicken meat over the time and can cause health problems to humans such as cancer and allergic reactions.

Understanding the use of antibiotics was one of the main problems portrayed in this study and elsewhere (Osaili *et al*., 2022; Sekyere, 2014). Farmers struggled to identify chicken diseases, although they were able to identify associated systems. This limits the ability to provide appropriate antibiotics to their chickens. But they indicated that they provide treatment based on the symptoms presented by chickens. Furthermore, symptom-based treatment alone is highly likely to result in misuse and overuse of antibiotics, which contributes to the development of AMR. The lack of knowledge regarding the specific indications and improper administration of antibiotics due to a lack of training in AMU increases the risk of antibiotic misuse and contributes to the development of MR in broiler chickens.

5.2. Knowledge, Attitudes, and Practices of Farmers Related to AMU and AMR

This study found a low knowledge of AMU and AMR, a negative attitude and poor practices towards AMU and AMR. The low level of knowledge could be associated with a lack of training in AMU and AMR. In this study, only a small proportion of the farmers were trained in AMU and AMR. This lack of appropriate training among poultry farmers is consistent with findings reported in (Chilawa *et al.*, 2023; Moffo *et al*., 2020; Tasmim *et al.*, 2023). The majority of poultry producers lack sufficient expertise, as evidenced by the poor knowledge scores in this study. These farmers are unaware of the potential negative effects of improper use of antimicrobials which can lead to the irrational use of these medications. Poultry producers who are ignorant about AMU and AMR run the risk of using antibiotics improperly, which exacerbates AMR (Sadiq *et al*., 2018). Hence, there is a need for these poultry farmers to be sensitized regarding correct AMU and AMR.

The farmers' low level of knowledge in AMR is concerning. Especially when the majority indicated that they were not aware that AMR which is a public health concern and that antibiotics could be used to treat any disease. To these farmers, antibiotics can be used for any illness, including anti-parasitic, anti-protozoa, and viral infections. The low awareness of AMR among poultry farmers calls for improved educational interventions and the strengthening of antimicrobial stewardship programs (Mpundu *et al*., 2022).

In this study, education was shown to play a big role in knowledge on antimicrobial use and resistance among broiler farmers. A significant relationship was found between the knowledge level and education level. Most poultry farmers who attained a tertiary education generally had high knowledge scores. This could be attributed to the fact that participants of the tertiary level and some from the secondary level may have learned about AMU and AMR or may have read about the same. Previous studies done in Sudan and Ethiopia reported similar findings that attributed the lack of knowledge on AMU and AMR was associated with level of education of the participants (Eltayb *et al*., 2012; Geta & Kibret, 2021).

In this study, a serious gap was revealed in agricultural systems in Mzimba, Malawi, where a shortage of veterinarians, leads to the administration of antibiotics by farmers without prescriptions and consultation. In some places, government veterinarians stopped visiting the farmers to assess the health status of their chickens. In areas where veterinarians are available, farmer’s lack of money to call veterinarians and loss of trust prevent them from accessing required services. Others reported that they have been doing chicken farming for years and claimed to have good experience in chicken farming that does not require the services of experts. Another fact that may be related to the failure to consult veterinarians is the ease of purchasing medications without a prescription and easily access antibiotics in Malawi in veterinary shops (Kainga *et al*., 2025; Mankhomwa *et al*., 2022).

In this study, most of farmers purchased antimicrobials over counter without consulting veterinarians, this is also similar studies conducted in Kenya, Nigeria, Tanzania, Serbia, Ghana and other LMICs where farmers were buying antibiotics over counter(Aworh *et al*., 2021; Chauhan *et al*., 2018; Kariuki, 2013; Mdegela *et al*., 2021; Vidović *et al.*, 2022). Normally, the procedure was supposed to be that the farmers should report disease cases to veterinarians, who would then diagnose the animals, purchase the drugs, and administer the recommended drugs to the animals (Schmerold *et al*., 2023). Normally, veterinary shops are supposed to sell drugs on the basis of a prescription to registered veterinarians, rather than directly to farmers.

Farmers who lack expertise in chicken production and antibiotic use may not be aware of the appropriate use of antibiotics, including the correct dosage, duration, and withdrawal periods(Phares *et al.*, 2020). The lack of understanding among farmers, as evidenced by the use of antibiotics for various animal diseases without proper knowledge, raises immediate concerns for animal health and public health. Using antibiotics without understanding how to use them properly, and not considering other ways to treat illnesses can be risky and could lead to AMR(Azabo *et al*., 2022). 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 practices in animals.

Interestingly, the majority of the farmers believed that the sale of non-prescription antibiotics should be outlawed because they frequently purchase expired medications, and some claim that antibiotics couldn't cure their chickens' illnesses. Due to high cost of the antibiotics, some farmers stop the dosage after the animal is cured and the antibiotics are reserved to be used in the future. The tendency to purchase non-potent and expired antibiotics could negatively affect the attitudes of farmers towards the use of drugs. The sale of non-potent and expired antibiotics is attributed to a lack of proper regulation of antibiotic use in Malawi. This increases the likelihood of using fake drugs or expired antimicrobials among farmers, which can lead to AMR(Mouiche *et al.*, 2019), hence significantly affecting public health. Although farmers in the study reported obtaining antibiotics from registered shops, the absence of regulatory oversight in antibiotic acquisition contributes to unmonitored and possibly inappropriate use.

Poor practices regarding AMU and AMR reported in this study is another stumbling block to the efforts put in place by the Ministry of Agriculture in Malawi, WHO and other international organizations aimed at controlling AMR(WHO, 2022b). This is similar to the practices of farmers in Cambodia regarding AMU and AMR (Chea *et al.*, 2022). Treating chickens without antimicrobial sensitivity testing and consultations with veterinarians is one of the common practices that can influence AMR(Abraham *et al*., 2025; Kainga *et al*., 2023; Mtila *et al*., 2024). This is commonly due to the lack of diagnostic tools, shortage of veterinarians, and high cost of sensitivity testing in Malawi, which hinders evidence-based treatment of bacterial infections in chickens. This might also be because most farmers are not trained on antibiotic usage and AMR.

