

**THE USE OF ANTIMICROBIAL AGENTS AND SMART REGULATIONS TO  
PREVENT ANTIMICROBIAL RESISTANCE IN POULTRY FARMS: TOWARDS  
ONE HEALTH**

**by**

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**THESIS**

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**2025**

## DECLARATION

I, Mathobela Caswell Kwena Kedishi, at this moment, affirm that the thesis I am submitting to the University of Limpopo for the Doctor of Pharmacy degree is original to me and has not been submitted for credit at any other academic institution. It is also my original work both in terms of design and implementation, and all cited materials have been properly cited.

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Mathobela Caswell Kwena Kedishi

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Date

## **DEDICATION**

To brother Kgwadi and my children Lethabo and Phuti, I dedicate this research to inspire them to make an informed decision that will both make learning fashionable and develop and brighten their futures. In addition, as my parents could not go further in receiving a better education, in honor of them and especially in memory of my father, I would want to devote this study. They wished for me to have the best possible education and success in life. To be in charge, you have to take charge of what people read, watch, and learn in schools.

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## DEFINITION OF TERMS

**Acceptable daily intake:** a veterinary medicine's threshold, which determines how much of a daily adult human dose can be consumed without significantly endangering health (Horel, 2017).

**Antibiotic:** An antimicrobial, which is a chemical called an antibiotic, which is made either synthetically or naturally by a fungus or other microbe and which either kills or prevents the growth of microorganisms that can make people or animals sick. An example of this is penicillin (Horel, 2017).

**Antimicrobial agent:** a semi-synthetic, synthetic, or naturally occurring substance that, at doses achievable in vivo, demonstrates antimicrobial action (kills or inhibits the development of germs) (Roth et al., 2019). In this study, a medication designed to cure harmful microorganisms such as fungi, viruses, and bacteria is referred to as an antimicrobial agent.

**Antimicrobial resistance** is the capacity of a microbe, such as bacteria, to thwart the actions of an antimicrobial, such as antibiotics (Horel, 2017). In the current study, it refers to the state where a microorganism is not susceptible to antimicrobial agents.

**Antimicrobial Stewardship (AMS):** is a methodical, individual, or multidisciplinary strategy to maximize the proper use of one or more antimicrobial drugs to ensure patient safety, enhance patient outcomes, and prevent the evolution of resistant microorganisms (Department of Health AMS guidelines, 2017).

**Bacteria:** single-celled microorganisms that are capable of existing as parasites that rely on another creature for survival or as autonomous, free-living entities (Horel, 2017).

**Biosecurity:** symbolizes a collection of precautionary practices and rules intended to shield a specific population (human or animal) from dangerous biological entities and goods (Department of Health AMS guidelines, 2017).

**Broiler chicken:** Any chicken (*Gallus gallus domesticus*) bred especially for meat production is referred to as a broiler [Global Animal Partnership (GAP), 2018].

**A commercial poultry farm** is a form of animal husbandry that raises domesticated birds such as chickens, ducks, turkeys, and geese to produce meat or eggs for food that are farmed in great numbers for commercial purposes (Horel, 2019).

**Growth promotion:** refers to the application of antimicrobial drugs for non-nutritional objectives with the goal of accelerating animal weight gain and/or improving feed utilization efficiency. Antibiotic agents are given to healthy animals with the main objective of boosting growth rates and improving feed conversion efficiency (Hoelzer et al., 2017).

**Hygiene:** circumstances and behaviours that support good health and stop the spread of illness, such as sanitizing water and sanitation systems, sterilizing equipment, maintaining hand hygiene, and safely disposing of waste (Department of Health AMS guidelines, 2017).

**A microorganism:** is a microscopic organism, such as bacteria, viruses, or fungi, that can have one or more cells (The Free Encyclopaedia on Microorganism).

**One Health approach/initiative:** an interactive endeavour including several government agencies, partners, and disciplines that works to attain the greatest potential health for people, animals, and the environment locally, nationally, or worldwide (Department of Health AMS guidelines, 2017).

**Smart regulations:** are regulations that aim to achieve public confidence in regulatory effectiveness, while limiting the burden on those being regulated, for example poultry farmers, particularly when it involves multiple stakeholders (Gunningham & Sinclair, 2017).

**Zoonosis:** is a contagious illness that can naturally spread between people and domestic or wild animals (Slingenbergh, Gilbert, De Balogh, Wint, 2004).

## ABBREVIATIONS AND ACRONYMS

ADG:	Average Daily Gain
ADI:	Acceptable Daily Intake
AFASA	African Farmers Association of South Africa
AMA:	Antimicrobial Agent
AMR:	Antibiotic Resistance
AMS:	Antimicrobial Stewardship
AMU:	Antimicrobial Use
ARGs:	Antibiotic Resistance Genes
AU:	Antibiotic Usage
CDC:	Centres for Disease Control and Prevention
CLSI:	Clinical and Laboratory Standards Institute
DLS:	Department of Live Stock
ECDC:	European Centre for Disease Prevention and Control
EU:	European Union
FDA:	Food and Drug Administration
FISH:	Fluorescent In Situ Hybridization
GAP:	Global Animal Partnership
GAP:	Global Action Plan
GC:	Gas Chromatography
GLASS:	Global Antimicrobial Resistance and Use Surveillance System
GRADE:	Grading of Recommendations Assessment, Development, and Evaluation
HPCIA:	Highest Priority Critically Important Antimicrobials
HPLC:	High-Performance Liquid Chromatography
IDSA:	Infectious Diseases Society of America
IPC:	International Poultry Council
LMICs:	Low and Middle-Income Countries
MDR:	Multidrug Resistance
MRF:	Mannan-rich fraction
MS:	Mass Spectrometry
NAP:	National Action Plan

NHLS:	National Health Laboratory Services
OIE:	World Organizational for Animal Health
PCR:	Polymerase Chain Reaction
PFA:	Phytogetic Feed Additives
SAHPRA:	South African Health Products Regulatory Authorities
SAPA	South African Poultry Association
SOP:	Standard Operating Procedure
UL:	University of Limpopo
UTIs:	Urinary Tract Infections
WAAW:	World Antimicrobial Awareness Week
WGS:	Whole Genome Sequencing
WHO:	World Health Organization
XLD:	Xylose Lysine Deoxycholate

## MANUSCRIPTS SUBMITTED

### International Journal of One Health

#### ANTIMICROBIAL USE, ANTIMICROBIAL RESISTANCE PREVENTION AND ONE HEALTH APPROACH: A STATE-OF-THE -ART IN SOUTHERN AFRICA REVIEW

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#### Background

In the past three decades, almost 75% of newly emerging infectious diseases, requiring the use of antimicrobial agents, among humans have been zoonotic diseases. COVID-19 caused by the SARS-Cov-2 virus, and Tuberculosis caused by *Mycobacterium bovis* are good examples. As a matter of fact, about 60% of human pathogens are zoonotic. Nonetheless, a crucial aspect that is sometimes disregarded is the reality that zoonoses are a two-way street, meaning that humans can infect animals and vice versa.

#### Material and method

The publications used for this review were those that were indexed before June 2023 in the Scientific Database, Web of Science, PubMed, Scopus, and Google Scholar databases. The online search included a combination of keywords such as antimicrobial use, antimicrobial resistance, antimicrobial stewardship, medicine for humans and animals, zoonosis, and the One Health concept. The literature was filtered to include only Southern African countries. The electronic database was searched for literature reporting anything on the keywords in sub-Saharan Africa to build a context for the continental perspective. Joint External Evaluation calculated the International Health Regulation (IHR), capability ratings depending on whether or not there are signs of being ready for a potential public health danger, such as AMR, a silent pandemic. A rating of 1 denotes no capacity, a score of 2 limited capacity, a score of 3 developed capacity, a score of 4 demonstrated capacity, and a score of 5 maintained capacity.

## **Results**

From the review, it was found that both human and veterinary antimicrobial use, in other words, the standard units per 1000/population in the SADC region is not well documented, Standard units per 1000/population is the amount of antimicrobial supplied by pharmaceutical manufacturers to the public and private sector. From the review of all literature on work done in the SADC region, One Health has paid little attention to the environmental effects of health management at the human-animal interface or to how human and environmental factors interact to affect animal health.

## **Conclusion**

One Health approach if embraced and fully implemented by all SADC region countries, will support the United Nations Sustainable Development Goals, SDGs 2, 13, and 14. Continual involvement of communities who are partly to blame for the injudicious use of antimicrobials is critical. Therefore, every community in each country must be aware of and actively participate in events during the 18-24 November, World Antimicrobial Awareness Week (WAAW) each year as part of the country action plan endorsed by the 68th WHA in May 2015. This is a week to Encourage the public, One Health stakeholders, and policymakers to adopt best practices since they all contribute significantly to halting the further emergence and spread of antimicrobial resistance.

**Keywords:** Usage of antimicrobial agents, Antimicrobial resistance, Antimicrobial Stewardship, Initiation of One Health approach, Zoonosis.

**Progress: under review**

**KNOWLEDGE, ATTITUDE, AND PRACTICES AMONG COMMERCIAL POULTRY  
FARM CHICKEN FEEDERS REGARDING ANTIMICROBIAL AGENTS USE:  
TOWARDS SMART REGULATIONS**

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**Background**

The poultry sector has adjusted by enhancing biosecurity and management procedures, environmental control of livestock facilities, improving selective breeding, and implementing adjustments to birds' composition and feeding schedules. It has been acknowledged that one key strategy for enhancing feed efficiency and growth performance in animals that produce food is the modulation of their gut microbiota with antimicrobial agents. Antimicrobial agents used in animals raised for food are subject to significantly stricter regulations due to public health concerns, particularly concerning antimicrobial compounds with human equivalents.

**Objectives**

To determine commercial poultry farm chicken feeders' knowledge, attitudes, and practices about the use of antimicrobial agents.

**Method**

A total of 175 chicken farm workers from three commercial poultry farms were included in the research. A pretested questionnaire was administered to chicken farm workers responsible for feeding the chickens in the presence of the poultry farmers. This was followed by transacting walk at each farm with the poultry farmers.

**Results**

Amongst the 175 chicken feeders at farms, 57% maintained that they do not use antimicrobial agents in poultry, 20% confirmed that they used these agents and 23% were not sure whether the antimicrobial agents are used or not. From the results of the questionnaire administered it was found that 67% of feeders indicated that penicillin was commonly used.

**Conclusion**

It was evident from the study that chicken farm factory workers needed education about the judicious use of antimicrobial agents in general and the consequences of their use as growth promoters. Therefore, any review by regulatory authorities in South Africa regarding the use of antibiotics in chicken farm factories to promote their growth needs to take into account the level of knowledge, attitude, and practice of every actor in the industry.

**Keywords:** Antimicrobial agents, antimicrobial resistance, poultry farm workers, chicken feeds

**Progress: under review**

## CONFERENCES AND PROCEEDINGS

### Online-oral presentation

Lekalakala M.R., Mathobela C.K.K., Nyazema N.Z. An audit of antimicrobial agents uses in surgical, orthopaedic, and gynaecology wards at a public hospital: a stepping stone towards antimicrobial stewardship University of Limpopo, South Africa, 2022.

### Online recorded presentation

Lekalakala M.R., Mathobela C.K.K., Nyazema N.Z. The prevalence of antimicrobial agents uses and resistance in poultry industry around Polokwane, University of Limpopo, South Africa, 2022. World antimicrobial awareness.

### Oral presentations

Mathobela C.K.K., Nyazema Z.N., Lekalakala M.R., Nlooto M., Ng'ambi J.

Development of guidelines on the usage of antimicrobial agents in poultry farms: towards one health, International One Health Organisation, University of Fort Hare, 2023.

Mathobela C.K.K., Nyazema Z.N., Lekalakala M.R., Nlooto M., Ng'ambi J.

Antimicrobial resistance, antimicrobial stewardship, zoonosis, one health: the role of SAMHS, University of Limpopo, South Africa, 2023. The Surgeon General of the South African Military Health Services awarded the paper presented the best paper and presenter.

Mathobela C.K.K., Nyazema Z.N., Lekalakala M.R., Nlooto M., Ng'ambi J.

The concepts of antimicrobial stewardship and resistance, zoonosis, Towards One Health, International One Health Organisation, University of Limpopo, South Africa, 2023.

Mathobela C.K.K., Nyazema Z.N., Lekalakala M.R., Nlooto M., Ng'ambi J.

Antimicrobial resistance is a silent pandemic: a situation analysis at two military health centres in Area Military Health Unit Limpopo, University of Limpopo, South Africa, 2023.

## ABSTRACTS SUBMITTED FOR CONFERENCES

### ABSTRACTS OF PAPERS PRESENTED AT THE UN-WAAW (WORLD ANTIMICROBIAL AWARENESS WEEK) - STELLENBOSCH, NOV 2022 CONFERENCE

#### THE PREVALENCE OF ANTIMICROBIAL AGENTS USE AND RESISTANCE IN THE POULTRY INDUSTRY AROUND POLOKWANE

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**Background:** Antimicrobial agents such as tetracycline, neomycin, and bacitracin are paramount in the poultry industry for prevention, treatment, and growth promotion. The recurrent use of antimicrobial agents has produced irrational use of antibacterial substances resulting in the development of the growing worldwide threat that antimicrobial resistance poses to the wellbeing of both humans and animals. This is much so if microorganisms, for example, *Salmonella* found in chickens and also capable of infecting humans become resistant. There is a need for stakeholders in the human and animal health sector to come together to curb AMR.

#### **Objective(s):**

- a. To identify the most commonly used antimicrobial agents in the poultry industry;
- b. To identify resistant microorganisms against the most commonly used antimicrobial agents.

**Methods:** A cross-sectional, experimental, and quantitative study design was conducted at three chicken farms in Polokwane, initially using a pretested questionnaire among chicken feeders. Chicken droppings and chicken gut contents were collected for microbiological investigation using standardized laboratory methods.

**Results:** The study showed that amoxicillin/clavulanic acid was most commonly used for growth promotion. *Salmonella* and *Shigella* were identified from the chicken droppings collected from the farms the susceptibility test indicated that all the *Shigella* strains were susceptible to ciprofloxacin and that amikacin is the most effective antibiotic against *Salmonella*. *Salmonella* strains were found to be resistant to gentamycin and amoxicillin which are the most widely used agents in humans.

**Conclusions:** From the results obtained, it was found that farm workers lacked knowledge about antimicrobial agents' use. Some of the AMA used were also those used in human *Salmonella* and *Shigella* infections calling for closer collaboration in controlling AMR.

**Keywords:** Antimicrobial agents, antimicrobial resistance, mortality, poultry

# **AN AUDIT OF ANTIMICROBIAL AGENTS USE IN SURGICAL, ORTHOPAEDIC, AND GYNAECOLOGY WARDS AT PUBLIC HOSPITALS: A STEPPING STONE TOWARDS ANTIMICROBIAL STEWARDSHIP**

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## **Background:**

The gravity of antimicrobial resistance (AMR), caused by the interplay of several factors, has increased significantly since research and development of newer antimicrobial agents (AMA) has significantly diminished. Promotion of judicious use of AMA in, for example, surgery is often considered easier a good starting point for initiating antimicrobial stewardship, AMS, activities in institutions. It has been shown that, following AMS activities, improvements occur in patients' outcomes in terms of, for example, reduced incidence of surgical site infections. A retrospective audit was carried out in the three wards at two public hospitals.

## **Objective(s):**

- a. To identify the frequently prescribed antimicrobial agents;
- b. To determine the resistant patterns of the mostly used antimicrobial agents;
- c. To investigate how AMS could be used as a strategy to rationalize the use of AMA and prevent AMR.

## **Method:**

A total of 79 medical records of patients from surgical, orthopaedic, and gynaecology wards who had developed surgical site infections were reviewed. A critical analysis of culture and sensitivity test results was done followed by interviews with the prescribers. A feedback meeting was also done with hospital staff involved in the three wards, including pharmacists.