There was a weak relationship between attitudes, practices, and knowledge. This study shows that despite people had knowledge on withdraw periods and knowledge of antimicrobial resistance, they were not practicing such knowledge on use antimicrobials in chicken farming which might contribute to antimicrobial resistance due to lack of training on AMU and AMR. This shows that a lack of training in AMU and AMR might be a contributing factor to low KAP.

5.3 Drug value chain

The study found that antibiotics are supplied through uncontrolled pathways that eventually reach end consumers. Veterinary retail stores, animal health professionals, and farmers that purchase veterinary antibiotics directly rather than through approved importers are examples of these channels. These uncontrolled routes provide a problem since the goods coming into the nation are not checked for quality and are not noted in AMU surveillance reports and this is similar to the study done in Cameroon(Azabo *et al.*, 2022)

The strong influence of demand on stocking decisions indicates a market-driven approach, where customer preferences may override clinical or scientific considerations. The low mention of disease-specific factors suggests limited focus on tailoring drug stocks to local disease patterns, which could contribute to inappropriate antimicrobial use. This is a similar finding found in Malawi by Mhone *et al.* (2024), who found that demand influence vet shop owners to stock particular antibiotics.

History by clients as reported by 96.4% was mostly used for selling antibiotic which could influence shop owners to use wrong dose as most recommendations were based on subjective assessments rather than formal prescriptions or laboratory confirmation at the same time most of them were not trained in terms of disease diagnosis and treatment. The heavy reliance on history and clinical signs, combined with widespread of sales and vet shop owners' decision-making without consulting veterinarians, points to a lack of professional oversight in drug dispensing. This is a critical concern for antimicrobial resistance (AMR), as the inappropriate selling or use of drugs can exacerbate resistance. On top of that most of shop owners were not trained for their job and this also support to similar study done by Kainga *et al.*, (2023) who found most of shop attendants were not qualified for job. Lack of training and qualification might cause shop owners to give wrong dosage. This misuse can lead to treatment failure, adverse reactions, or the development of antibiotic resistance.

There was high rate of advice provision by vet shops to farmers, but the advice was focused on dosage and duration, with less emphasis on storage or AMR-related guidance, suggesting gaps in educating farmers about responsible antimicrobial use. The variability in advice frequency indicates potential inconsistencies in customer education. While refrigeration was commonly used, the reliance on open shelves and varied storage methods suggests inconsistent adherence to optimal storage conditions (e.g., cold chain for vaccines). This could compromise drug efficacy and safety.

Farmers and vet shop owners indicated that they were keeping veterinary antibiotics on open shelves and disposing of expired antibiotics in pit latrines. This open-shelf storage exposes antibiotics to light, heat, and moisture, which can degrade the active ingredients, reducing their efficacy and increasing the risk of antibiotic resistance (Khan *et al*., 2021). A study in Blantyre found that between 19 and 44% of amoxicillin, ciprofloxacin, flucloxacillin and sulfadoxine/pyrimethamine in drug stores were substandard due to poor storage (Chikowe *et al.*, 2018). When antibiotics are disposed of in the environment via pit latrines, they can be transported through surface runoff and infiltrating water into surface water bodies or groundwater. This can lead to the emergence, selection, and persistence of (ARGs) (Gothwal & Shashidhar, 2015; Huo *et al*., 2024) and bioaccumulation. Therefore, correct storage and disposal of antibiotics are essential.

The widespread selling of antimicrobials for prophylaxis, particularly in poultry, is a major concern for AMR, as it promotes unnecessary drug exposure. The major clients were poultry farmers reflects the intensive nature of poultry farming, where prophylactic use is common but risky. Prescribing antibiotics as prophylaxis by vet shop owners is public health concern as it can lead to antimicrobial resistance in chicken farming that will lead infections difficult to treat as well as can lean to antimicrobial residues as a lot of antibiotics will be used to treat infections.(Tufa *et al*., 2018).

The low reported incidence of counterfeit drugs is encouraging, but the practice of keeping counterfeits in store is concerning. The tendency to change drugs rather than investigate underlying issues (e.g., resistance) suggests a reactive approach to treatment failures. It is important to understand the underlying cause first and important to consult veterinarian first before making any decision about changing veterinary antibiotics to farmers. According to the PMRA Act of 2014(Government, 2022) and the Veterinary and Para-Veterinary Act of 2001 (Government, 2001), a veterinary surgeon or a para-veterinary surgeon who holds a degree, diploma, certificate or other qualification recognized by the Board of Veterinary Surgeons are only allowed to prescribe and sell veterinary drugs. However, this is not the case, from this study it was observed that most of shop owners selling antibiotics don't have a qualification related to animal health, and don't know the pharmacokinetics and pharmacodynamics of antibiotic hence there is a possibility that they are giving wrong antimicrobials to farmers, which can lead to AMR in chickens.

The reliance on routine inspections for monitoring, as reported by 45%, and the lack of robust information-sharing channels as only 32% reported, indicate weak engagement between veterinary shops and regulators. This limits the ability to enforce policies and address AMR effectively. To prevent conflicting mandates, it is critical to explicitly define the roles and duties of various government agencies. To address AMR, a formal interagency task group comprising members from the environmental, human health, and trade sectors should be established.

5.4 Laboratory experiment

5.4.1 Preverance of E. coli, Salmonella spp, and Enterobacter spp.

The positive result *to E.coli*, *Enterobacter,* and *Salmonella* spp is serious public concern as these bacteria are Extended-spectrum beta-lactamase (ESBL) which are capable of breaking antibiotics such as penicillin's and cephalosporin's (Husna *et al.*, 2023).

The prevalence was found to be 69%. The high prevalence of *E. coli* can be because this bacteria is gut flora bacteria or can be due to fecal contamination of meat (Schwaiger *et al*., 2012). Such a high prevalence can also be due to environmental contamination. The prevalence was greater than the study done by (Emiliana *et al*., 2024) which was 57% and also higher than the prevalence found by Ramatla *et al*. (2022) which was 66.8%.

The high prevalence might also be due to contamination by vendors during slaughtering process, the ruptured digestive tract causes *E. coli* which were initially present in the digestive tract might contaminate chicken meat (Dugassa, 2022). It was evident that meat handlers were not cleaning their hands frequently enough when killing chickens. When delivering meat to customers, most meat workers did not wear gloves. Additionally, they were handling meat with their bare hands, and they frequently used a piece of cloth to wipe their hands rather than washing them. This also supports the study done by Tresse, (2017), who stated that chicken meat is easily contaminated by meat handlers during handling. When *E. coli* prevalence data are compared with literature findings, it is clear that the prevalence data can be affected due to numerous reasons.