## **Results:**

The most commonly prescribed AMAs were metronidazole, amoxicillin, augumentin, cefoxitin, meropenem, and ertapenem for infection due to mainly *E. coli*, *Klebsiella*

*pneumoniae*, and *Staphylococcus aureus*. It was discovered that *Staphylococcus aureus* was resistant to  $\beta$ -lactam, whereas *E. coli* and *Klebsiella pneumoniae* were resistant to carbapenems, fluoroquinolone, and  $\beta$ -lactam. Results obtained from interviews with surgical staff and pharmacists seemed to suggest there were different perceptions regarding AMA use and levels of AMR.

### **Conclusion**

The results of the AMA use, AMR, and the perceptions identified can be used as evidence to initiate AMS at the institutions.

**Keywords:** Antimicrobial agents, antimicrobial resistance, surgical site infections.

# PROBIOTICS/ESSENTIAL MICROBES AN ALTERNATIVE FOR ANTIMICROBIALS IN CHICKEN GROWTH PROMOTION: THE NEED FOR SMART REGULATIONS FOR RESISTANCE PREVENTION.

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**INTRODUCTION:** Probiotics or Effective Microbes (EMs), are products of biotechnology. When administered in adequate amounts, as an alternative to antimicrobial agents (AMA), they are thought to confer a health benefit on chickens, for example. AMAs are used for growth promotion in chickens. Antimicrobial resistance (AMR) has been identified by FAO, the World Organisation for Animal Health (OIE), and WHO as a risk to the public's health. The shift from AMAs to EMs requires enough information on the effects of EMs on chicken production and health. Two separate studies were carried out to inform how this could be achieved.

## OBJECTIVES

- a. To determine chicken feeders' knowledge, attitude, and practice (KAP) regarding antimicrobial use.
- b. To compare the effects of *Lactobacillus planetarium*, *Candida valida*, *Actinomyces*, *Streptomyces albus* and *Aspergillus oryzae*, and oxytetracycline and coccidiostat on broiler chicken growth rate.

**METHODS:** A questionnaire to capture KAP was administered to 9 chicken feeders at 2 farms. For a total of 150 days old chicks on the grower diet were assigned 5 different treatments (Control, 30, 50, or 100 ml of EMs/l of water or AMAs) replicated thrice, and the growth rate was monitored weekly for 21 days.

**RESULTS:** Chicken feeders had very little knowledge about AMAs and AMR. No difference in chicken growth rate between chickens supplemented with EMs or AMAs was observed.

**CONCLUSION:** There is need to educate chicken feeders on effects of AMAs and AMR in chicken production. EMs can replace AMRs in broiler chicken feeds without any adverse effect on growth and health of the chickens.

**KEYS WORDS:** Probiotics, Antimicrobials, Chickens

# **ANTIMICROBIAL RESISTANCE, ANTIMICROBIAL STEWARDSHIP, ZONOSIS, ONE HEALTH: THE ROLE OF SOUTH AFRICAN MILITARY HEALTH SERVICES**

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## **Background**

Benefits of rational use of antimicrobial agents include: maximizing patients' health status and reducing unnecessary prescribing, reducing the chances of antimicrobial resistance, cost-effectiveness, shorter duration of antimicrobial treatment, and the use of narrow-spectrum antimicrobial agents whenever possible. Antimicrobial agents are of paramount importance in growth promotion, the management, and avoidance of infectious diseases such as listeriosis in poultry. Antimicrobial resistance strains such as *Salmonella* and *Campylobacter* are contaminated and spread from chickens to people when they handle or eat poultry meat. Antimicrobial resistance has resulted from this, and it is now posing a worldwide concern to the health of people and animals. To safeguard the health of both humans and animals, the promotion of the prudent use of antimicrobial agents needs a transdisciplinary, multidisciplinary, collaborative approach which is what the One Health approach is all about.

## **Aim**

To demonstrate the importance and the need for antimicrobial stewardship within SAMHS towards One Health.

## **Objective(s)**

To identify steps that SAHMS can take to establish effective antimicrobial stewardship to stop and manage the spread of zoonotic illnesses and the emergence of antibiotic resistance.

## **Methods**

A mixed-method approach was used for data collection at 1 Military Hospital and three poultry farms around Polokwane. Medical records at 1 Military Hospital were examined and studied for surgical site infections. Chicken gut contents were collected from the

farms for laboratory culture and sensitivity testing at NHLS and genotyping of the microorganisms.

## **Results**

In humans, the majority of common microorganisms identified were *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, reported to be 100%, 99.3%, and 92.7% resistant to ampicillin respectively.

In chickens, *Salmonella* and *Shigella* were the most common microorganisms. The susceptibility testing indicated that *Shigella* was susceptible to ciprofloxacin and that amikacin was the most effective antibiotic against the *Salmonella* strain. *Salmonella* strains were resistant to gentamycin and amoxicillin which are the most widely used agents in humans. *C. freundii* was also 100% resistant to piperacillin. It was discovered that *Staphylococcus aureus* was resistant to  $\beta$ -lactam, whereas *E. coli* and *Klebsiella pneumoniae* were resistant to carbapenems, fluoroquinolone, and  $\beta$ -lactam.

**Conclusions:** The rational use of antimicrobial agents needs to be maintained with critical care as no new antimicrobial agents are produced. Antimicrobial stewardship and one health initiative are needed to eradicate antimicrobial resistance and rationally use antimicrobial agents.

**Keywords:** antimicrobial agents, antimicrobial resistance, antimicrobial stewardship.

# **ANTIMICROBIAL RESISTANCE, ANTIMICROBIAL STEWARDSHIP, ZONOSIS, ONE HEALTH: THE ROLE OF SOUTH AFRICAN MILITARY HEALTH SERVICES (AUSTRALIA)**

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## **Background**

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farms for laboratory culture and sensitivity testing at NHLS and genotyping of the microorganisms.

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**Conclusions:** The rational use of antimicrobial agents needs to be maintained with critical care as no new antimicrobial agents are produced. Antimicrobial stewardship and one health initiative are needed to eradicate antimicrobial resistance and rationally use antimicrobial agents.

**Keywords:** antimicrobial agents, antimicrobial resistance, antimicrobial stewardship.

# ANTIMICROBIAL RESISTANCE IS A SILENT PANDEMIC: A SITUATION ANALYSIS AT TWO MILITARY HEALTH CENTRES IN AREA MILITARY HEALTH UNIT LIMPOPO

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## Background

Drug-resistant diseases are becoming a greater threat to everyone, irrespective of socioeconomic status, age, or gender. They put individuals in peril not only in less developed countries but also in wealthy, industrial economies such as the United States. The microbes that cause meningitis, ear infections, and pneumonia are among the examples of clinically significant germs that are quickly becoming resistant to the antimicrobials that are now on the market. Bloodstream infections, skin, bone, lung, and streptococcus pneumoniae, for example. *Staphylococcus aureus*, infections spread in hospital settings (such as *Enterococci*, *Pseudomonas aeruginosa*, and *Klebsiella spp.*), foodborne diseases (such as *Salmonella* or *E. coli* obtained from meat, eggs, nuts, and fresh produce), and urinary tract infections (such as *Escherichia coli*). Military personnel bear a heavy financial burden from treating antimicrobial-resistant diseases, a burden that is only going to get worse under the radar. A study looking into the degree of antibiotic resistance in military hospitals in particular military health centres in Area Military Health Unit Limpopo was carried out to have an idea of the severity of the problem.

## Aim

To ascertain the degree of antimicrobial resistance at the two health centres in Area Military Health Unit Limpopo.

## Objectives

Assessing antimicrobial resistance's degree of severity in military health centres.

## **Method**

An antimicrobial agent angiogram from the two health centres in Area Military Health Unit Limpopo was examined and analyzed for antimicrobial resistance patterns from a period from January to August 2023. The angiogram contained all culture and sensitivity results of all samples sent to National Health Laboratory Services.

## **Results**

From the analysis *E. coli* was 94%, 50%, and 42% resistant to erythromycin, amoxicillin, and ampicillin respectively, *Morganella morganii* was 35% resistant to cefuroxime, *Enterobacter cloacae complex* and *Citrobacter freundii* were 12% resistant to cefoxitin, and *Proteus mirabilis* was 33% and 11% resistant to tetracycline and tigecycline respectively. Furthermore, most of the microorganisms were 94% resistant to erythromycin.

## **Conclusion**

There is a need for beseeching antimicrobial stewardship committees to do surveillance on the most prevailing microorganism, to aid judicious usage of antimicrobial agents, and to curb antimicrobial resistance within health centres. The window of opportunity is quickly closing since newly discovered multidrug-resistant germs are spreading alongside well-known, older infections. If we don't take action now, there won't be a cure tomorrow, which would bring us back to the time before antibiotics agents.

**Keywords:** Antimicrobial agents, microorganisms, antimicrobial resistance.

# THE PREVALENCE OF ANTIMICROBIAL AGENTS USE AND RESISTANCE IN THE POULTRY INDUSTRY AROUND POLOKWANE (ELEPHANT)

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## **Background**

Antimicrobial agents such as tetracycline, neomycin, and bacitracin are of paramount importance in the poultry industry for growth promotion, treatment, and prevention. The recurrent use of antimicrobial agents has led to irrational use of antimicrobial agents resulting in the development of antimicrobial resistance, which is increasingly becoming a global threat to both human and animal health. This is much so if microorganisms, for example, Salmonella found in chickens and also capable of infecting humans become resistant. There is a need for stakeholders in human and animal health together to curb AMR.

## **Aim**

To demonstrate the importance and the need for collaboration to eradicate antimicrobial resistance.

## **Objective(s)**

- To identify the most commonly used antimicrobial agents in the poultry industry;
- To identify resistant microorganisms against the most commonly used antimicrobial agents.

## **Methods**

A cross-sectional, experimental, and quantitative study design was conducted at two chicken farms in Polokwane, initially using a pretested questionnaire among chicken feeders. Chicken droppings were collected for microbiological investigation using standardized laboratory methods.

## **Results**

The study showed that amoxicillin/clavulanic acid was most commonly used for growth promotion. Salmonella and Shigella were identified from the chicken droppings collected from the farms the susceptibility test indicated that all the Shigella strains were susceptible to ciprofloxacin and that amikacin is the most effective antibiotic against Salmonella. Salmonella strains were found to be resistant to gentamycin and amoxicillin which are the most widely used agents in humans.

## **Conclusions**

From the results obtained, it was found that farm workers lacked knowledge about antimicrobial agents' use. Some of the AMA used were also those used in human Salmonella and Shigella infections calling for closer collaboration in controlling AMR.

**Keywords:** Antimicrobial agents, antimicrobial resistance, mortality, poultry

## **ABSTRACT**

**Background:** A quiet pandemic known as antimicrobial resistance (AMR) coexists with the COVID-19 pandemic and is caused by the widespread, careless use of antimicrobial medicines in self-medication and unsupervised therapy regimens by a huge number of people. Similar circumstances can be found in the livestock and poultry farming industry, which has resulted in AMR problems like mastitis caused by Methicillin-resistant *Staphylococcus aureus* (MRSA). Treatment and infection prevention in both humans and animals may become challenging shortly if AMR is not tackled, which is a concerning issue. Animal husbandry procedures need to be changed to lessen the utilization of antibiotics indiscriminately and the problems associated with AMR in the industry. Documenting the field level using antibacterial substances and the amount of AMR knowledge is a prerequisite for developing such intervention strategies. Unfortunately, there was a dearth of information, inadequate documentation, and in many cases non-existent data on AMR concerns at the field level in South Africa. Recent publications from the World Health Organization (WHO) have also highlighted the danger that humanity faces from reverting to pre-antibiotic times, highlighting the pressing necessity of using all monetary and scientific resources at our disposal to reduce this risk. Farmers' ignorance of antimicrobial agents, a lack of oversight and regulatory services, a high number of unofficial animal health service providers, and antimicrobial resistance (AMR) have resulted in the overuse and misuse of antimicrobial agents and, ultimately, the evolution of antibiotic-resistant genes and bacteria in commercial poultry farming are some of the predisposing factors.

**Methods:** A cross-sectional, experimental, and quantitative study design was conducted at three chicken farms in Polokwane. Initial research involved using a pretested questionnaire among chicken feeders. Chicken droppings and gut contents were collected for microbiological investigation using standardized laboratory methods.

**Results:** The study showed that amoxicillin/clavulanic acid was most commonly used for growth promotion. *Enterobacter cloacae complex*, *Salmonella*, and *E. coli* were identified from the chicken droppings collected from the farms and gut contents from

chickens and the susceptibility test indicated that all the *Enterobacter cloacae complex* strains were susceptible to amoxicillin, augumentin, ertapenem and that amikacin is the most effective antibiotic against *Salmonella*. *Salmonella* strains were found to be resistant to gentamycin and amoxicillin which are the most widely used agents in humans.

**Conclusions:** It was evident from the study that chicken farm factory workers needed education about the judicious use of antimicrobial agents in general and the consequences of their use as growth promoters. Therefore, any review by regulatory authorities in South Africa regarding the use of antibiotics in chicken farm factories to promote their growth needs to take into account the level of knowledge, attitude, and practice of every actor in the industry. Some of the AMA used were also those used in humans e.g. for *Salmonella* infections which calls for closer collaboration in controlling AMR.

**Keywords:** Antimicrobial agents, antimicrobial resistance, microorganisms.

## CHAPTER 1 INTRODUCTION

Antimicrobial agents (AMA) are necessary to keep animals healthy in systems that produce livestock, but misuse and/or abuse of these treatments can be a factor in the phenomenon of antibiotic resistance (AMR), in which microorganisms become resistant to one or more of the drugs used to treat microbial diseases (Caudell et al., 2020). Antimicrobial agents are primarily used in individuals to handle a range of infectious diseases. However, in veterinary medicine antibiotics are mainly used for growth promotion; this is very common in the poultry industry (Poole and Sheffield, 2013).

In poultry, three main uses for antibiotics are typically observed: (1) therapeutic use, which involves administering large dosages of antimicrobial agents to animals (individually or in small groups) for relatively short periods; (2) prophylactic use, which entails exposing animals to moderate dosages of antibiotics for longer periods; and (3) growth promotion is the practice of administering antibiotics for an extended period or for the duration of the animals' lives at subtherapeutic doses, such as 10 or 100 times smaller than the recommended therapeutic dosages (Marshall and Levy, 2011).

These antimicrobial agents are given via feed or water, stabilizing the gut flora and lowering illness susceptibility (Ferdous et al., 2019). The authorities responsible for food regulation and health have identified excessive use of antimicrobial medications as illegal and prohibited due to their tendency to collect residues found in the treated animals' tissues and organs that ultimately find their way into the food pyramid (Mund et al., 2017).

By 2030, it is anticipated that the use of antimicrobial substances in agriculture will have increased by 67%, which could further jeopardize the efficacy of AMA for the health of humans and animals (Van Boeckel et al., 2015). Due to the extensive use of antimicrobial agents, food originating from animals has significant levels of antibiotic residues, which can be transferred from animal products to humans (Landers et al., 2012). Regrettably, improper and excessive use of these antimicrobial agents leads to resistance to antimicrobial agents emerging in pathogenic bacteria and the endogenous

flora of exposed individuals, such as humans and animals (Tripathi, Hasan, Verma, 2017).

The global public's health, food security, and animal welfare are at risk due to antimicrobial resistance (AMR) growth, which is causing more and/or longer treatment cycles and therapeutic failures. Because of resistant microorganisms, AMR in animals may have an effect on the frequency of infectious diseases in humans. Microorganisms, such as bacteria, viruses, and parasites, can move across the human-animal interface through a variety of paths, including sharing water supplies, eating animal products, and direct contact (Caudell et al., 2020; Landers et al., 2012). World Health Organization [WHO (2015)] states that there is a serious risk of treatment failure and widespread transmission. There is an additional risk because nine of the fourteen drug classes that are deemed "critically important" for public health also have uses in livestock systems.

The usage of antibiotics in the production of feed for animals and the potential for antibiotic resistance to move up the food chain are issues that are causing increasing concern. Antimicrobial agents' usage for therapeutic purposes without veterinary oversight is more common in Bangladesh's small-scale commercial broiler farms (Sikder et al., 2019). In 1982, the Bangladesh Veterinary Practitioners Ordinance was passed, limiting the practice of medical prescription and surgery to registered veterinarians alone. Nevertheless, antimicrobials were utilized by farmers, non-veterinarian staff, and registered veterinarians without a confirming diagnosis or to promote growth (Wadoum et al., 2016).

Antimicrobial agents are also administered to feed and water at subtherapeutic dosages for risk management, prophylaxis, and growth enhancement. In human medicine and animal husbandry, suboptimal antibiotic prescription and usage are common risk management strategies. Antimicrobial agents are forbidden to be added to feed, according to Bangladesh's Animal Feed Act. Anecdotally, though, chicken growers get around the rule by giving broiler water that has antibiotics in it. Furthermore, there is little oversight and governance of small-scale producers (Sikder et al., 2019).

The Drug Act of 1940 states that only registered pharmacists with valid prescription paperwork may sell antimicrobial agents. However, there are currently no regulations in Bangladesh regarding the use of antimicrobial agents in laying hens. Antimicrobial agents are indiscriminately used to address infectious illnesses in chickens and as growth enhancers (Hasan et al., 2011).

The most frequent sources of *Salmonella* infections have been animal dietary products, particularly eggs and chicken meats (Greig and Ravel, 2009). The bacterial pathogen *Salmonella*, which plays a significant role in determining the safety of chicken and raw meat, will continue to get attention as the world's consumption of poultry meat rises. Globally, antimicrobial resistance has been on the rise recently, particularly for *Salmonella* strains from food animals that are multidrug-resistant (MDR) according to Xie et al. (2019).

Antimicrobial agents are utilized more often in China than in most other nations; according to a 2007 assessment, the country generated 210,000 tons of antibiotics, of which roughly half were used as medicinal medications and feed additives for livestock (Zhua et al., 2017). The overuse use of antimicrobial drugs in animal agriculture is thought to be one of the primary causes of *Salmonella's* resistance to antimicrobial agents. This resistance can be passed on to humans through animal products, which is extremely dangerous for everyone's health. Animal-origin *Salmonella* has been found to exhibit multidrug-resistant phenotypes more frequently in China (Zhua et al., 2017).

Tetracycline, gentamicin (Filazi et al., 2013), ceftiofur, bacitracin, erythromycin, virginiamycin, tylosine, and neomycin are the most often used antimicrobial agents. These agents are typically beneficial in lowering and preventing infections caused by necrotic enteritis and respiratory illnesses; gastroenteritis, options for gastroenteritis, skin issues, or soft tissue infections include quinolone compounds and/or fluoroquinolones. Sulfonamide drugs are used as both chemotherapeutic and preventive measures against pullorum disease, coryza, poultry typhoid, and coccidiosis. Meanwhile, coccidiosis is treated with piperazine, oxytetracycline, amoxicillin, amprolium, ciprofloxacin, and sulfa drugs (Kolaczek et al., 2014). Moreover, various anticoccidial medications such as nicarbazin, amprolium, clopidol, sulfonamides, and

salinomycin and vaccines (made from either attenuated or non-attenuated oocysts of coccidia strains) are widely used in poultry feed to prevent and treat coccidiosis. It is well known that the parasitic disease coccidiosis, caused by several *Eimeria species*, hinders the growth and expansion of the chicken industry (Chapman and Jeffers, 2014). Table 1.1 lists some of the antibiotics used in chickens, along with their derivatives, mechanisms of action, and withdrawal periods.

Table 1.1: Commonly used antimicrobial agents in the poultry industry (Mund et al., 2017)

Antibiotics	Derivatives	Mechanism
Beta-Lactams	Amoxicillin (aminopenicillin), penicillin, cephalosporin, ampicillin, monobactam, carbapenem	Bactericidal (prevents bacterial cell synthesis, disrupts cell wall integrity, inhibit transpeptidase)
Tetramisole	Levamisole	Effective against lungworms and gastrointestinal nematodes, mimics thymic hormone thymopoietin which affects many components of immune system
Quinolones/ Fluoroquinolones	Oxolinic acid, nalidixic acid, flumequine, enrofloxacin, norfloxacin, ciprofloxacin	Arrest bacterial cell growth via inhibiting DNA gyrases involved in DNA replication, recombination and repairing
Tetracyclines	Chlortetracycline, tigecycline, minocycline, oxytetracycline, chlcardine	Bacteriostatic and bactericidal, inhibit protein synthesis
Sulfonamides	Sulfacetamide, sulfamethoxyipyridiazine, sulfamethoxydiazine, sulfamethoxazole, sulfadimidine, suflamethoxine, sulfadiazine, sulfafurazole,	Act as competitive antagonists in microbial cells, block formation of folic acid
Aminocyclitol	Spectinomycin, apramycin	Bacteriostatic and bacteriocidal, irreversible inhibition of protein synthesis
Amphenicols Coccidiostats	Chloramphenicol, thiamphenicol, florfenicol Salinomycin, diclazuril, robenidine, naduramicin, lasalocid, toltrazuril, halfoginone, nicarbazin, narasin, monensin, clopidol, ionophores, amprolium	Inhibit protein synthesis Disrupt ion gradients across parasite cell membrane, inhibit parasite mitochondrial respiration, folic acid pathway, competitive inhibition of thiamine uptake
Macrolide	Erythromycin, tylosin, spiramycin	Act on 50S ribosomal subunit and inhibit protein synthesis
Nitrofurans	Furazolidone, furaltadone, nitrofurantoin, nitrofurazone	Bacteriostatic or bacteriocidal (at high doses)
Aminoglycosides	Neomycin, canamycin, gentamycin, netilmycin	Irreversible inhibition of protein synthesis

Different degrees of resistance to medications that contain beta-lactam antibiotics, such as piperacillin, amoxicillin, cefazolin, and penicillin, were demonstrated by *Salmonella* from eggs, reported the findings (Xie et al., 2019). Following these antimicrobial agents were gentamicin, kanamycin, streptomycin, minocycline, and tetracycline among the aminoglycosides and tetracyclines.

Due to fierce competition in the sale of veterinary medications, developing nations, particularly Indonesia, have recently experienced issues with poultry. Numerous

domestic and foreign animal health businesses in Indonesia compete with one another to market their goods (Nugroho et al., 2020). This results in poultry producers misusing veterinary medications, particularly in the production of commercial broiler and layer chickens. Farmers rarely follow the usage instructions, which leads to this misuse. Furthermore, animal health organizations' technical assistance frequently overlooks post-purchase services, like providing accurate information regarding medicine usage. The reason this issue is so serious is that improper use of veterinary medications, including overdosing, can directly harm hens and pose a risk to human health due to residues that may find their way into finished goods (Sikkema and Koopmans, 2016).

For example, it has been demonstrated that there are genotypic commonalities among resistant enteric bacteria in Tanzania that affect humans, animals (such as livestock and wildlife), and the environment (such as water supplies). These same patterns have been discovered in *Salmonella* isolates in Uganda derived from animal and human sources (Katakweba et al., 2015).

Due to antimicrobial resistance at the farms, there is a chance that procedures at commercial poultry farms in South Africa could contaminate carcasses, which could then contaminate processed chicks. National action plans were created in response to the growing threat of antimicrobial resistance (AMR). Among their five strategic goals are reporting local, regional, and national resistance patterns and optimizing empirical and targeted antibiotic choices through enhanced surveillance and early detection of AMR (Mokgophi et al., 2021). Few reports of bacterial pathogens, such as the frequency and molecular characterisation of *Staphylococcus aureus* isolates and the isolation of *Salmonella*, have been made on chickens from the outlets of the informal markets in Gauteng Province. The antibiotic resistance profiles of *Salmonella* in hens processed at both commercial and informal poultry farms in South Africa, however, have not yet been published (Mokgophi et al., 2021).

But there's no denying that antibiotics, especially those used on humans as well as poultry, need to be protected. This is in spite of the fact that probiotics, also known as vital bacteria, appear to be gaining popularity (Wang et al., 2017). Because of its excellent nutritional value, low-fat level, and unique flavour, poultry meat is a highly

sought-after food commodity not only in Limpopo but across the globe (Wang et al., 2017).

AMR is the "quintessential One Health challenge" of our day, according to Robinson et al. (2016), who claim that to effectively manage AMR in wildlife, humans, and the ecosystem partnership between public and private sectors, disciplines, and scales (locally, globally) is necessary. This is due to the connection between the use of antimicrobial agents in public health and agriculture. Low- and middle-income countries (LMICs) have a higher risk of contracting AMR due to the disproportionately high loads of infectious diseases, as well as lifestyle choices and living conditions that promote frequent contact between humans and animals. Similarly, genetic research conducted in LMICs demonstrates that AMR is transmitted through people, animals, and the environment (Katakweba et al., 2015).

### **1.1. PROBLEM STATEMENT**

In commercial poultry farms, unquantified and multidrug usage of antibiotic agents is generally provided for treatment, disease prevention (metaphylaxis), and growth promotion to the whole flock (Poole and Sheffield, 2013; Sikder et al., 2019). Antimicrobial agents are more frequently utilized for therapeutic purposes on large-scale commercial broiler farms without veterinarian supervision. The use of such products could be more harmful to human health, particularly if they cause resistant bacteria to grow in animals like chickens and spread zoonotically with their genes responsible for resistance, to people through the environment or the food chain, thereby limiting treatment options in humans (Van Vuuren, 2001). Therefore, it is imperative to safeguard the antibiotics used in commercial poultry farms in Limpopo, as there seems to be a dearth of information regarding bacterial related zoonotic illnesses and the use of antimicrobial agents in chicken farms (Maduane et al., 2018). One Health approach has therefore been proposed to address the situation by WHO in 2018.

There's mounting evidence that the paucity of available antimicrobial substances and the development of novel antimicrobial agents make antimicrobial-resistant infections more expensive and difficult to treat (The European Consumer Organisation, 2013). Antimicrobial resistance is another epidemic that goes unnoticed, according to the

United Nations Inter-Agency Coordinating Group (IACG) study (United Nations Report, 2019).

Policies pertaining to antibiotics that limit the unwarranted or improper use of antibiotic agents are important since poor antibiotic consumption of animals raised for food exacerbates antibiotic resistance which is a problem (Tang et al., 2015; Hoelzer et al., 2017). Unfortunately, such policies, to a large extent, are hardly implemented because data and information regarding the extent of antimicrobial agents used in agriculture are lacking (United Nations Report, 2019).

Concerns regarding antibiotic-resistant organisms, which are ubiquitous worldwide and responsible for approximately 75% of newly emerging infectious diseases impacting humans, are raised by zoonotic diseases in poultry (Petrovska et al., 2016).

### **Study purpose**

The investigation was conducted to close the gap in the use of antibiotics in both animals, chickens in particular, and humans that lead to antibiotic resistance and to preserve the available antibiotics. At the poultry farms that are included in the study, there appeared to be the usage of antibiotics for growth promotion for commercial purposes rather than treatment.

## CHAPTER 2

### LITERATURE REVIEW

#### **2.1. Poultry farming and antibiotic use**

The use of antimicrobial agents in food animals has gained significant global attention, particularly regarding the class of medications that are critical to human medicine (Ma et al., 2021). Additionally, overuse and misuse of these antimicrobial medicines raise selection pressure, which helps to spread resistant genes throughout the commensal and pathogenic microbiota and promotes the establishment of resistant bacteria colonizing people. Antibiotics are classified by Hoelzer et al. (2017) as medications that are primarily utilized in a variety of contexts, such as veterinary clinics, farms, and feedlots, in addition to hospitals, long-term care institutions, and outpatient clinics. Despite being necessary for treating illnesses in plants, animals (aquatic and terrestrial), and humans, antimicrobial medicines are progressively losing their efficacy. Thus, antimicrobial resistance develops and spreads more quickly when people, animals, and plants misuse or overuse the current antimicrobial agents (United Nations Report, 2019).

The disagreement could impede the implementation of rules meant to ensure the wise and responsible use of antibiotics in animal agriculture. It also detracts from the discussion surrounding the responsible use of antibiotics in animal agriculture. Notably, the idea of prudent, responsible antibiotic usage in animal husbandry has been rejected by several organizations, including the American and British Veterinary Medical Associations, the World Health Organization for Animals, and the U.S. Food and Drug Administration (Hoelzer et al., 2017). The same author, Hoelzer et al. (2017), concluded that, despite some definitional variations, maximizing antimicrobial resistance and increasing therapeutic efficacy are important goals behind all of these classifications.

Many nations have undertaken initiatives to lower the amount of antibiotics used in chicken production and agriculture. As shown in Figure 2.1, in 1986, Sweden outlawed the use of any antibiotics that encourage growth. Additionally, the use of virginiamycin and avoparcin was prohibited in Denmark in 1998 and 1995, respectively. Namibia was among the first nations in Africa to forbid the widespread use of antibiotics in livestock

(Khan and Rahman, 2022; Medina, Legido-Quigley and Hsu, 2020). Prior to the Food and Drug Administration (FDA) outlawing such applications at the beginning of 2017, in order to promote growth in livestock, several antimicrobial agents were given to them in the United States (Khan and Rahman, 2022; Wallinga et al., 2022).

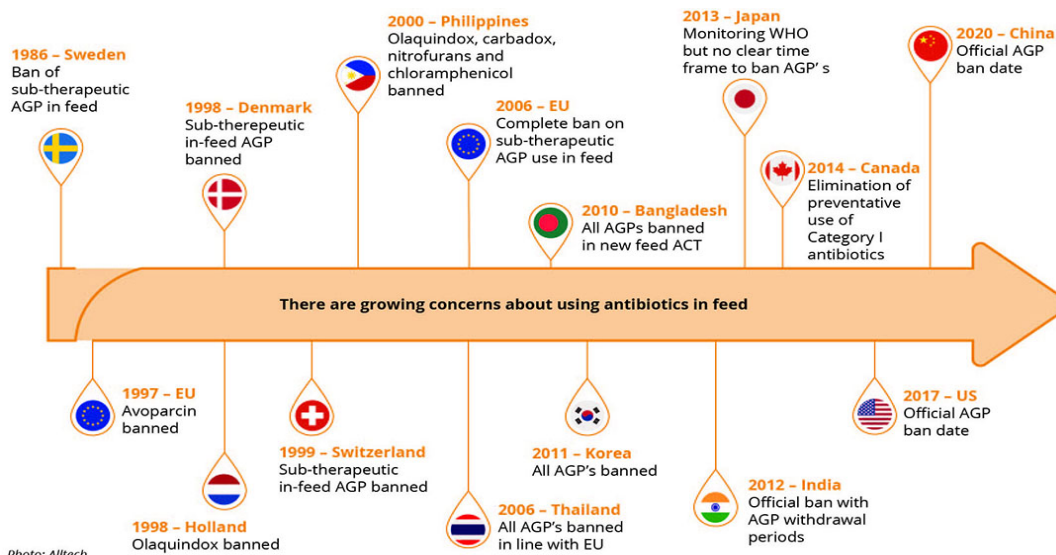


Figure 2.1: Countries that banned the use of routine antibiotics in animals (Walker, 2022)

However, it took nearly another 20 years for governments to take any action, and responses to the issue vary greatly throughout nations. In 1986, Sweden banned the use of any antimicrobial medication for the purpose of promoting growth, and in 1995, Denmark outlawed the use of avoparcin and virginiamycin, two medically significant antimicrobials, for the same purpose. The four antibiotics that were still considered medically significant by the World Health Organization namely bacitracin, spiramycin, tylosin, and virginiamycin were prohibited from being used for growth promotion in 1999. In 1997, avoparcin was outlawed in the EU for issues related to growth promotion (Marshall and Levy, 2011).