Lack of biosecurity during rearing can influence preverance of *E.coli* because can cause chicken to harbor these pathogens in the intestines, feathers, and skin, which can contaminate meat during slaughtering(Lichtner *et al.*, 2024; WHO, 2023b). The other way is cross-contamination, in which contaminated equipment with bacteria used to different chickens during slaughtering can contaminate meat in the process (Lichtner *et al.*, 2024; Mazengia *et al.*, 2015; Munther *et al.*, 2016). The other way which can influence high preverance of *E.coli* is environmental exposure, in which chicken meat can be contaminated with feed or water which contains bacteria (Sajid *et al.*, 2023; Sinhamahapatra, 2022; WHO, 2023).

E.coli is considered a heat-sensitive bacterium that is destroyed with proper cooking methods; however Entertoxigenic *E. coli* (ETEC), enterotoxins, and verotoxins produced by *E. coli* O157: H7 can remain in food commodities, causing food-borne diseases, a potential public health risk(Dinu & Bach, 2011). Chicken meat contamination by *E.coli* was high and this support to the study by (Saliu *et al.*, 2017) who reported that poultry contamination by E.coli is associated by feacal contamination. The high prevalence in this study could support this. Moreover, the survival of *E. coli* in raw or undercooked food products can lead to diarrheal diseases and related complications since they are directly consumed without preliminary heat treatment(Wei & Zhao, 2018). Ajayi *et al.* (2024) stated that cooking meat over 100 degrees Celsius is crucial to preventing the spread of resistant *E. coli* to humans.

The prevalence of *Enterobacter* spp was 12 %, which might be because the meat was not highly contaminated with Enterobacter. The results is lower than the study found by(Amer *et al*., 2011) and by (Schwaiger *et al.*, 2012) who found the prevalence was 20.8% and 22% respectively but higher than findings found by (Tekiner & Özpınar, 2016) which was 6.9%.

The prevalence of *Enterobacter* spp in Meat is a serious concern as it can lead to infections in immunocompromised people.

The prevalence of *Salmonella* spp was 2%. Such a low prevalence may be because the meat in most markets was not contaminated by Salmonella spp. The lower prevalence might also be because the meat sellers were using chemical antimicrobials such as chlorine-containing compounds and organic acids which could eliminate *Salmonella* spp. The prevalence of *Salmonella* spp. was lower than that reported by Procura *et al*., (2017), who found a prevalence of 4.8%. The preverance is higher than the results found by Muligisa-Muonga et al., (2021) in Zambia which was 0.005%. The lower preverance of *Salmonella* spp in meat indicates that people are not of high risk to *Salmonella* spp.

5.4.2 Prevalence of AMR

The presence of resistance in food is worrisome. Antibiotic resistance in pathogenic bacteria that may infect people is one of the implications of the improper overuse of antibiotics in poultry farming, as reported by (Agyare *et al*., 2019; Z. I. Kimera *et al.*, 2020; Kumwenda *et al.*, 2021*;* Osaili *et al.*, 2022*)*. The high resistance might also because farmers were using antibiotics at low dose in animal feed for disease prevention or were not following proper dosage and duration of antibiotics in chicken which might cause the natural balance of microflora to be upset, leading to a decrease in sensitive bacterial groups and an increase in resistant bacterial groups and this might support study by (Manyi-Loh & Lues, 2023).

There is some evidence that a significant amount of resistant bacteria and their resistance genes are present in foods derived from different animal sources, regardless of the level of processing (Marshall and Levy, 2011). The results showed that the resistance to ampicillin was 91.3%, which could be because some farmers were using penicillin in the treatment of chicken diseases. The results showed that the ampicillin resistance rate was lower than the 100% resistance rate found by Reddy *et al*., (2021) and the resistance rate found by Munim *et al*., (2024) where E.coli resistance to ampicillin was 94%, and the results are also higher than the 75% resistance rate reported by Lee *et al*., (2018) for E. coli. All these results reported that the resistant rate was due to overuse of penicillin and ampicillin. The *Enterobacter* spp isolates from samples also showed high resistance against ampicillin, this could be due to use of penicillin.

The prevalence rates of *E.coli* to Meropenem is 82.6% and *Enterobacter* spp resistant to meropenem was 75% lower than for *E.coli* resistance. Meropenem is not used as a growth therapy, growth promotion, or therapeutic in poultry, however such result could be due to cross-contamination of meat or horizontal gene transfer from *E. coli* infecting humans, or the usage of antibiotics in chicken. This resistance could be caused by the fact that most farmers keep their chickens indoors together with humans to prevent them from theft so such poor biosecurity could lead to meropenem resistance *E. coli* and *Enterobacte*r spp from humans to be transmitted to chickens, , the other cause could be because due to co-selection in which *E. coli* can develop resistance due to the chemicals which they use as disinfectant which contain metals and such resistance metals can coexist with *E. coli and Enterobacter* spp leading to meropenem resistance. The result from this study can be supported by various studies which reported that resistance to antibiotics such as meropenem may occur due to the cross-resistance, co-resistance, or co-selection of one or more resistant bacteria to antibiotics in one or more classes (Huo *et al.*, 2024; Murray *et al*., 2024; Silva *et al*., 2021; Wales & Davies, 2015).

Resistance to gentamicin, chloramphenicol, ceftriaxone, and cefepime may also be due to use of antibiotics in chicken or due to cross-resistance which can occur due to gene transfer. It can also occur due to the environment can contribute to such resistance of *E. coli* to antibiotics e.g the cage that they were using might contain resistant for *E.coli*. From this study, it was also found that most farmers were not following proper way of disposing of expired antibiotics as most of them were burying in the ground, others were throwing in bins, these might pollute environment hence bacteria can develop resistance genes(Murray *et al*., 2024) and resistance rate in this study can be contributed by bad disposition of expired antibiotics.