Since 2006, the use of any antimicrobial medication for promoting growth including those from classes of antimicrobial agents not used in human medicine has been prohibited in Europe due to the precautionary principle. In the U.S., the use of medically important antimicrobial drugs (as defined by the U.S. Food and Drug Administration) for growth promotion was phased out on January 1, 2017. In reality, almost 70% of member nations that participated in the survey did not approve antimicrobial medications for use

in growth promotion, according to data gathered by the OIE for 2015 (Moulin et al., 2016). However, preventing the growth promotion of medically significant antimicrobial medications is merely the first step in ensuring their responsible use in animal husbandry.

Marshall and Levy (2011) state that the U.S. Food and Drug Administration recently announced plans to look into possible measures to that effect. Several European nations have taken proactive measures to limit the emergence and spread of antimicrobial resistance by ensuring that antimicrobial drugs are used sparingly and only when necessary to ensure the health and well-being of the animal.

Antimicrobial agents are unfortunately inefficient in the clinical therapy of illnesses because their overuse contributes to the emergence and spread of antimicrobial-resistant bacteria. Furthermore, employing horizontal gene transfer processes, resistant determinants can spread to additional diseases, strengthening resistance in environmental pathogens. Areas used for chicken production are a major source of AMR genes, according to current trends in antibiotic resistance (Agyare et al., 2018). It's concerning that colistin, a medication used as a last resort to treat serious bacterial infections in humans is no longer effective in treating illnesses affecting poultry (Khan and Rahman, 2022; Islam et al., 2020). *Salmonella* discovered on the surfaces of chicken eggs in Bangladesh, according to Khan and Rahman (2022), showed 100% resistance to amoxicillin and ampicillin, then tetracycline, ciprofloxacin, and colistin (Mahmud et al., 2016).

Roth et al. (2019) state that the usage of antibiotics is thought to be the most unique and significant factor contributing to the emergence of resistant organisms. Critics in the United States continue to dispute the connection between antibiotic usage on farms and antimicrobial-resistant diseases in humans, despite decades of research and a substantial body of scientific evidence to support their claims.

i. Development of guidelines i.e. smart regulations

The following are the guidelines/ smart regulations to concentrate on in poultry farming:

a. Stockmanship and training,

- b. Biosecurity: internal environment,
- c. Chicken environment,
- d. Hygiene houses,
- e. Nutrition,
- f. Antibiotic stewardship principles (refine, reduce and replace antibiotic usage), antibiotic medication- responsible use, and
- g. Growth promotion.

Concerns over the utilization of antimicrobial agents in poultry growth promotion have been expressed by several, including the United Nations Interagency Coordination Group (United Nations Report, 2019). The following are important management techniques that, according to the International Chicken Council (IPC), Horel (2019), assist in preventing infections in flocks, maintain the health and well-being of birds, and lessen the need for antibiotics across the entire poultry production process:

- ii. Stockmanship and training. The three components of sound stockmanship are as follows:
  - a. Farm workers should be well-versed in both animal husbandry and animal biology, including illness prevention and the optimal ways to meet the demands of the animals.
  - b. Farm workers with a background in animal husbandry should be able to demonstrate their abilities in problem-solving and observation as well as managing, caring for, and treating animals.
  - c. Along with patience and determination, farm workers need to possess other personal attributes including empathy and sympathy for animals.

All of these are vital to guarantee that all employees working on farms and hatcheries possess the knowledge, abilities, and training needed to effectively address the requirements of the birds at every stage of the production cycle and actively promote sensible management techniques. By learning, assessing, and implementing new husbandry procedures, staff and management can reduce the need for antibiotics while maintaining the flock's health and well-being.

iii. Biosecurity. External environment:

- a. The only people who should be allowed access to the live bird areas are farm employees, necessary guests, and qualified experts.
- b. Litter should be kept covered to keep rodents and wild birds from contaminating it.
- c. Make sure that any feed spills are cleared up right once to prevent rodents and wild birds from entering.
- d. To stop the transmission of disease, farms shouldn't share farm equipment.
- e. A good distance away from human habitation and activities.
- f. Preferably higher land to avoid water logging.

iv. Biosecurity. Internal environment:

- a. Farms ought to implement a comprehensive biosecurity policy to mitigate the potential for introducing pathogens into the chicken house and minimize the likelihood of disease transmission between houses.
- b. It's advised that a double barrier system be put in place at the poultry house's entrance to lower the possibility of infections being brought in by shoes or other fomites.
- c. The devoted farm apparel, in other words, personal protective equipment, ought to be worn constantly and kept on the farm.
- d. Every hatchery needs access to clean water for hand washing or hand sanitizing facilities.
- e. Following each production cycle, all equipment needs to be cleaned and disinfected.

v. Bird environment. By giving birds the best possible habitat throughout the whole production chain, the disease problems can be lessened and the resulting need to treat the flock:

- a. The first step in reducing the requirement for antimicrobial agents' use is to secure viable, high-quality chicks from flocks of healthy parents. There should be no health hazards, such as *Salmonella* or mycoplasma, in the parent flocks.
- b. In order to prevent diseases from spreading across flocks, the houses should be cleaned and disinfected before the chicks arrive at the farm. Additionally, any litter

- should be cleansed before being used again, and the interior of each house should be ready for the arrival of the new chick.
- c. For chickens housed indoors, optimal environmental conditions require efficient ventilation of the housing. To achieve the best outcomes for the health and welfare of birds, avoid accumulating carbon dioxide and ammonia levels.
  - d. Every flock, house, and farm should have records that include details on the flock's history, mortality, daily culls, feed and water intake, and medications given.
- vi. Hygiene. Houses and hatcheries:
- a. To prevent water-borne illnesses, fresh, clean drinking water must be supplied to birds.
  - b. Sanitizing drinking water systems following each flock is necessary to avoid biofilm accumulation.
  - c. Maintaining strict cleanliness standards in the hatchery is essential to minimizing the risk of bacteria and viruses affecting the eggs and chicks.
  - d. After each batch, the hatchery's surfaces and equipment should be completely cleaned and sanitized.
- vii. Plans for health and well-being that flock in consultation with the veterinary services including the way antibiotics are to be used which include the following:
- a. To maintain the effectiveness of antimicrobial drugs in humans and animals in the event of disease transmission, they should not be used frequently or without a thorough evaluation of disease risk by the veterinarian.
  - b. To address the flock's health and wellbeing, use the drug at the ideal dosage and for the recommended amount of time.
  - c. It is not advisable to employ antimicrobial agents in a program designed to manage *Salmonella* in chicken flocks.
  - d. The farmer, veterinarian, and senior manager in charge of integrated companies should keep a record of all medications used and review them on a regular basis to determine the rationale behind their use and to discuss potential modifications to management practices that could lower the demand for antibiotics.

- e. To protect their use in human medicine, the usage of any WHO Highest Priority Critically Important Antimicrobials (HPCIA) in chicken production is strongly prohibited and should be avoided.
- f. No antimicrobial agent listed as Critically important, highly important, or Important for Human Medicine by the WHO should ever be used to promote growth. In the absence of risk analysis, all other antimicrobial drugs used as growth promoters ought to be gradually phased out.

viii. Possible solutions are:

- a. Treatment outcomes with antimicrobial medications should be objectively assessed, documented, and reviewed on a regular basis.
- b. An effective technique for analysing the types and quantities of antimicrobial agents used is the on-farm gathering of consumption data.
- c. Farm-specific active component quantities (mg/kg) for each approved product type should be placed into an easy-to-use database.
- d. Reviewing the justifications for the usage of antimicrobial agents should be done using the data. Comparative data sharing can assist in guiding optimal practices.
- e. It is recommended that national industry groups work with the national veterinary services to gather, validate, and disseminate real data about the use of antimicrobial agents as well as data analysis about the number of birds killed (Horel, 2019).

ix. Preparation before the chicks' arrival:

- a. Apply disinfectants to the litter and the entire henhouse.
- b. Trash should be removed, and household appliances should be cleaned.
- c. Apply certain disinfectants with a spray.
- d. Clean water pipelines.
- e. Using the appropriate chemicals to fumigate a chicken coop (Chatterjee and Rajkumar, 2015).

**2.1.1. Antibiotics used in livestock, poultry in particular, and in humans**

2.1.1.1. Horel (2017) scientifically defined individual antimicrobial compounds within a class which can be grouped according to usage:

- i. Important for human medicine:
  - a. Antimicrobial agents are substances that are solely utilized by humans; they are not employed in the treatment of animals.
  - b. Antimicrobial agents with shared class usage are substances or groups of substances that are employed in both human and veterinary medicine.
- ii. Not crucial for human health:
  - a. Antimicrobial agents are substances that are exclusively utilized by animals; they are not used in human medicine.

2.1.1.2. In general, the application of antimicrobial agents is described as:

Therapeutic use includes:

- a. Any specific process intended to cure or improve a disease is referred to as disease therapy (curative) usage.
- b. Use of disease control (metaphylaxis): procedures used to lessen the occurrence or spread of disease and
- c. Use of disease prevention (prophylaxis): lowering the risk of an illness if a susceptible population has a high probability of contracting the illness.

### 2.1.2. **Judicious use of antibiotics in poultry**

- 2.1.2.2. Only use antimicrobial drugs in conjunction with a veterinarian or other appropriately competent individual when medically essential to reduce or prevent pain and suffering.
- 2.1.2.3. To maintain their effectiveness, antimicrobial medicines shouldn't be used frequently or without a thorough evaluation of illness risk by the veterinarian.
- 2.1.2.4. To address the flock's health and well-being, use the antimicrobial agents at the ideal dosage and for the recommended amount of time.
- 2.1.2.5. It is not advisable to employ antimicrobial drugs in chicken flock management programs to control *Salmonella*.
- 2.1.2.6. The farmer, veterinarian, and senior managers of integrated organizations should keep a record of all medications used and examine them on a regular basis to understand the rationale behind their usage and consider modifying management practices to decrease the requirement for antimicrobial agents.

2.1.2.7. To protect their use in human medicine, the use of any WHO Highest Priority Critically Important Antimicrobials (HPCIA) in chicken production is strongly prohibited and should be avoided (Horel, 2019).

Changing the microflora in the digestive tract to a better balance of helpful and harmful bacteria will increase nutrient usage to support healthy growth and enhanced performance, which is known as growth promotion/production use. To keep antibiotics as effective as possible, farmers must continuously improve their methods to guarantee that antibiotics are administered sensibly and only when absolutely necessary to treat a health or welfare issue involving birds. This is made evident by the IPC standards (Horel, 2019). Horel (2019) went on to state that no antibiotics on the WHO list of Antimicrobials Critically Important, Highly Important, or Important for Human Medicine should ever be used to treat growth-promoting conditions. Without conducting a risk study, other antibiotics that are utilized as growth promoters ought to be gradually phased out.

In the mid-1950s, farm animals were provided antibacterial medicines for the goal of promoting growth. To encourage growth and egg production, the chicken industry has since widely used diets enriched with tetracycline, chloramphenicol, and procaine penicillin at subtherapeutic levels (Kabir et al., 2004). Natural and synthetic hormones are commonly administered to food-producing animals raised for food in underdeveloped countries, along with antimicrobial agents since hormones play a role in improved development and feed efficiency. For example, some authors have reported that young birds were castrated formerly subcutaneously or as a feed addition using oestradiol, the female sex hormone (Passantino, 2012).

The most common illnesses, including typhoid, mycotoxicosis, *E. coli* infections, coccidiosis, Salmonellosis, enteritis, ascites, Newcastle disease, Marek's disease, hydropericardium syndrome, Gumboro, and growth promotion, can be treated with antimicrobial drugs in nations that produce chicken. These illnesses have a significant impact on the growth and productivity of chickens, but they also significantly increase the financial losses incurred because of the high mortality rate of the flocks (Chapman and Jeffers, 2014).

For many years, the use of antibiotics in commercial chicken companies was considered standard practice and was unrestricted by rules or oversight. Nevertheless, until the development of antibiotic resistance, the detrimental consequences of these growth promotants were not recognized. The advent of a rising number of resistant bacteria is the primary cause of worries over the therapeutic and growth promotion usage of antibiotics (Marshall and Levy, 2011).

Despite being against the law, a large number of broiler chickens and layers (egg-laying hens) receive overdoses or improper dosages of antimicrobial agents for medicinal, preventative, and non-therapeutic uses throughout their lives. In the event that these medications are not absorbed or are broken down by the animal, there won't be any issues for the items. Sadly, things don't always work out like that. As a result, toxic medication residues often build up in different amounts in the animal parts that humans eat. The majority of these residues are made up of parent and derivative compounds, such as conjugates, metabolites, and residues that are either partially or completely attached to macromolecules (Alm-EI-Dein and Elhearon, 2010).

### 2.1.3. Antibiotic Stewardship Principles

The key principles of stewardship on antibiotic use are:

- a. **Refine:** The List of Antimicrobials of Veterinary Importance, Organization for Animal Health (OIE), and its recommendations are taken into consideration when choosing the most effective antibiotic for use. Resistance development that could pose the greatest threats to global public health should be minimized.
- b. **Reduce:** Lower the number of birds receiving treatment by using a production system that effectively monitors each flock (breeder, grower, indoor, free range, organic). In order to help farms utilizing higher doses of antibiotics improve their flock management systems and lessen the need for antibiotics, monitoring should be implemented to determine the causes for use and an Action Plan should be followed.
- c. **Replace:** analyzing the use of antibiotics and how management strategies that successfully prevent sickness can take their place. These methods can lessen the need for antibiotics and include immunization, immunological balancing for overall

health, non-antibiotic additions for gut health, enhanced biosecurity, alterations to husbandry, stress management, and coccidiosis control (Horel, 2019).

#### **2.1.4. The driving forces on the usage of antibiotics in poultry**

In broiler hens, heat stress impairs immunity and raises the possibility of bacterial infection. Additionally, it causes symptoms like decreased appetite and diarrhoea that, especially in less experienced farmers like many of those who are transitioning to chicken farming, might be mistakenly classified as illnesses that respond to drugs (Cole and Desphande, 2019).

#### **2.1.5. Types of chicken feeds additives**

Feed additives are added to feed to enhance its quality, colour, consistency, and shelf life by preventing mould growth, oxidation, mustiness, and additional physical, chemical, and microbiological deterioration; to increase feed efficiency through improved nutrient digestion, absorption, and utilization; and to lower mortality and morbidity from various illnesses and stressors (Prabakaran, 2003). According to their characteristics and purposes, additives and non-nutrient feed additives are categorized as follows:

- i. Feed supplements with antimicrobial compounds are used to reduce subclinical bacterial infections and improve overall health. Tetracycline, tiamutin, erythromycin, flavomycin, virginiamycin, bacitracin, lincomycin, tylosin, colistin, and so forth are a few examples. Antibiotics that are not used to cure illnesses in humans or animals ought to be applied. Utilization of antimicrobial agents in feed has now decreased as a result of the development of probiotics containing beneficial bacteria.
- ii. Feed supplements with non-antimicrobial agents: prevent bacterial infections and enhance performance. Furazolidone and chlorhydroxy-quinoline are two examples.
- iii. Antimycotic agents: stop the growth of mould and the generation of poisons. Examples include sodium benzoate, propionic acid, calcium propionate, gentian violet, and copper sulphate.
- iv. Coccidiostats: stop coccidiosis outbreaks. Maduramycin, salinomycin, robenichne, nicarbazine, monensin, and dinitro-ortho-toluamide are a few examples.

- v. Check for different parasite infestations using anti-parasitic chemicals. Examples include niclosamide, praziquantel, and dichlorophan (Prabakaran, 2003).

Roth et al. (2019) reported that France has been tracking the sales of active antimicrobial agents used in chicken farming since 1999. For example, it is reported that 106 metric tons of active antimicrobial agents accounting for 20% of all veterinary antimicrobial agents were marketed for use in poultry in 2016. According to the French Agency for Veterinary Medicinal Products and the French Agency for Food Environmental and Occupational Health & Safety, 47 mg of active ingredients were consumed annually on average per kilogram of chicken produced (Roth et al., 2019).

#### 2.1.6. **Microorganisms in poultry**

In certain instances, antibiotics are used to treat intestinal infections in hens, including colibacillosis, necrotic enteritis, and other illnesses commonly caused by *Salmonella*, *E. coli*, or *Clostridium spp.* Poultry farmers in the United States, at least, are extremely concerned about these infections because they can cause substantial monetary losses (United States Department of Agriculture, 2015). It's highly possible that things in South Africa may be worse.

A greater selection pressure for microorganisms resistant to antibiotics is brought about by the utilization of antimicrobial agents in chicken production, claim Diarra and Malouin (2014). Furthermore, according to Roth et al. (2019), because *E. coli* is a highly available commensal bacterium that is present in all animals and humans, monitoring and comparing the impacts of selective pressure across all pertinent groups is made possible by commensal microorganisms. When tracking newly developing resistance in cattle and potential transmission to foods produced from animals, including chicken meat, it is thought to be helpful as an early warning system.

The antibiotic usage (AU) and AMR data on *E. coli* in broiler chickens are displayed for the leading global producers of broiler chicken meat, which include the US, Brazil, China, the EU, Poland, the UK, Germany, France, and Spain (Roth et al., 2019).

Among the most significant zoonotic illnesses salmonellosis is one of the main foodborne illnesses that occurs worldwide (Pławińska-Czarnak et al., 2021; Petrovska et al., 2016). *Salmonella* is a rod-shaped, facultatively anaerobic, gram-negative, mobile, harmful bacteria that is most well-known for its capacity to induce a variety of illnesses in both humans and animals, typhoid fever, septicaemia, salmonellosis, and chicken typhoid (Khan and Rahman, 2022). According to Khan and Rahman (2022), people can contract *Salmonella* from animals by a faecal oral method, which involves direct interaction with sick animals, as well as through the ingestion of food or water contaminated with animal waste.

*Salmonella* can be transmitted to people via the faecal oral route, animal feed, and poultry processing facilities, infected hatcheries, and continuously contaminated livestock settings. It can also enter the food chain by vertical transmission. Salmonellosis in people can appear 12–72 hours after eating food tainted with the *Salmonella* bacteria. According to Scallan et al. (2011), and WHO (2015), out of all the diarrheal disease agents, *Salmonella spp.* is the foodborne infection that, up to 2015, was responsible for 59,000 deaths out of 420,000 casualties in the United States, due to foodborne hazards. More than 2,600 *Salmonella enterica* serotypes have been found recently, with *Salmonella Typhimurium* being the second most often isolated serotype globally after Enteritidis (Hendriksen et al., 2011). Hardly any serotyping of this magnitude to understand the prevalence and development of antibiotic resistance occurs in developing countries according to IPC.

Khan and Rahman (2022) assert that antibiotics are usually recommended for the medical intervention of salmonellosis. MDR *Salmonella spp.* unfortunately arise and spread as a result of antibiotic misuse. Management of infections brought on by MDR strains will become increasingly challenging because of drug inefficiency. As a potential substitute for antibiotics in the fight against AMR, scientists are now giving phage treatment more consideration. While the commercial application of phages for *Salmonella* control in chicken is still in its early stages, lytic phages are regarded as an affordable, ecologically benign, and sustainable microbiological approach. Additionally, it has benefits over antimicrobial agents in terms of genetic amenability, development expenses, resistance, and specificity (Khan and Rahman, 2022).

More than 95% of human illnesses caused by *Campylobacter jejuni* or *C. coli* are caused by this bacterium, which is found in poultry and is a primary factor in sickness in humans worldwide. Cross-contamination of food items or ingestion of tainted chicken is linked to the majority of human diseases. Primarily, ciprofloxacin and erythromycin are the recommended main drugs for treating human campylobacteriosis (Lutgen et al., 2009). Furthermore, Lutgen et al. (2009); Jacobs-Reitsma (2000), deduced that *Campylobacter* is a commensal bacterium that can contaminate processed products and carcasses when it colonizes chicken and enters the processing facility.

#### 2.1.7. Antibiotic resistance and gene strains in poultry and humans

The 2019 antimicrobial resistance threats in the United States report from the Centres for Disease Control and Prevention (CDC) notes that generally, antimicrobial-resistant bacteria caused more than 2.8 million illnesses and over 35,000 deaths annually from 2012 through 2017. The Infectious Diseases Society of America (IDSA) recognized that the lengthy processes required to create updated or new clinical practice recommendations that are predicated on thorough literature reviews and adhere to strict Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria limited the organization's capacity to address quickly changing topics like AMR (Tamma et al., 2022). Jacoby (2009) outlined that numerous gram-negative species, such as *E. cloacae*, *Klebsiella* (previously *Enterobacter*) *aerogenes*, *C. freundii*, *S. marcescens*, *Providencia stuartii*, *P. aeruginosa*, *Hafnia alvei*, and *Morganella morganii*, have been found to possess chromosomally encoded AmpC genes. According to Tamma et al. (2019), AmpC  $\beta$ -lactamases belong to class C enzymes in the Ambler classification scheme because they have serine residues for catalysis at their active site. The mechanisms of AmpC  $\beta$ -lactamase resistance are divided into 3 categories: (1) inducible resistance via chromosomally encoded AmpC genes (e.g. *Enterobacter cloacae*, *Serratia marcescens*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, etc.), (2) non-inducible chromosomal resistance due to promoter and/or attenuator mutations (e.g. *E. coli*, *Shigella species*, *Acinetobacter baumannii*), (3) or plasmid-mediated resistance (e.g. *Klebsiella pneumoniae*, *E. coli*, *Salmonella species*, etc.). Even infections produced by originally susceptible isolates can develop considerable AmpC synthesis and  $\beta$ -lactam resistance as a result of exposure to  $\beta$ -lactams. Treatment

considerations are complicated by species and  $\beta$ -lactam variations in the possibility of generating AmpC formation (Jacoby, 2009).

Worldwide, antimicrobial resistance (AR) poses a severe risk to food security, animal welfare, and human health (United Nations report, 2019). The overuse or misuse of antimicrobial agents in humans, animals, and plants, along with their uncontrolled release into the environment, has led to a problem known as antimicrobial resistance, a natural phenomenon in bacteria. This problem now poses a threat to the advancements made in contemporary human and veterinary medicine. This requires multi-sectorial cooperation under a One Health approach (WHO, 2018).

When bacteria undergo modifications that lessen or completely eradicate an antibiotic's potency, antibiotic resistance arises. Antibiotics may no longer be able to treat bacterial infections, and common diseases like *Streptococcal* throat infections may once again become lethal. This makes it among the most difficult public health issues of our day (The European Consumer Organisation, 2013). The European Consumer Organization (2013) states that consumer organizations have been fighting antibiotic resistance from the patient side alone for some years, and it is only recently that they have chosen to confront the issue from the perspective of food safety. Furthermore, it was discovered that antibiotic-resistant bacteria are widely present in meat products following a battery of testing.

In addition to other commensal flora bacteria, *E. coli* can serve as repositories for AR genes that can be passed across different bacterial species, including ones that can infect people and animals. Additionally, People can get infected with *E. coli* through the land-based transmission of animal faeces or by direct interactions between humans and animals through the food supply chain (Marshall and Levy, 2011). Compared to food-borne pathogens, this indicator bacteria allows for a more accurate investigation of the impact of antimicrobial agents used and the patterns in the incidence of AR in animals raised for food (Roth et al., 2019; Deak et al., 2016).

*Escherichia coli* isolates may be non-pathogenic commensals of pathogens that affect humans or animals. Depending on the presence of particular virulence factors, the

pathogenic ones are categorized as enterotoxigenic, enteropathogenic, entero-invasive, or enterohaemorrhagic. Certain isolates, such as *E. coli* shiga-like toxin producers, are zoonotic and can result in severe intestinal disorders, including haemorrhagic colitis, haemolytic uraemic syndrome, and diarrhoea (Guerra et al., 2003). *E. coli* from livestock is subject to strong selection pressure as, in certain nations, animals raised for food production use more than half of the antimicrobial drugs used in livestock. As a result, resistance is rising, and several resistance factors have been identified (Guerra et al., 2003).

It was discovered that the Class 1 integrons encoding gene in chickens was resistant to antibiotics (Asgharpour et al., 2018). Genes that code for antibiotic resistance can be expressed via integrons (Kheiri and Akhtari, 2016). On transposons, integrons are found that have inserted gene cassettes. Integrons are defined as having gene cassettes encoding resistance to antibiotics (Domingues et al., 2012; Permatasari et al., 2020). Class 1 integrons are generally found in high frequency in gram-negative bacteria, such as *Citrobacter freundii*. It has been discovered that Class 1 integrons are pathogens and cause serious bacterial illness in cattle (Hidayatullah et al., 2020).

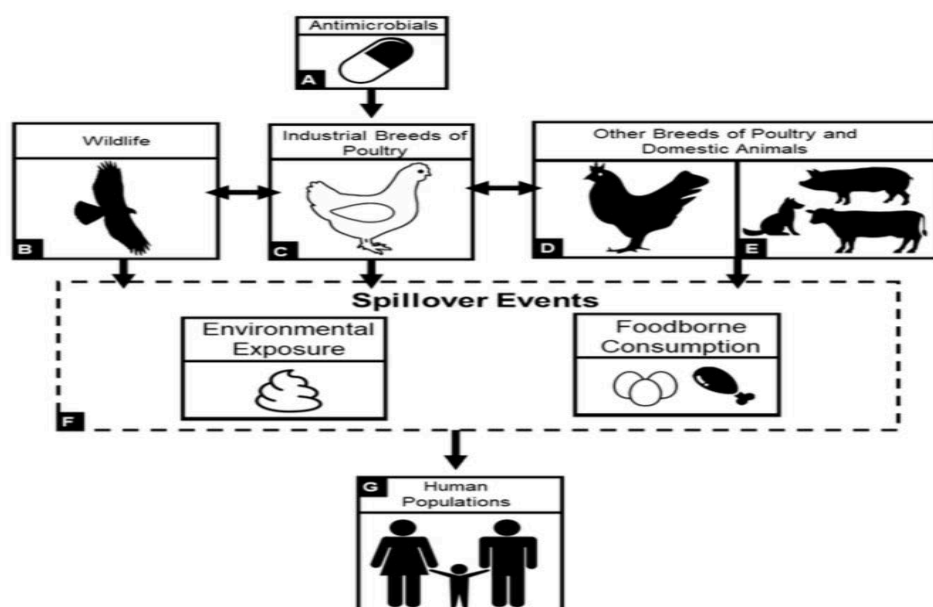


Figure 2.2: Antibiotic resistance associated with intensive poultry production (Hedman et al., 2020)

According to Projahn et al (2018), *Citrobacter freundii* is a common *Enterobacteriaceae* strain discovered in poultry. In food, soil, water, and the intestines of both humans and animals, *Citrobacter freundii* is a kind of facultative anaerobic bacteria that is motile via flagella and gram-negative (Liu et al., 2018). *Citrobacter freundii* exists in gastrointestinal and widespread animal food-borne illnesses (Bai et al., 2012). Treatment with numerous medications led to drug resistance in bacterial infections, and antibiotics impeded the growth of microorganisms. As such, proof of the MDR's proliferation in chicken farms must be provided via a molecular detection method. Application of the MDR-coding Class 1 integron gene *Citrobacter freundii* in poultry detection is a new step in the correct direction (Prota et al., 2015). Pathogen and foodborne infections in broiler farms were caused by *Citrobacter freundii* contamination, according to (Aminharati et al., 2019).

*Citrobacter freundii* was found in broiler chickens, based on a study carried out by Witaningrum et al. (2021). *Citrobacter freundii* with multidrug resistance was confirmed in 18 out of 22 cases (81.82%) of the study, and 5 out of 22 cases were positive for the Class 1 Integron encoding gene by PCR testing (22.72%). In addition, the findings showed that, as Figure 2.2 illustrates, multidrug-resistant gram-negative bacteria arise in chicken farms and require management plans to be evaluated in order to stop the spread of these bacteria to humans and the environment (Hedman et al., 2020).

According to Zimmern and Kroese (2007), conventionally, genetic disorders are defined as those that carry a high risk of inheritance to other family members and are inherited in accordance with recognized patterns of heredity. Furthermore, the phrase "genetic test" should be used as a colloquial word to refer to an assay designed to identify:

- (i) a specific genetic variation (or set of variants),
- (ii) for a certain illness,
- (iii) within a specific demographic, and
- (iv) for a specific objective.

There are several reasons to conduct genetic tests. These include screening, diagnosis, prediction, carrier screening, and prenatal testing. Furthermore, studies known as

pharmacogenetics can determine whether a person has a certain genetic variant that may affect how they react to a drug.

According to Mokgophi et al. (2021) antimicrobial resistance develops more quickly when antimicrobial agents are continuously overused or misused in the fields of human and animal health. Mokgophi et al. (2021) noted in the European Centre for Disease Prevention and Control (ECDC) that there have been notable variances among Member States in the decreases observed in the usage of antimicrobial drugs for human health. It is crucial to stop and manage the spread of antibiotic-resistant bacteria among patients in healthcare settings, including long-term care homes and hospitals. Making certain that good infection prevention and control procedures, like cleaning, patient segregation, contact precautions, and hand hygiene, can be difficult, though as shown in Figure 2.3. Thousands of healthcare workers in the EU must strictly implement these procedures, which call for oversight, resources, and training throughout healthcare facilities. Furthermore, according to ECDC data, hospital basic diagnostic tests which are essential for determining the best course of treatment are not being performed as frequently as they should throughout the EU. This also contributes to the overuse of antibiotics with a broad spectrum of activity (European Union report, 2019).