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Mtila *et al*., (2024) also reported that most farmers in Lilongwe, Malawi were not following biosecurity measures and reported that such behaviours act as drivers of AMR and this correlates to this study as it was observed that most farmers were keeping broiler chicken in their houses where they sleep and there is possibility that resistant *E.coli* from humans can infect chickens and at same time resistant *E.coli*, *Salmonella* spp and *Enterobacter* spp from chicken can easily affect human. Such resistance in Mzimba might increase the risk of disease transmission, serious illness, disability, and death by making antibiotics and other antimicrobial medications ineffective, which makes it harder or impossible to treat infections as reported by WHO (2023). The *E. coli* resistant to tetracycline was 71%, the results was lower that the resistant pattern found by Chisembe *et al*., (2024), and Jaiswal *et al*., (2024) in Malawi and India which was 90 and 78% respectively, and high than study done in south Korea which was 69%.(P. Lee *et al*., 2019). The high resistance of tetracycline could be due to overuse or use of drugs as growth promoters and treatment as most farmers reported that they are using egocine (which contain oxytetetacycline). *Enterobacter* spp resistant to tetracycline was 83.3% higher than the resistant rate *to E. coli*.

The resistance rates of *E. coli* to gentamycin was 10.1% and resistance to chloramphenicol was 31.9%. The resistance rates of *Enterobacter spp* to gentamycin was 25%, ceftriaxone was 0% and resistance to chloramphenicol was 24.6%. According to certain recent studies conducted worldwide, the occurrence of antibiotic-resistant pathogenic bacteria in chicken meat may be caused by the poultry industry's unregulated use of antibiotics, which leads to bacterial resistance through a variety of methods.(Abdelkarim *et al*., 2020; Ayinla & Mateus, 2023; Tufa *et al*., 2018)

Resistance to ciprofloxacin may be could be due to the increased use of quinolones in poultry farms, and this result supports the possible reasons for the high resistance to ciprofloxacin in human specimens to be due to the colonization of ciprofloxacin-resistant *E. coli* in the human gut (Sa *et al*., 2001). In clinical settings worldwide, ciprofloxacin resistance in the Enterobacteriaceae family is becoming a growing concern. The usage of neomycin, a substance frequently used in poultry farming that shares structural similarities with gentamycin, or the fact that farmers are using gentamycin to treat infections in their chickens could be the cause of antimicrobial resistance to gentamicin.

The MAR index of *E. coli* showed that 68 samples have an index above 0.2. This is big concern. This shows that almost all samples collected were taken from area that are of high risk. This MAR index threshold indicates that chickens in these areas are exposed to selective pressure which promotes multiple antibiotic resistance (Tama *et al.*, 2021). These selective pressures can be due to overuse of antibiotics in the area or the use of chemicals (such as disinfectants for cleaning chicken houses) which contains metals that coexist with bacteria, hence cause resistance of due to mutations where by in the past years the area were using a lot of antibiotics and have developed some mutations (Chukwu *et al*., 2023). The difference interms of MAR index among 4 locations is associated with how farmers were using antibiotics, and the hygiene practiced by chicken farmers.

The MDR in *E.coli* and *Enterobacter* spp could be because these bacteria were frequently exposed to antibiotics and had to develop a resistance against them because they are able to protect themselves against the effects of the drugs by developing cell membranes that block the entry of antibiotics into bacterial cells as reported by Islam *et al*. (2024). The use of uncontrolled antibacterial will lead to changes like resistance in these bacteria, such as incorrect medicine selection, duration of injection, and dose.

6 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

a. Farmers had low mean levels of knowledge, attitudes, and practices on AMU and AMR.

b. The prevalence of *E. coli* was high among all isolated bacteria

c. *E. coli* and *Salmonella* spp showed high resistance patterns towards ampicillin, meropenem and tetracycline.

d. Farmers and agrovets shop owners were involved in an unregulated medicine supply chain.

e. Factors such as easy access to antibiotics, inadequate guidance from veterinary professionals on proper administration and antibiotic selection, limited health and national policies related to AMU and AMR, and poor government oversight of antimicrobial acquisition and use were risk factors of AMU and AMR in chicken production

6.2 Recommendations

6.1.1 Recommendations to farmers

a. Farmers need to consult veterinary professionals about the need to use antimicrobials in any case in their production.

b. To lessen the need for antibiotics in chicken production, farmers must employ alternative therapies, including vaccinations, probiotics, and prebiotics. .

c. Farmers need to respect guidelines in connection to antimicrobial use and antimicrobial resistance.

6.1.3 Recommendation to vet shop owners

Vet shop owners need to follow regulation and policies related to selling antimicrobial, storage, disposition and stocking antimicrobials.

6.1.3 Recommendation to Veterinarians

a. Veterinarians should educate farmers about the use of antibiotics in poultry production.

b. Veterinarians must work with farmers, governmental organizations, and other interested parties to develop and execute strategies that promote responsible antibiotic use and avoid antibiotic resistance.

6.1.4 Recommendations to government agencies

a. For the sale and disposal of unneeded or expired veterinary antibiotics, government authorities and the pharmaceutical industry must establish a reverse logistics program that uses eco-friendly techniques like burning.
b. They should put in place regulations and rules regarding the use of antibiotics in chicken production to make sure farmers follow ethical standards.
c. They should monitor antibiotic use and instruct farmers and veterinary shops on antibiotic stewardship.
d. Government should enforce regulations to poultry farmers to avoid using human antibiotics in chicken production.

6.3 Limitations of study

This study had a number of limitations. Firstly, only broiler farmers and one district were included in the study. The current study might not be representative of the whole Malawi and all livestock animals, the short time span of the research may not reflect long-term dynamics and also the sample was drawn from broilers farmers from February to May 2025, which limits the generalizability of the results to broader populations/contexts**.** Potential recall and social desirability bias may also affect the results, lack of residue analysis to complement self-reported practices and the study used cross-sectional design which limits causal inference. The study also did not do cover any molecular techniques on isolated bacteria to identify specie's and resistance genes. Nonetheless, the results of this study may offer a baseline comparison with the other districts of Malawi. Therefore, other studies must be conducted to assess KAP among other livestock farmer on AMU and AMR, accessibility of antimicrobials, and consultation of vetenerians on use of antimicrobials. Future research needs to include all other districts, and also need assess antibiotic residues in chicken meat. The results highlight that more research is required to understand the interactions between veterinarians and farmers, especially on the use of antibiotics in chicken farming. Filling the gaps in these interactions will inform antimicrobial stewardship training.

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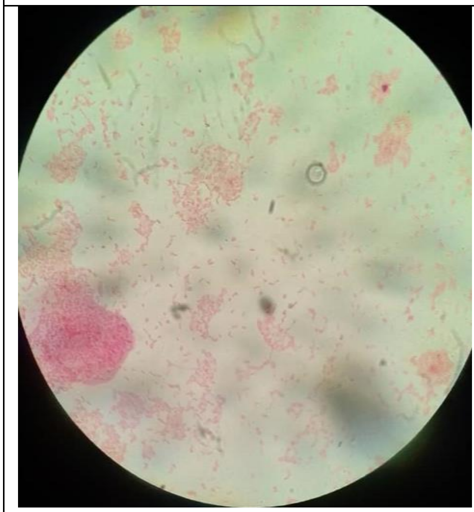
8.0 LIST OF APPENDICES

Appendix 1: Synthetic and non-synthetic antibiotics used by chicken farmers

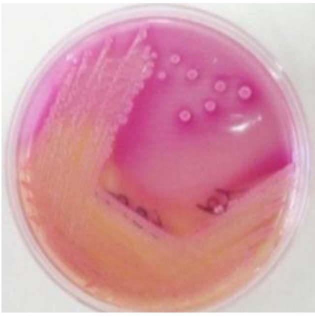
Synthetic antibiotics

Herbal medicine

Appendix 2 Gram staining and biochemical test

Gram-negative bacteria after gram staining	**Biochemical test results**
	

Appendix 3 : *Salmonella* spp, *E.col*i and *Enterobacter* spp on media

Salmonella spp on XLT 4	*Enterobacter* spp on Mankokey agar	*E.coli on Mankokey agar*
		

Appendix 4: AST of E. coli

Appendix 5: Knowledge of farmers on AMU and AMR

Variable	yes	No
Do you know that antimicrobial resistance is a serious public health problem?	22 (25%)	67 (75%)
Does the Government of Malawi have a policy/framework for antimicrobial use in animals?	32 (36)	57 (64%)
Heard of AMR	84 (94%)	5 (6%)
Heard of AMU	87 (98%)	2 (2%)
Do you know that antimicrobials can be passed on to humans through the consumption of chicken products?	85 (96%)	4 (4%)
Do you know that inappropriate use of antimicrobials can lead to the development of the AMR?	81 (91)	8 (9%)
Do you know that it is wrong to sell your animal products (meat)before the withdrawal period is over after administering?	86 (97%)	3 (3%)
Do you know that antimicrobial residues in poultry animals could be hazardous for public health?	84 (94%)	5 (6%)
Do you know that the use of antimicrobials in feed formulation is inappropriate?	24 (27%)	65 (73%)
Do you know who controls the sale of antimicrobials?	56 (63%)	33 (37%)
Do you know that lack of control in the sales of antimicrobials contributes to AMR?	62 (70%)	27 (30%)

Appendix 6: Attitudes of farmers on AMU and AMR

Variable	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	
Antimicrobials are safe, so they are commonly used in human and animals.	87 (98%)	0 (0)	1 (1%)	1 (1%)	0 (0%)	
Antimicrobials are needed for the treatment of any type of illness in animals.	81 (91%)	2 (2%)	3 (3%)	2 (2%)	1 (1%)	
It is better to make sure that animals are cured by broad-spectrum antimicrobials?	83 (93%)		2 (2%)	3 (3%)	1 (1%)	0 (0%)
Antimicrobials are needed to prevent only serious illness?	73 (82%)		2 (2%)	10 (11%)	2 (2%)	2 (2%)
Non-prescribed antimicrobial sales should be prohibited?	84 (94%)		3 (3%)	0 (0%)	0(0%)	2 (2%)
When weather/seasons change, antimicrobials are needed for animals?	51 (57%)		4 (4%)	26 (29%)	2 (2%)	6 (6%)
When animals have a fever/cold, antimicrobials are needed?	56 (62)		19(21%)	6 (7%)	4 (4%)	4 (4%)
Once the animals are cured, it's important to stop the dosage so that they are kept safe in case the animals get sick again in the future and can be re-used?	70 (79%)		6 (7%)	10 (11%)	3 (3%)	0 (0%)
It's safe to eat an animal if it dies in the course of treatment	2 (2%)		0(0%)	4 (4%)	14 (16%)	69 (78%)
Overdosing can lead to AMR	29 (33%)		2 (2%)	11(12%)	12 (13%)	35 (39%)
Missing a dose of antimicrobials may contribute to antimicrobial resistance?	85 (96.5%)		1 (1%)	1 (1%)	1 (1%)	2 (2%)
Vaccination can reduce the use of antimicrobials in animal farms?	55 (62%)		2 (2%)	25 (28)	1 (1%)	6 (7%)
Antimicrobials should be added to feed at any time to prevent animals from becoming sick.	17 (19%)		3 (3%)	22 (25%)	16 (18%)	31 ((35%)
Expired antimicrobial can be given to animals when they are sick rather than waste/dispose it?	0(0%)	5 (6%)	0	14 (16%)	70 (79%)	

Appendix 7: Practices of farmers on AMU and AMR

Variable	Category	Frequency (%)
What do you do when animals sick	Treat without sensitivity tests	84 (94%)
	Treat after carrying out sensitivity tests at the laboratory	5 (6%)
Whom do you consult when animals are sick?	No body	46 (51%)
	Assistant veterinarian	25 (28%)
	Farmer technician	10 (11%)
	Veterinarian	8 (8.99)
Whom do you consult for selection of antimicrobials and its dosage?	No body	56 (63%)
	Assistant veterinarian	23 (26%)
	Farmer technician	6 (7%)
	Veterinarian	4 (4%)
Whom do you consult for the preparation and administration of antibiotics	No body	61 (69%)
	Assistant veterinary	17 (19%)
	Farmer technician	7 (8%)
	Veterinary	4 (4%)
Do you allow your animals to complete the entire course of antimicrobials as prescribed by the veterinarian?	No	8 (9)
	Yes	81 (91%)
Do you check the expiry date of the antimicrobials before administering them/selling them	No	14 (16%)
	Yes	75 (84%)
Do you continue issuing antibiotics when the symptoms disappear	No	82 (92%)
	Yes	7 (8%)
What do you do when you realize that the antimicrobials have expired?	Burn/bury them	82 (92%)
	Throw them	57 (64%)
	Put them in animal feed	5 (6%)
	Return to veterinary labs	2 (2.2%)
	Use them	2 (2.2%)
Do you increase the dose and frequency of antimicrobials when there are clinical symptoms?	No	17 (19%)
	Yes	72 (80%)