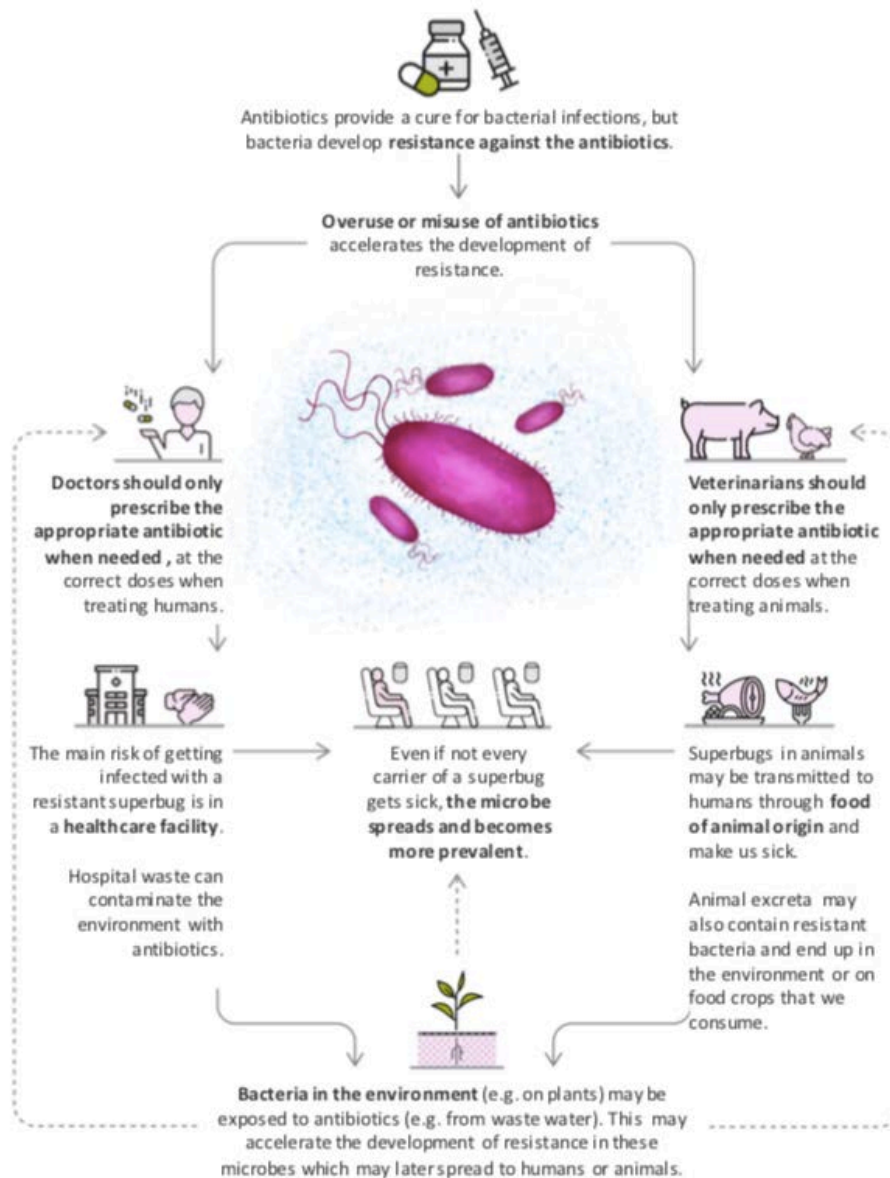


Figure 2.3: How antibiotic resistance spread (European Union report, 2019)

Human campylobacteriosis is usually self-limiting, however, severe cases necessitate antibiotic therapy, such as ciprofloxacin and erythromycin, which are frequently prescribed medications, according to Lutgen et al. (2009). When a microbiological diagnosis is not possible, fluoroquinolones like ciprofloxacin have been employed as the first line of treatment for bacterial gastroenteritis. Nonetheless, Worldwide, there has been a rise in reports of fluoroquinolone-resistant *Campylobacter* infections in people, which may be related to the utilization of fluoroquinolones in food animals (McDermott, 2004). The U.S. Food and Drug Administration withdrew its approval of the fluoroquinolone enrofloxacin for use in poultry in 2005, however as (Nelson et al., 2007;

Lutgen et al., 2009) noted that it is still feasible that *Campylobacter* resistant to fluoroquinolones will continue to exist in poultry flocks. The recommended course of treatment for *Campylobacter* infections has been macrolides like erythromycin; nevertheless, it has been shown that *Campylobacter*, especially *C. coli*, is becoming more resistant to erythromycin. Patients infected with *Campylobacter* infections resistant to macrolides or quinolones have been demonstrated to have longer disease durations, a higher risk of invasive infections, or worse treatment outcomes (Gibreel and Taylor, 2006).

The possibility of antibiotic-resistant bacteria spreading from treated animals into the food chain was brought to light in the Swann (1969) study, but little action was taken until vancomycin-resistant enterococci were found in animals fed avoparcin, a similar glycopeptide. Later, the problem and other instances of resistant bacteria spreading up the food chain like enterococci resistant to quinupristin-dalfopristin or to everninomicin, campylobacters resistant to fluoroquinolones and multi-resistant *Escherichia coli*, and *Salmonella*, like *Salmonella Typhimurium* became more widely recognized (Barton, 2000).

According to Blazejewska et al. (2022), fewer tetracycline prescriptions result in lower rates of resistance detection. Large areas that produce chicken are permitted to use fluoroquinolones, third-generation cephalosporins, macrolides, and polymyxins the WHO's "highest priority critically important" antibiotics used in human medicine except the US and the EU (Roth et al., 2019).

All evaluated countries, including China, the US, Brazil, Germany, France, Spain, and EU member states, have allowed the use of tetracyclines, aminoglycosides, sulphonamides, and penicillin in poultry (2019). Globally, *E. Coli* average resistance rates more than 40% of members of these antibiotic groups, with the US having the exception of ampicillin (Roth et al., 2019). While fluoroquinolone use is permitted in Brazil, China, and the EU, where the average rate of resistant *E. coli* is above 40%, the resistance rates to fluoroquinolones and quinolones are fewer than 5% in the United States, where the utilization of fluoroquinolones is not authorized. The emergence of

resistant populations has not completely disappeared, despite the prohibition of quinolones and fluoroquinolones.

A growing number of human diseases caused by strongly resistant organisms are being treated using medications of the last option, which frequently do not fall into the usual therapeutic regimen. For example, nations with high rates of antimicrobial resistance (AMR) are using a lot of even colistin, an antibiotic that was hardly ever administered to patients a few years ago because of its nephrotoxicity and neurotoxicity, to treat Gram-negative bacterial infections that are resistant to carbapenem (Amin et al., 2020). Furthermore, in the past, the chicken industry employed colistin, and from samples taken as early as the 1980s, colistin-resistant bacteria have been found. It was formerly thought that colistin resistance was mostly caused by mutations in chromosomal genes such *pmrAB*, *phoPQ*, and *mgrB*. However, colistin resistance in human infections has never been documented. According to Amin et al. (2020) since China's initial report on the appearance of *mcr-1*, at least 30 other nations have reported seeing the same gene appear in both human and animal sources. This fast migration of the *mcr-1* plasmid-mediated colistin resistance gene, through horizontal transmission of plasmids that carry resistance genes, has completely changed the paradigm of AMR (Islam et al., 2017).

Among the sources of several species that produce *mcr-1* and have been isolated from various phases of the supply chain and poultry production, poultry is by far the most prevalent (Joshi et al., 2019).

#### **2.1.8. Presence and distribution of drug residues in poultry products**

According to Mund et al. (2017), intensive and conventional backyard poultry farming are common practices in numerous developing countries. Veterinary medications are also easily accessible to farmers and they frequently use higher doses of antimicrobial drugs inappropriately and indiscriminately, which over time accumulates harmful residues in the poultry's edible tissues.

Unintentional or unintended cross-contamination of feed in feed mills, recirculation via litter, and feeding of feed components or water tainted with hazardous chemicals, pesticides, or metals, among other circumstances, are the other scenarios in which

poultry may have hazardous traces in their items made from meat and eggs. Due to low and constant exposure to antimicrobial agents, all of these residues change the microflora and may provide a direct hazard to humans. This could lead to the growth of resistant bacteria and the ineffectiveness of antibiotic treatment (Lawal et al., 2015).

The fact that practically everyone eats eggs raises serious concerns regarding the potential for drug residues to accumulate in different parts of eggs. Antibiotics are absorbed into the chicken's intestines and then travel throughout the body via blood plasma to the ovaries (follicles and oviducts), which are in charge of forming and secreting egg contents. This increases the likelihood that drug residues will accumulate in the albumen and yolk of the egg (Alaboudi, Basha, and Musallam, 2013). Drugs have been shown to deposit more quickly in both the yolk and albumen, as demonstrated by Barbosa et al. (2012). However, the distribution and pattern of residue deposition vary according to the composition, physiochemical characteristics of antimicrobial agents, hen physiology, egg development, and egg compartment type.

Amiri et al. (2014) conducted experiments that show indications of drug residue contamination in varying amounts in poultry eggs. For instance, it was verified that nitrofurans (3-amino-2-oxazolidone), which are used to treat bacterial diseases such as salmonellosis, were deposited in eggs. It was discovered that the eggs contained 268.25 ng kg<sup>-1</sup> of amino-2-oxazolidone metabolite, indicating a high dosage of furazolidone medications. Similarly, Mehtabuddin et al. (2012) revealed that eggs were contaminated with 103–230 ng g<sup>-1</sup> of sulfonamide residues (sulfadiazine) above maximum residue levels. Additionally, it has been determined that a variety of medication residues, such as oxytetracycline, amprolium, furaltadone, furaprol (nitrofurans-amprolium combination), soluvite (vitamins and minerals supplement), tylosin, streptomycin, and sulfaquinoxaline, are deposited within eggs (Kabir et al., 2004). Similarly, dinitrocarbanilide deposits found in egg samples have demonstrated the presence of nicarbazin residues. Furthermore, the transfer of amoxicillin from chickens that lay eggs to the eggs and its accumulation in both the yolk and the egg white have been determined by (Khattab et al., 2010).

Similarly, Alaboudi (2013) reports that even after the drug's dosage was stopped, differences in the residues deposited between egg whites and yolks were observed when gentamycin was administered at varying doses, either subcutaneously or intramuscularly. In contrast to the albumen, these residues accumulated in the yolk at much higher concentrations of 90%. Nevertheless, it has also been projected that egg whites will contain higher than yolk levels of sulfanilamide and chlortetracycline residues (above MRLs). It is therefore clear that drug residues (beyond MRLs) accumulate in different egg compartments and that eating contaminated eggs poses a significant risk to the health of the consumer.

In terms of cost and nutritional value, poultry meat is a superior replacement for mutton and beef. However, excessive medication use and a lack of appropriate biosafety protocols for their withdrawal have contributed to a decline in meat quality (Mehtabuddin et al., 2012). For example, noticeably greater quantities of different antimicrobial agents' residues in the edible tissues of chickens taking prophylactic or therapeutic antibiotics without adhering to specified withdrawal periods (Hind et al., 2014).

It has also been documented that the accumulation of oxytetracycline, enrofloxacin, quinolones, chloramphenicol, and oxytetracycline residues leaves residues in chicken flesh (Er et al., 2013). Sattar et al. (2014) found that the meat of broilers had significantly increased enrofloxacin concentrations (because of their lipophilic nature), while Adewuyi (2011) reported that amoxicillin (26%) was deposited in the muscles of the thighs, in the breast muscles of egg-layers and broilers were ciprofloxacin (30%), tetracycline (24%), amoxicillin (22%), and enrofloxacin (18%). Karmi (2014) further verified that fresh, locally frozen, imported frozen, and tetracycline samples from chickens (breast and thighs) supplied for human consumption contained traces of amino-glycoside, tetracycline, quinolone, and sulphonamide. Furthermore,  $\beta$ -lactam and/or tetracycline (75.81%), macrolide and/or  $\beta$ -lactam (44.35%), sulphonamide (36.29%), and aminoglycoside (13.71%) residues were discovered to be contaminated in poultry meat samples. Similarly, oxytetracycline residual levels can be detected in chicken fillets as a result of widespread antimicrobial agent overuse and noncompliance with withdrawal periods (Hakem et al., 2013).

Using various methods, researchers have looked into the possibility of drug residues in the edible tissues and/or offal of treated animals, such as the heart, liver, kidneys, and gizzard (Aslam et al., 2015). Mund et al. (2017) discovered that broiler chicken liver has higher levels of levamisole residues than other body tissues, such as thigh muscles since the drug is lipid-soluble. Furthermore, oxytetracycline and chloramphenicol residue deposition were found in considerably higher concentrations in the liver and kidney samples from broiler chickens. Similar to this, larger levels of ciprofloxacin and enrofloxacin residues, as well as significantly lower levels of flumequine, have also been found in the livers and kidneys of broiler chickens.

According to Sattar et al. (2014), the livers of boilers and egg layers had the highest concentrations of leftover tetracycline (48%), ciprofloxacin (44%), amoxicillin (42%), and enrofloxacin (40%), while the kidneys had 24, 42, 30, and 34 percent of the corresponding antibiotic substances. Amoxicillin and sulphaquinoxaline residues were found to contaminate chicken giblets, according to Hassan et al. (2014). According to the study, only 10% and 13.33% of the gizzard and liver samples were found to be contaminated with higher concentrations of sulphaquinoxaline residues, out of the 90 heart, liver, and gizzard samples (30 each) that were examined (Mund et al., 2017). Approximately 3.33%, 13.33%, and 10% of the heart, liver, and gizzard samples contained higher levels of amoxicillin residues.

#### **2.1.9. Risks to human health posed by drug residues**

According to Mund et al. (2017), consuming contaminated edible tissues by humans may cause veterinary medicine residues to be incorporated into chicken products. This could lead to several pathological outcomes that are considered to be serious health risks. Specifically, drug residues result in the emergence of antimicrobial resistant bacteria, allergic reactions, or shifts in the useful microflora of the digestive system to harmful or non-healthy microflora, all of which pose serious health hazards to the general public. For example, it has been documented that consuming contaminated poultry products might result in anaphylaxis, dermatitis, cutaneous eruptions, and gastrointestinal problems in humans due to residual  $\beta$ -lactams, such as cephalosporin and penicillin (Mund et al., 2017).

Due to the fact that a significant number of allergic individuals, penicillin residues are thought to be the most dangerous. Additionally, eating chicken containing penicillin residues can result in severe anaphylactic reactions, and eating eggs that have sulphonamide residues in them at greater percentages can cause skin allergies (Babapour et al., 2012).

Remaining tetracycline levels in food meant for human consumption have been linked to gastrointestinal problems, immunopathological, pro-inflammatory, and cytotoxic effects, as well as impaired foetus development and teeth discoloration in young children (Lawal et al., 2015). In a similar vein, Eslayed et al. (2014) clarified that lingering levels of tilmicosin impact biochemical parameters including concentrations of total protein, albumen, cholesterol, and triglycerides as well as haematological measurements, such as red blood cells (RBC) and white blood cells (WBC). Similarly, it is known that human residues of sulphamethazine, oxytetracycline, and furazolidone can have immunopathological effects (such as autoimmunity and carcinogenicity), and that gentamicin and chloramphenicol can be mutagenic, nephropathic, and hepatotoxic, which can result in toxicity to the bone marrow or reproductive abnormalities (Eslayed et al., 2014).

Since nitrofurans metabolites may have deleterious, mutagenic, or carcinogenic consequences when consumed through poultry products, as well as increase antibiotic resistance in human microbiota, the use of it is forbidden to use nitrofurans in poultry (Amiri et al., 2014). Conversely, certain medication residues, like 3-nitrofurans and nitroimidazole, induce various malignancies in people. It is also known that continuous human dosages of ciprofloxacin residues can be hazardous, interfere with cytochrome (CYP1A2)-mediated metabolism, and raise the concentration of the drug in the systemic circulation as a result of decreased renal clearance (Khan et al., 2015). Furthermore, consuming drug residues from chicken products may cause drug-resistant bacteria to grow and proliferate in humans, which may result in treatment failures for those who are ill (Khan et al., 2015).

#### 2.1.10. Threats posed by antimicrobial resistant bacteria spreading among people

The use of antimicrobial compounds in chicken feed has been demonstrated, according to Mund et al. (2017), to prevent pathogenic bacteria that cause disease; however, prolonged, intensive use of these substances at low dosages as growth promoters has resulted in the emergence of strains of antibiotic resistance. For instance, it has been documented that certain strains of *Salmonella* and *Campylobacter* have developed resistance to third-generation cephalosporins and fluoroquinolones, which are applied to treat illnesses in humans. Similar to this, *S. intermedius* strains (221), which were isolated from the canine family, demonstrated varying rates of resistance to amoxicillin/clavulanic acid, ciprofloxacin, clindamycin, gentamicin, cephalotin, and mupirocin. This could make it difficult to treat human diseases effectively (Chrobak et al., 2011). Furthermore, several studies have examined the frequency of microorganisms resistant to antibiotics in broiler chickens raised on farms.

Roughly 525 strains of avian *E. coli* that were isolated from broiler chickens have been reported to be resistant to ciprofloxacin and trimethoprim-sulfamethoxazole, among other antimicrobial treatments (Mund et al., 2017). Furthermore, it was revealed that broiler chickens with colibacillosis had a considerably greater prevalence of *E. coli* resistance to ciprofloxacin and erythromycin compared to the control group (Moniri and Dastehgoli, 2007). The 468 bird *E. coli* strains that were isolated had far greater levels of resistance to fluoroquinolones and trimethoprim-sulfamethoxazole; additionally, it was indicated that these antibiotics might cause cross-resistance in human enteric infections. *S. enterica Indiana* was identified by the isolation of 293 strains obtained from poultry farms and slaughterhouses. *S. enterica Indiana* strains were resistant to ciprofloxacin, enrofloxacin, and norfloxacin. Additionally, gene alterations were reported in these strains (Lu et al., 2015).