Appendix 8: Comparison of MAR index of E.coli among Markets

Location	Coefficients	t	P>t	[95% Conf. Interval]
CHIBAVI vs AREA 1B	-.0916667	-1.43	0.937	-.3070382 .1237049
EKWENDEN vs AREA 1B	-.0555556	-1.02	0.994	-.2375777 .1264666
ENUKWENI vs AREA 1B	-.25	-3.25	0.064	-.5074183 .0074183
KAFUKULE vs AREA 1B	-.3125	-5.14	0.000	-.516007 -.108993
KAVIWALE vs AREA 1B	-.0916667	-1.43	0.937	-.3070382 .1237049
LUWINGA vs AREA 1B	-.1510417	-2.70	0.227	-.3386658 .0365825
MCHENGAUTUWA vs AREA 1B	-.25	-3.25	0.064	-.5074183 .0074183
MZUZU TOWN vs AREA 1B	-.0984848	-1.90	0.714	-.2720362 .0750665
NKHOLONGO vs AREA 1B	-.1979167	-2.86	0.163	-.4299503 .034117
ZOLOZOLO vs AREA 1B	-.2291667	-3.77	0.015	-.4326737 -.0256597
EKWENDEN vs CHIBAVI	.0361111	0.56	1.000	-.1792604 .2514827
ENUKWENI vs CHIBAVI	-.1583333	-1.88	0.726	-.4403209 .1236542
KAFUKULE vs CHIBAVI	-.2208333	-3.16	0.080	-.4546451 .0129784
KAVIWALE vs CHIBAVI	0	0.00	1.000	-.2442084 .2442084
LUWINGA vs CHIBAVI	-.059375	-0.90	0.998	-.2795015 .1607515
MCHENGAUTUWA vs CHIBAVI	-.1583333	-1.88	0.726	-.4403209 .1236542
MZUZU TOWN vs CHIBAVI	-.0068182	-0.11	1.000	-.2150798 .2014434
NKHOLONGO vs CHIBAVI	-.10625	-1.37	0.950	-.3652721 .1527721
ZOLOZOLO vs CHIBAVI	-.1375	-1.97	0.669	-.3713117 .0963117
ENUKWENI vs EKWENDEN	-.1944444	-2.53	0.309	-.4518627 .0629738
KAFUKULE vs EKWENDEN	-.2569444	-4.23	0.004	-.4604514 -.0534374
KAVIWALE vs EKWENDEN	-.0361111	-0.56	1.000	-.2514827 .1792604
LUWINGA vs EKWENDEN	-.0954861	-1.71	0.827	-.2831103 .0921381
MCHENGAUTUWA vs EKWENDEN	-.1944444	-2.53	0.309	-.4518627 .0629738
MZUZU TOWN vs EKWENDEN	-.0429293	-0.83	0.999	-.2164806 .1306221
NKHOLONGO vs EKWENDEN	-.1423611	-2.06	0.612	-.3743948 .0896726
ZOLOZOLO vs EKWENDEN	-.1736111	-2.86	0.163	-.3771181 .0298959
KAFUKULE vs ENUKWENI	-.0625	-0.77	0.999	-.3355333 .2105333
KAVIWALE vs ENUKWENI	.1583333	1.88	0.726	-.1236542 .4403209
LUWINGA vs ENUKWENI	.0989583	1.27	0.971	-.1624511 .3603678
MCHENGAUTUWA vs ENUKWENI	0	0.00	1.000	-.3152717 .3152717
MZUZU TOWN vs ENUKWENI	.1515152	2.02	0.637	-.0999846 .4030149
NKHOLONGO vs ENUKWENI	.0520833	0.59	1.000	-.2428263 .346993

ZOLOZOLO vs ENUKWENI	.0208333	0.26	1.000	-.2522 .2938666
KAVIWALE vs KAFUKULE	.2208333	3.16	0.080	-.0129784 .4546451
LUWINGA vs KAFUKULE	.1614583	2.59	0.276	-.0470743 .3699909
MCHENGAUTUWA vs KAFUKULE	.0625	0.77	0.999	-.2105333 .3355333
MZUZU TOWN vs KAFUKULE	.2140152	3.66	0.021	.0180481 .4099822
NKHOLONGO vs KAFUKULE	.1145833	1.54	0.900	-.1346608 .3638275
ZOLOZOLO vs KAFUKULE	.0833333	1.25	0.973	-.1395974 .3062641
LUWINGA vs KAVIWALE	-.059375	-0.90	0.998	-.2795015 .1607515
MCHENGAUTUWA vs KAVIWALE	-.1583333	-1.88	0.726	-.4403209 .1236542
MZUZU TOWN vs KAVIWALE	-.0068182	-0.11	1.000	-.2150798 .2014434
NKHOLONGO vs KAVIWALE	-.10625	-1.37	0.950	-.3652721 .1527721
ZOLOZOLO vs KAVIWALE	-.1375	-1.97	0.669	-.3713117 .0963117
MCHENGAUTUWA vs LUWINGA	-.0989583	-1.27	0.971	-.3603678 .1624511
MZUZU TOWN vs LUWINGA	.0525568	0.98	0.996	-.1268612 .2319748
NKHOLONGO vs LUWINGA	-.046875	-0.66	1.000	-.2833288 .1895788
ZOLOZOLO vs LUWINGA	-.078125	-1.26	0.973	-.2866576 .1304076
MZUZU TOWN vs MCHENGAUTUWA	.1515152	2.02	0.637	-.0999846 .4030149
NKHOLONGO vs MCHENGAUTUWA	.0520833	0.59	1.000	-.2428263 .346993
ZOLOZOLO vs MCHENGAUTUWA	.0208333	0.26	1.000	-.2522 .2938666
NKHOLONGO vs MZUZU TOWN	-.0994318	-1.48	0.921	-.3248816 .126018
ZOLOZOLO vs MZUZU TOWN	-.1306818	-2.23	0.491	-.3266489 .0652852
ZOLOZOLO vs NKHOLONGO	-.03125	-0.42	1.000	-.2804941 .2179941