In a similar vein, *E. tenella* which was isolated from chicken fillets also showed signs of developing salinomycin resistance (Abbas et al., 2008). The continued and careless use of these antimicrobial medicines, according to Krishnasamy (2015), could be facilitating the development of antimicrobial resistance in human diseases and/or foodborne microorganisms, as well as the decline in the efficacy of antibiotics against diseases in poultry and humans.

*Salmonella* sp., *C. jejuni*, and Verotoxigenic *Escherichia coli* were identified by Koluman and Dikici (2013) as the three main emerging foodborne pathogens affecting humans. According to Luber et al. (2003), antimicrobial resistant *C. jejuni* has contaminated poultry meat found in supermarkets, which can cause campylobacteriosis when consumed. It was found that several antibiotics could not be overcome by strains of *C. jejuni* recovered from samples of chickens, including ampicillin, cephalothin, and sulfamethoxazole/trimethoprim (Siddiqui et al., 2015). Furthermore, Sison (2014) reported that strains of *Campylobacter* (both *C. jejuni* and *C. coli*) found in fresh chicken flesh showed resistance to ampicillin after exposure to ciprofloxacin, tetracycline, erythromycin, and gentamicin. This is a result of antimicrobial agent abuse or misuse leading to the evolution of antimicrobial resistance to several antibiotics.

Correspondingly, greater rates of resistance to tetracycline, sulphonamides, and quinolones were seen in chicken carcass-derived *C. jejuni*, *S. enterica*, serotype *Enteritidis*, *L. monocytogenes*, and *E. coli* (Dan et al., 2015). Multi-drug resistant *E. coli* was found in chickens by Ali et al. (2014); this finding is significant since it can result in major public health problems. Furthermore, it was discovered that air, water, litter/manure, and feed for chickens all had high concentrations of vancomycin, kanamycin, clarithromycin, ampicillin, gentamicin, and erythromycin-resistant non-enterococci and enterococci.

Several strains of *E. coli* that have demonstrated resistance against  $\beta$ -lactamase with an expanded spectrum were recovered from poultry carcasses by Koga et al. (2015). The primary reason for concern regarding these strains is their possible spread to humans through meat intake. Mund et al. (2017) came to the conclusion that it is really concerning when residual concentrations of these antimicrobial compounds are found in poultry products beyond maximum resistance levels (MRLs). In addition, the co-occurrence and evolution of antibiotic resistance in pathogenic, mutualistic microbes, and commensal bacteria poses a hazard to human health. Therefore, stringent laws and regulations should be put in place at both the national and municipal levels to regulate the manufacture, distribution, and use of veterinary medications for therapeutic purposes or as feed additives. Furthermore, the relevant food and drug administration, additional regulatory bodies, and certified veterinary professionals are required to

establish and implement the recommended intervals of time between medicine administration and slaughtering. To protect consumers, there should be routine testing and evaluations for the presence of medication residues in poultry edible tissues.

Table 2.1: The most commonly used antimicrobial agents in animal-food production (Aleida et al., 2014)

<b>Antibiotic</b>	<b>Treatment objectives</b>	<b>Animals treated</b>	<b>Human administration</b>
Lincomycin	Feed effectiveness, stimulant, and prevention	growth and illness Pork and chicken	Used as IV in humans
Tylosin	Feed effectiveness, stimulant, and prevention	growth and illness Poultry and cattle	Not used by humans
Penicillin	Feed effectiveness, stimulant, and prevention	growth and illness Pork and chicken	Used as oral and IV in humans
Virgiamycin	Feed effectiveness, stimulant, and prevention	growth and illness Pork, chicken, and cattle	Not used by humans
Tetracycline	Feed effectiveness, stimulant, and prevention	growth and illness Pork, chicken, and cattle	Oral use in humans
Bacitracin	Growth promotion and disease control	Poultry, and rabbits	Not used by humans
Erythromycin	Disease control	Pork, poultry, cattle and sheep (IV)	Used as tablets

According to Aleida et al. (2014), as shown in Table 2.1, certain antimicrobial drugs are used on both humans and animals. This could show that once there is a resistance development in animals it could possibly lead to resistance in humans since the antimicrobial agents used will be ineffective.

#### **2.1.11. Poultry veterinarians' opinions about the use of antimicrobial agents**

According to the World Organization for Animal Health (2020), veterinarians play an essential role in antibiotic prescription and their use in animals. Understanding of their prescription practices and assistance in developing policies and initiatives targeted at lowering the use of antimicrobial agents may be gained from a deeper comprehension of their attitudes and knowledge regarding antibiotics use and AMR (Padda et al., 2021). The majority of veterinarians reported in the Torres et al. (2022) study that when clinical indicators are found, antimicrobial agents are administered for every animal kept within the barn, regardless of their health status.

#### **2.1.12. Guidelines and policies on antibiotic use in poultry**

Global policy initiatives to guarantee the usage of antimicrobial agents in animal husbandry with caution have typically started with limiting the use of those antibiotics that are crucial for human health as growth promoters (Hoelzer et al., 2017). On substantiation, the Swann Report (1969) from the Joint Committee of the United Kingdom on the application of antibiotics in animal husbandry called for the prohibition of medically important (i.e., shared between humans and animals) antimicrobial agents for growth promotion due to worries about the emergence of antimicrobial resistance. The guideline acts as a legally mandatory framework for the application of antibiotic monitoring in the breeding and fattening of poultry (Nienhoff, 2020). The EU urged and instituted some guidelines to safeguard the efficiency of antibiotics:

- a. Limiting the use of antibiotics for medical purposes, improving reporting and regulation of the use of metaphylaxis, and gradually ceasing prophylactic use.
- b. Limiting the use of oral therapy and making individual treatment the rule rather than the exception. It is important to consider the possibility of outlawing the use of antibiotics in medicated feed.
- c. Enhancing animal health through biosecurity protocols, illness prevention, and sound management techniques in order to decrease the need for medications.
- d. Eliminating veterinarians' dual authority to prescribe and sell antibiotics results in the removal of all financial incentives.
- e. Limiting the use of vitally important antibiotics like macrolides, fluoroquinolones, and new cephalosporins. These antibiotics ought to be prohibited for use in therapeutic groups, metaphylaxis, and species where a high risk of resistance transfer has been

noted. We think their use should be tapered out when there are better options for therapy available. As long as strict regulations are required to guarantee the medication is not used for livestock production, carbapenems should likewise remain illegal in veterinary medicine.

- f. Imposing severe limitations on "off-label use," specifically cascade. To achieve a considerable decrease in the usage of antibiotics, concrete and aggressive reduction targets should be defined concurrently.
- g. Assessing the presence of microorganism resistant bacteria to antibiotics in food products using meat product testing. Policymakers in the EU should undertake this task in addition to consumer organizations.

Relying on reliable statistics to track developments and determine whether the EU is headed in the right direction. All member states should gather data on consumption by species. Gathering information about antibiotic usage on farms turned out to be a useful method for contrasting methods and identifying top performers (The European Consumer Organisation, 2013). All the above is an effort not to waste time in securing the future from antibiotic-resistant infections, which is now a worldwide emergency that could jeopardize a century's worth of health advancements and the accomplishment of sustainable development goals.

According to Al Amin et al. (2020), The National Action Plan (NAP) aims to reduce antibiotic resistance in humans, animals, and environmental sectors is being implemented in Bangladesh using the "One Health" approach, which calls for a concerted effort and a strong commitment from each implementation partner to both ensuring one health and combating AMR in Bangladesh. The Department of Live Stock (DLS) has been tasked by the NAP with carrying out all required tasks in order to accomplish strategic goals for controlling AMR issues in veterinary practices. Even though the DLS is carrying out these tasks, significant policy gaps remain. These consist of precise operational, monitoring, and assessment frameworks; recommendations for the veterinary industry's antimicrobial stewardship; and a well-defined funding structure (Al Amin et al., 2020).

## 2.2. Alternatives on the usage of antibiotics in poultry

Researchers are looking at alternatives to the use of antimicrobial agents due to consumer pressure, concerns regarding the adverse effects of antibiotic use, and the EU's prohibition on antibiotic use (Diarra and Malouin, 2014; Mehdi et al., 2018). These substitutes sought to protect the environment and the health of the public while maintaining a low death rate and a high degree of animal productivity. In an attempt to identify natural chemicals with the same beneficial effects as growth promoters, numerous investigations have been carried out. In fact, there are a lot of non-therapeutic substitutes that chickens can utilize in place of antibiotics. The most commonly employed of these include phytogetic feed additives, phytocides, probiotics, prebiotics, organic acids, enzymes, immune stimulants, bacteriocins, bacteriophages, nanoparticles, and essential oils.

### 2.2.1. Additives in feed that are phytogetic

Mehdi et al. (2018) state that phytogetic feed additives (PFA) are additives used to feed animals that are derived from plants, spices, and herbs. Their favourable impact on growth strengthened defense mechanisms, and lowered stress response have made them extremely effective. According to recent findings, PFA was an effective substitute for antibacterial agents used for growth promotion in broiler chickens. Mehdi et al. (2018) provided an example of how adding 2g/kg of cinnamon to the diet improved the growth performance of the animals at 28 days old (974 g as opposed to 850 g) and 42 days old (2,111g as opposed to 1,931g). Additionally, adding 5g/kg of *Lippia javanica* to broiler feed improved the average daily increase during the grower period (67g vs. 30g), the fatty acid composition of the chicken meat utilized in broiler production, as well as the slaughter weight (2,213g versus 1,967g) (Mpofu et al., 2016). According to Mpofu et al. (2016), glycolysis can be aided by phytogetic extracts derived from *L. javanica* leaf meal which boost energy generation use, and eventually become growth promoters. Black pepper powder (1g/kg) and garlic powder (5g/kg) also exhibited favourable impacts regarding the Index of consumption for broiler chickens and weight gain (Kirubakaran et al., 2016). Certain herbs, particularly *Cratoxylum formosum*, have been shown in a study one by Jarriyawattanachaikul et al. (2016) to exhibit antibacterial action against *E. coli*, *C. jejuni*, and *S. aureus* that have been isolated from chicken caecum. When it came to controlling inoculated *Clostridium*, *Salmonella*, and *E. coli* in broiler chicken, after 28

days, PFA containing extracts from the following plants proved to be just as effective as bacitracin methylene disalicylate: clove (*Syzygium aromaticum* L.), anise (*Pimpinella anisum* L.), peppermint (*Mentha arvensis* L.), fennel (*Foeniculum vulgare* var. *dulcemil*), oak (*Quercus cortex*), thyme (*Thymus vulgaris* L.), and Melissa balm (*Melissa officinalis* L.) (Wati et al., 2015).

### 2.2.2. Essential oils

A liquid that is hydrophobic and that contains volatile aromatic and odoriferous chemicals from a plant is called an essential oil (Mehdi et al., 2018). Essential oils come in two varieties: synthetic and organic (of vegetable origin). Few essential oils have any real antibacterial properties. Thymol, trans-cinnamaldehyde, carvacrol, and eugenol are the most popular. Their mechanisms of action involve interfering with the bacterial enzymatic system, as well as modulating immunological responses and inflammation. Numerous experiments have proved the benefits of essential oils (Peng et al., 2016; Mehdi et al., 2018) to be viable substitutes for growth-promoting antimicrobial agents such as avilamycin in enhancing chicken production. Additionally, essential oils have the potential to both prevent and treat necrotic enteritis disease in broiler chickens (Jerzsele et al., 2012). The usage of essential oils improves chicken health, growth, and the quality of the meat and carcass. According to Peng et al. (2016), broiler chicken feed containing 300 and 600 mg/kg of oregano essential oil (*Origanum* genus) boosted Average Daily Gain (ADG). The authors hypothesize that this result could be related to the broiler chickens' jejunum's increased villus height and decreased crypt depth. Moreover, the percentage of thigh muscle growth of broiler chickens and their percentage of belly fat dropped when 600 mg/kg of oregano essential oil was added to their diet. However, peppermint (*Mentha piperita*) worked wonders as a virginiamycin alternative in broiler chicks (Khodambashi Emami et al., 2012).

### 2.2.3. Probiotics

According to the definition of probiotics, "live microorganisms confer a health benefit to the host when administered in sufficient concentrations" (Acar and Rostel, 2001). Supplementing feed with probiotics enhances growth in poultry, feed effectiveness, and gut health (Ghasemi et al., 2014; Giannenas et al., 2014; Samli et al., 2007). This improvement is attributed to a reduction in intestinal pH, the composition of intestinal

bacteria, and digestive activity (Mehdi et al., 2018). Probiotics work by stimulating endogenous enzymes, decreasing metabolic reactions that result in the formation of harmful compounds, and producing vitamins or antibacterial molecules (Hassanein and Soliman, 2010). Bacteriocins, which prevent the formation of toxins and the attachment of pathogens, are produced by probiotic bacteria (Pan and Yu, 2014). Probiotics, on the other hand, boost the immune system and promote resistance to bacterial colonization (Hassanein and Soliman, 2010).

Administration of *Enterococcus faecium* in chicken feed had an antibacterial effect on bacterial microflora in the small intestine (Levkut et al., 2012). Similar results were reported with *Streptomyces* sp. (Latha et al., 2016) and *Bacillus subtilis* (Zhang et al., 2013). In a study (Zhang et al., 2013), comparing *B. subtilis* with enramycin, widely used as a feed additive for chickens to prevent necrotic enteritis, administration of 105 cfu of *B. subtilis* UBT-MO2/kg in broiler feed increased body weight by 4.4% and relative weight of the thymus. Additionally, the treatment decreased the concentrations of NH<sub>3</sub> and H<sub>2</sub>S in chicken excretions, which lowered smell emissions. The quality of poultry meat is enhanced by probiotics (Popova, 2017; Mehdi et al., 2018). They improve pH, colour, fatty acid profile, chemical composition, water retention capacity, and oxidation stability (Popova, 2017).

The quality of meat is impacted by the probiotics' effects on the protein and fat content of meat. According to Abdurrahman et al. (2016), one of the primary reasons feed quality deteriorates is lipid oxidation. Additional research demonstrating that the addition of *Aspergillus awamori* and *Saccharomyces cerevisiae* to chicken feed decreased blood saturated fatty acids and increased polyunsaturated fatty acids supports this idea (Saleh et al., 2013). According to a related study done by Liu et al. (2012), treatment with *Bacillus licheniformis* considerably enhanced the amount of protein and essential and aromatic amino acids present in the treated samples. *B. licheniformis*-containing feed enhances the colour, juiciness, and flavour of the broiler chickens' meat (Liu et al., 2012). Regarding customer enjoyment, these elements are crucial, particularly the colour. Probiotics might also act as an anticoccidial agent.

According to Giannenas et al. (2014), probiotic medicine could lessen the consequences of parasite infection in chickens when anticoccidial infections are absent. The use of probiotics affected *Eimeria tenella* in a coccidiostatic manner. This can protect gut health and lessen the chance of coccidiosis spreading.

#### 2.2.4. Organic acids

According to Kum et al. (2010), organic acids are preservation agents that guard feed against the growth of bacteria and fungi. The majority of these acids are carboxylic acids, like tartaric, lactic, and malic acids, that have an alpha-carbon hydroxyl group. Organic acids that are simple monocarboxylic include acetic, formic, butyric, and propionic acids. Organic acids have an antibacterial effect on bacteria because non-dissociated acids can permeate through lipophilic bacterium membranes and obstruct enzymatic operations and transport systems (Mehdi et al., 2018; Cherrington et al., 1991). The addition of organic acids to broiler feed was demonstrated in several experiments (Hassan et al., 2010; Nava et al., 2009) to improve the usage of feed, growth, and feed conversion rate.