Appendix 9: KAP survey questionnaire

1. Questionnaire ID	
2. District	
3. Location	Geopoint
4. Date of Survey	DD/MM/YYYY
5. Enumerator's name	
6. Name of the respondent	
7. Sex of the respondent	☐1 Male, ☐2 Female
8. Age of respondent (years)	○ 18-30 ○ 30-40 a. 40-50 b. Above 60
9. Number of years in practice	1. 0-2 2. 1-4 3. 4-10 4. More than 10
10. Highest Qualification of the respondent	a. Degree b. No formal education c. High School d. Primary school
11. How many animals/birds to you keep?	
12. What do you do in case of diseases in your animals?	○ Self-treat ○ Call a veterinarian/AHA ○ Use Herbs ○ Other (specify
13. How long have you been involved in poultry farming	○ Less than 1 year ○ 1-5 years ○ 6-10 years ○ more than 10 years
Have you used antimicrobials on your chicken in the past year?	○ Yes ○ No
What are the main reasons for using antimicrobials?	○ Treatment ○ prevention ○ Growth promotion ○ Other (specify),specify other reseaons
How do you decide which antimicrobial to use?	○ Veterinary prescription ○ Advice from an agro-dealer ○ Personal experience ○ Internet or online advice ○ Other (specify)
Can you provide the names of antimicrobials currently used on your farm?	
Do you have any antimicrobial products stored on your farm,If yes, can you show us the bottles/packages for verification?	○ Yes ○ No
How do you administer antimicrobials to your livestock? (Select all that apply	○ Oral (via feed or water) ○ InjectioTopical application ○ Other (specify
Where do you purchase antimicrobials?	○ Veterinary clinic ○ Agro-dealer ○ Pharmacy ○ Other (specify):
Do you follow the recommended dosage and duration for antimicrobial use?	
How do you store antimicrobials on your farm?	○ In a locked cabinet ○ In the animal shed ○ Throw them in gabbage

	○ Other (specify):
How do you dispose of unused or expired antimicrobials?	○ Burn them ○ Burry them
Diseases currently on farm	
How do you usually treat these diseases? (Select all that apply	○ Veterinary consultation ○ Self-treatment using antimicrobials ○ Advice from other farmers ○ Herbal or traditional remedies ○ Other (specify
Are vaccines used on your farm?	○ Yes ○ No If yes, specify which vaccines
Are you aware of any guidelines or regulations on antimicrobial use in livestock?	○ Yes ○ No If yes, specify
Do you receive any training or advice on responsible antimicrobial use?	○ Yes ○ No
If yes, which ones	○ NGO ○ Government ○ Friends through discussion
What are the main challenges you face in reducing antimicrobial use on your farm?	○ Lack of affordable alternatives ○ Limited knowledge about antimicrobial resistance ○ Difficulty accessing professional veterinary services ○ High prevalence of animal diseases ○ Pressure to maintain productivity and profit ○ Limited availability of vaccines or preventive measures ○ Other (specify):
Are you aware of any national policies or strategies addressing AMU and AMR	○ Yes ○ No
Do you think these policies are effective?	○ Yes ○ No If no, explain why
KAP SURVEY	
Have you ever heard about AMR in humans or animals?	○ Yes ○ No
What is Antimicrobial Use	○ A way to improve animal nutrition ○ A type of animal vaccine ○ Not in the given list ○ Drugs used to treat, prevent and promote growth in animals
Have you ever been trained on AMR?	○ Yes ○ No
What is the common source of advice on the use of antimicrobials in this areas?	○ Self ○ Veterians ○ CAHW/lead ○ Farmers ○ AHSA ○ Vet shop owners
How do farmers select which antimicrobials to use when the animal is sick?	○ From shops using a prescription ○ From farmers ○ Through vetenerians ○ Through lead farmers

How easy is it to access antimicrobials?	○ Very easy ○ Easy ○ Neutral ○ Difficult
Are the antimicrobials affordable to the farmers?	○ Yes ○ No
What is the meaning of withdrawal period after administering antimicrobials?	○ The time between administering the antimicrobial and when the animal can be slaughtered or milk can be consumed. ○ The period when antimicrobials are no longer effective ○ The period when antimicrobials are no longer effective ○ The time farmers must wait before reusing antimicrobials ○ I don't know
Is the withdrawal period respected?	○ Yes ○ No
Do you know that antimicrobial resistance is serious public health problem?	○ Yes ○ no
Does the Government of Malawi have policy/framework for antimicrobial use in animals?	○ Yes ○ no
Do you know that antimicrobials can be passed on to humans through the consumption of animal products	○ Yes ○ no
	○ Yes ○ no
Do you know that it is wrong to sell your animal products (meat) before the withdraw period is over after administering	○ yes ○ no
Do you know that antimicrobial residues in poultry animals could be hazardous for public health?	○ Yes ○ no
Do you know that use of antimicrobials in feed formulation is inappropriate?	○ Yes ○ no
Do you know who controls the sale of antimicrobials?	○ Yes ○ no
Do you know that lack of control in the sales of antimicrobials contribute to AMR?	○ Yes ○ no
Who has the authority to write a prescription of antibiotics for animals?	○ Malawi poison board ○ DAHLD ○ MALAWI BUREA STANDARD ○ PHAMANCY
Are the prescriptions written?	○ YES ○ NO
Do the Veterinary medicine stores request for prescriptions for some antimicrobials like antibiotics?	○ Yes ○ no
Who is the important stakeholder in monitoring the responsibility of AMU in farms? (Please specify	○ Registered Veterinarian ○ Vetenarinarian para professional ○ Agro vets ○ anyone
Altitudes	
Antimicrobials are safe, so they are commonly used in humans and animals?	○ strong agree ○ neutral ○ disagree ○ strong disagree

Antimicrobials are needed for treatment of any type of illness in animals?	○ strong agree ○ neutral ○ disagree ○ strong disagree
It is better to make sure that animals are cured by board spectrum antimicrobials?	○ strong agree ○ neutral ○ disagree ○ strong disagree
Antimicrobials are needed to prevent only serious illness?	○ strong agree ○ neutral ○ disagree ○ strong disagree
Non-prescribed antimicrobials sale should be prohibited?	○ strong agree ○ neutral ○ disagree ○ strong disagree
When weather/seasons changes, antimicrobials are needed for animals?	○ strongly agree ○ neutral ○ disagree ○ strongly disagree
When animals have a fever/cold, antimicrobials are needed	○ strong agree ○ neutral ○ disagree ○ strong disagree
Once the animals are cured, its important to stop the dosage so that they are kept safe in case the animals get sick again in future and can be re-used	○ strongly agree ○ neutral ○ disagree ○ strongly disagree
It is safe to eat animal if it dies incourse of treatment	○ strong agree ○ neutral ○ disagree ○ strong disagree
Missing dose may contribute to antimicrobials resistance	○ strong agree ○ neutral ○ disagree ○ strong disagree
Overdosing the antimicrobial usage can lead to AMR?	○ strong agree ○ neutral ○ disagree ○ strong disagree
Missing a dose of antimicrobials may contribute to antimicrobials resistance?	○ strong agree ○ neutral ○ disagree ○ strong disagree
Vaccination can reduce the use of antimicrobials in animal	○ strong agree ○ neutral ○ disagree ○ strong disagree
Antimicrobials should be added to feed at any time to prevent animals from becoming sick?	○ strong agree ○ neutral ○ disagree ○ strong disagree
Expired antimicrobial be given to animals when sick rather than waste/dispose of it?	○ strong agree ○ neutral ○ disagree ○ strong disagree
PRACTICES	
What do you do when your chickens are sick?	○ Treat without sensitivity tests ○ Treat after carrying out sensitivity tests at the laboratory
Whom do you consult when chickens are sick?	○ Veterinarian ○ Assistant Veterinary Officer ○ Assistant Veterinary Officer

	○ farmers technician ○ Nobody
Whom do you consult for selection of antimicrobials and its dosage?	○ Consult a community animal health work, ○ Consult the AVO/veterinarian ○ , Consult the AVO/veterinarian ○ Consult shop ownersothers specify
Whom do you consult for preparation and administration of antimicrobials?	○ Veterinarian, ○ Assistant Veterinary Officer ○ Farmer technician ○ Nobody
Do you allow your animals to complete the entire course of antimicrobial as prescribed by veterinarian?	○ Yes ○ No
Do you continue issuing antibiotics when the symptoms disappear?	○ Yes ○ No
Do you check the expiry date of the antimicrobials before administering them/selling them?	○ Yes ○ No
What do you do when you realize that the antimicrobials have expired?	○ Yes ○ No
Do you increase the dose and frequency of antimicrobials when there are clinical symptoms?	○ Yes ○ No

Appendix 10:Drug supply chain

Drug supply chain	
Questionnaire ID	
District	
Location	Geopoint
Date of Survey	DD/MM/YYYY
Enumerator's name	
Name of the respondent	
Sex of the respondent	☐1 Male, ☐2 Female
Age of respondent (years)	c. 18-30 d. 30-60 e. Above 60
Number of years in practice	5. 0-2 6. 1-4 7. 4-10 8. More than 10
Highest Qualification of the respondent	○ Degree ○ No formal education ○ High School ○ Primary school ○ Certificate in animal health ○ Degree in animal health ○ Degree in another qualification ○ diploma in animal health
How long have you been involved in working	○ Less than 1 year ○ 1-5 years ○ 6-10 years ○ more than 10 years
Who are the main clients of your shops	○ Poultry farmers ○ Cattle farmers ○ Goat farmers ○ Pig farmers

How are you prescribing antimicrobials?	○ Treatment ○ prevention ○ Growth promotion ○ Other (specify),specify other reseaons
Which drugs do you commonly sell? (Upon listing the drugs, researcher will classify the drugs to respective class of drugs)	○ Antibiotics ○ Anthelmintic ○ Antiviral ○ Antifungal ○ Antiprotozoal ○ Analgesics ○ Sedative
Where do you buy your drugs from	○ Veterinarian/ animal health practitioner ○ Agrovet ○ Distributing company ○ Others (specify)
What determines your choice of drug to stock	○ quantity/availability ○ quality/ brand ○ cost/price ○ Condition ○ other (specify)
Do you give advice to farmers on their use?	○ Yes ○ No
If yes, which advice?	○ Dosage ○ Duration for use ○ .Route of administration ○ Withdrawal period ○ Other (specify)
What is the most frequent way of buying the drugs?	○ Proportion/measured ○ Whole package for whole course of treatment ○ Other (specify)
What advice do you give to farmers incase chickens are not recovering from antibiotics they bought at first?	○ call a vet/AHA ○ purchase another drug ○ use the same drug at a higher dose ○ other (specify)
How do you store your drugs	○ open shelves ○ refrigeration ○ cool boxes ○ cabinet ○ Other specify
What do you do with expired drugs?	○ Dispose in pitlatrines ○ Discard off ○ Return to the retailer ○ Other (specify)
Do you encounter any challenges when sourcing drugs	○ Yes ○ No
If yes, which ones	○ Availability ○ Quality ○ Quantity (doses) ○ Cost ○ Other (specify)
Do you encounter counterfeit drugs	○ Yes

	○ No
If yes, what do you do	
Are you aware of the institutions that govern drug use	○ Yes ○ No
If yes, list them	
Are you aware of any policies governing drug use/storage/handling	○ Yes ○ No
If yes, which ones	
Is there information sharing and interactions between you and	○ internet/press ○ workshops/trainings ○ background training ○ other (specify)
Do you think that the existing policies are effective	○ Yes ○ No
If no, what should be done	○ Enforcement of regulations ○ Training ○ Sensitization ○ Punshment
Do you attend any stakeholder meetings the last 6 months??	○ Yes ○ No
If yes, who were the organizers	
Which other stakeholders attend such meetings	
Is there any information sharing between you and regulatory agencies	○ Yes ○ No
Is there any interaction between you and institutions	○ Yes ○ No