Young chicks are given a protective efficacy against *Campylobacter* illness by adding organic acids to their drinking water (Chaveerach et al., 2004). Additionally, these acids offer defence against *Escherichia coli* (Izat et al., 1990). Therefore, it has been demonstrated by (Mohammadagheri et al., 2016) that supplementing with 2% citric acid can enhance the gastrointestinal tract's cell proliferation, epithelium, and villi height. Supplementing drinking water with an organic acid blend, formic and propionic acid (0.0525%), and increasing the colonization of *Lactobacillus* spp. in chicken ileum results in more uniform and unique populations of intestinal microbiota (Nava et al., 2009). These alterations in the intestinal microbiota and the rise in *Lactobacillus* populations demonstrate that using organic acid as a substitute for antibiotics (in this study, bacitracin) to eliminate harmful bacteria in the gastrointestinal tract is feasible (Nava et al., 2009).

#### 2.2.5. Prebiotics

Certain components of non-digestible feed called prebiotics may be healthy for the host because they include fermentable qualities that encourage bacterial activity and/or

growth in the ileum and caecum (Mehdi et al., 2018; Gibson and Roberfroid, 1995). Oligosaccharides and short-chain polysaccharides make up the majority of it. Yeast cell walls and fermentation products yield a variety of prebiotics (Mehdi et al., 2018). According to Mehdi et al. (2018) and Józefiak et al. (2008), even while commensal gut bacteria can metabolize prebiotics to make short-chain fatty acids like propionate, acetate, and butyrate, the host is unable to absorb prebiotics. These prebiotic substances improve poultry productivity, support a healthy gastrointestinal tract, and offer an antibiotic-free alternative (Morales-Lopez et al., 2009; Zhang et al., 2005). Prebiotics change the caeca microbial makeup when consumed, changing the genus and family of bacteria, the proteobacteria, and the proliferation of those bacteria (Park, 2019). When a mannose- and mannoprotein-rich product was added to chicken feed, the amount of intestinal villus cells rose dramatically (Baurhoo et al., 2007).

In addition, feeding chickens a diet high in mannanoligosaccharide (0.2%) improved intestinal health more than giving them antibiotics. These benefits manifest as a decrease in harmful bacteria, morphological growth (increased villus height and goblet cell count), and an increase in the colonization of helpful bacteria (Baurhoo et al., 2009). As per the findings of Bednarczyk et al. (2016), consuming prebiotics in ovo within the chicken embryo represents an extra delivery method (Mehdi et al., 2018). This is a practical method that can be used instead of putting antibiotics in the water after the hatch. The doses of prebiotics utilized in ovo are 10 times lower than those administered post-hatching.

#### **2.2.6. Amino acids and enzymes**

Enzymes used as feed additives are created by fermentations involving bacteria and fungus. To maximize feed conversion, they are employed. Proteins, phytates, and glucans are just a few of the components that enzymes help degrade. For example, to improve broiler digestion, the introduction of  $\beta$ -1,3,4-glucanases and endo- $\beta$ -1-4-xylanases to wheat and barley diets (Mehdi et al., 2018; Cowieson et al., 2006). Furthermore, phytase enzyme can improve ADG by enlarging the villus and shallow crypt (Mohammadagheri et al., 2016).

Bacteriophage endolysins, or lysins, are a cutting-edge alternative therapeutic approach for antibacterial. Lysins are peptidoglycan hydrolases encoded by phages that, when supplied exogenously to gram-positive bacteria, cause bacterial cell lysis (Fenton et al., 2010; Rios et al., 2016). In poultry, Volozhantsev et al. (2011) stated that administering a mixture of peptidases, amidases, and lysozymes from a group of lysins results in an antibacterial action against *C. perfringens*, claim. For instance, an enzyme called Ply3626 lysine has been shown to exhibit lytic activity against multiple strains of *C. perfringens*, a major cause of food poisoning that also results in financial losses for the poultry industry (Fenton et al., 2010; Zimmer et al., 2002).

### 2.2.7. Bacteriophages

Bacteriophages, the most prevalent creatures on the planet, are frequently referred to as bacteria and archaea viruses (Sulakvelidze, 2001). It is believed that there are roughly 1031 times more phages in the biosphere than there are bacteria (Abedon, 2011). The majority of phages have dsDNA as their nucleic acid, and the protein sheath that encases nucleic acids gives phages their morphological form (Mehdi et al., 2018). Based on their cycle of replication, bacteriophages are divided into two groups. According to Mehdi et al. (2018), lysogenic or temperate phages proliferate by incorporating their nucleic acids into the host's DNA with every new generation of hosts. At the conclusion of replication, they do not destroy the host (Ackermann, 2009). Additionally, lytic or virulent phages connect to the host, insert their genome into the host genome, multiply using the host replication machinery, assemble, and then kill the host cell using an enzyme provided by the phage.

Lytic bacteriophage (phage) therapy could be a viable supplement to or substitute for traditional antibacterial therapies in the management of infections with microorganisms. Given the global issue of antibiotic resistance, research into phage therapy is particularly crucial (Pelfrene et al., 2016). Before the discovery of antibiotics, according to Twort (1915), phages were discovered and started being employed in medicine in 1919. Additionally, bacteriophage therapy continued to be widely used throughout the 20th century in Eastern Europe (Poland) and the former Soviet Union, although antibiotics mainly replaced it in Western nations after the Second World War (Georgia, Russia).

Phage therapy has been envisioned as either a "personalized" treatment option given to a specific patient (as a tailored bacteriophage after the isolation and identification of the causative pathogen) or as a "ready to use" cocktail made up of several different phage strains that are targeted at one or more pathogenic bacterial species (Wittebole, De Roock, and Opal, 2014). Despite the well-known issue of antibiotic multidrug resistance and the paucity of antibacterial drugs with novel modes of action in development, (Pelfrene et al., 2016) suggested that bacteriophage therapy might provide an alternate approach to address this problem.

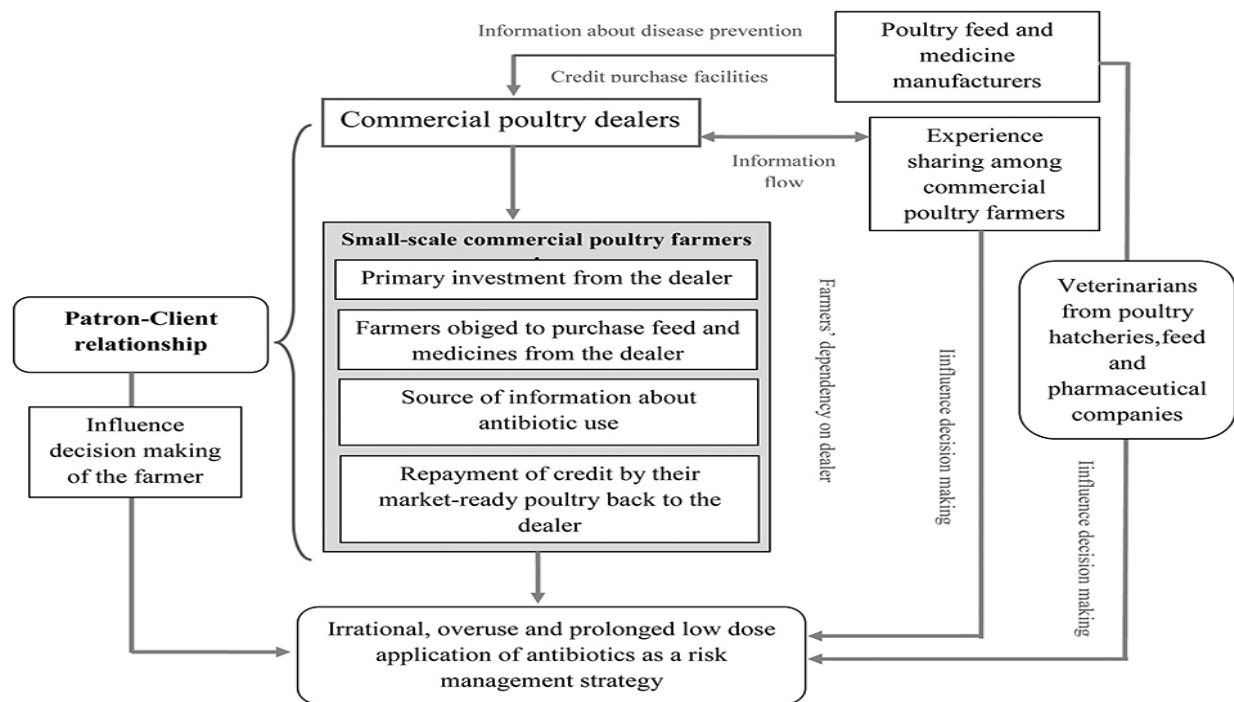


Figure 2.4. Factors impacting farmers' decisions on the use of antimicrobial agents in broiler industry (Masud et al., 2020)

Figure 2.4 stipulates how the responsibilities of farmers, dealers, and sales representatives from pharmaceutical companies were closely related to one another in the poultry-rearing industry; a conceptual framework for the patron-client interaction was developed using these data. Dealers make initial investments in poultry farms through credit purchases with the prerequisite that the farmers purchase all feed and medications from them for the duration of the production cycle. The transfer of data from pharmaceutical firms about preventing disease and promoting growth is managed by

the dealers (Masud et al., 2020). Moreover, the key informant described this arrangement as a "gentleman's agreement," in which both dealers and chicken farmers safeguard their interests. It appears that there is no harm in the relationship between chicken growers and traders. Because of the aforementioned "gentleman's agreement," farmers and dealers always consider their interests and never voice grievances against one another. No authority, not even the government, may access the link because it is based on a gentleman's agreement (Masud et al., 2020).

#### Mannan-Rich Fraction (MRF) in poultry diets

Many countries have banned the usage of antimicrobial drugs as growth promoters in animal feeds due to increased awareness and pressure about antimicrobial resistance and the implications of using antimicrobial agents in animal feed. These prohibitions, however, are insufficient to halt the growing emergence of antimicrobial resistance. Mannan-oligosaccharides from *Saccharomyces cerevisiae* have been used more frequently in chicken feed after subtherapeutic antimicrobial agents were outlawed in many countries due to their capacity to bind pathogens, lessen gut colonization, and improve the health and performance of the bird. Improved advantages of mannan-rich fractions have been obtained through further refining of mannan-oligosaccharides. *Saccharomyces cerevisiae* is the source of Antigen, a distinct MRF bioactive product of the second generation (Walker, 2022).

According to Walker (2022), MRF have been studied for their ability to alter the general composition of the bacterial population as well as their ability to boost microbiota diversity. It has been shown that broiler bacterial communities respond consistently to MRF supplementation. Furthermore, MRF in chicken feeds may cause the microbiota to change, favouring Bacteroidetes over Firmicutes. Antimicrobial resistance persists globally even in the face of international prohibitions on the use of antibiotics in feed. Antibiotic-resistant bacteria can become more sensitive and the requirement for antibiotics can be decreased when MRF is added to chicken diets (Walker, 2022).

Antibiotics cannot be added to feed and there is a need to find alternatives to synthetic growth promoters due to the risk that poultry meat products pose to human health. There have been attempts to feed animal root tubers like garlic, onions, and ginger to enhance

poultry performance and health (Malematja et al., 2022). According to Malematja et al. (2022), numerous phytochemical substances, flavonoids, and phenolic acids found in root tubers have been shown to have anti-inflammatory, antibacterial, antioxidant, and growth-promoting qualities. Furthermore, the growth performance of animals is influenced by bioactive chemicals found in root tubers, which can improve digestibility and modify intestinal health. These tubers may serve as an antibiotic substitute because research on their use and extraction has demonstrated that they have no harmful or infectious effects on birds. As an alternative to synthetic growth promoters, the onion bulb exhibits great potential as a phytochemical natural feed addition and natural growth promoter. Aditya et al. (2017) state that onions include phytochemical substances with anti-inflammatory, antibacterial, and antioxidant effects. They also mention that there is evidence that adding onion bulbs to a bird's diet can enhance the bird's growth performance. According to Goodarzi et al. (2013), extracts from onion bulbs have an impact on chicken growth performance. According to Goodarzi and Nanekarani (2014), there have been claims that onion extracts in drinking water and powder in cattle feed have anti-pathogen and growth-promoting qualities. Aditya et al. (2017) stated that broiler productivity and performance may be enhanced by adding onion meal or extracts in the proper amounts. Evidence suggests that adding onion meals to broiler chickens' diets may increase feed consumption because processed onions have a pleasant flavour, which may increase the hens' body weight (Aditya et al., 2017).

### **2.3. Observed the behavioural manner of farmers on the use of antibiotics in other countries**

A lot of information was kept under wraps about how much antibiotics were given to chickens during the drug audit of the food and water in North American poultry troughs, according to Webster (2009). Furthermore, In Canada, although a federal food inspection agency gathers drug-use data from poultry farmers, it refuses to release that data publicly. Even federal researchers tracking antibiotic use on farms were blocked from access to these data, according to a Public Health Agency of Canada veterinary epidemiologist who runs the Canadian Integrated Program for Antimicrobial Resistance Surveillance.

The US Department of Agriculture's expert on antimicrobial agents' usage attests to the confidentiality of information on the usage of antimicrobial agents in poultry farms. Secretive as the poultry industry information may be in the US and Canada a substantial headway in probing the usage of one highly controversial category of antibiotics on factory farms. In Canada, both the Food and Drug Administration (FDA) and the Public Health Agency are concerned about the use of cephalosporin antibiotics, which are both popular on farms and categorised as top priority drugs in human medicine. Cephalosporin-resistant bacteria were found to have spread from hospitals to the general public in Spain and Canada in 2004. This rapid spread of the bacteria was causing a rapid decline in the use of one of the few classes of antibiotics still in use for the treatment of gonorrhoea, pneumonia, and urinary tract infections (Webster, 2009).

Since 2002, the Public Health Agency of Canada has made significant investments in tracking cephalosporin resistance in bacteria isolated from animals raised for slaughter, in retail meat and poultry products, and in humans due to growing concerns about the increasingly rapid spread of cephalosporin resistance in both hospitals and urban communities. According to Webster (2009), there is a startling correlation between the use of cephalosporin antibiotics in Quebecian chicken hatcheries and the subsequent rise in cephalosporin resistance in bacteria extracted from both retail chicken products and humans. This finding raises doubts about the coincidence of the discovery of increased cephalosporin resistance in chicken products found in grocery stores and in humans.

#### **2.4. Resistant gene transfer**

Figure 2.5 shows How manmade sources like hospitals, sludge, sewage, and animal waste have been a major source of antimicrobial resistance genes (ARGs) in freshwater, estuaries, soil, and marine environments (Tang et al., 2015). Green et al. (2017) suggest that the continuous production of these novel microbial pollutants could increase background resistance levels, which would increase the likelihood that ARGs could diffuse, and spread by wind, and water vapour loop into air. Xie et al. (2018) argue that ARGs can be released into the atmosphere as biological aerosols by burning biomass, wind-borne dust, and wastewater treatment facilities. These aerosols are subsequently dispersed throughout the planet by jet streams.

Due to the tremendous selection pressure for antibiotic resistance in poultry germs, a significant fraction of the bacteria in the faecal flora is resistant. However, when chickens are killed, the resistant strains from their gut readily contaminate their carcasses, leading to the multi-resistant bacterium contamination of poultry flesh. As a result, poultry-derived resistant faecal coliforms can infect people through food, populating their gastrointestinal tracts and introducing resistant genes into their endogenous flora. The majority of gene transfer between pathogenic bacteria and bacteria that live in the gastrointestinal system occurs in vivo because comparable resistance genes are present in a range of bacteria species from different hosts (Nhung, Chansiripornchai, and Carrique-Mas, 2017). Furthermore, the majority of pathogenic pathogens that could endanger human health may eventually become resistant to every antibiotic currently in use (Mathur and Singh, 2005).

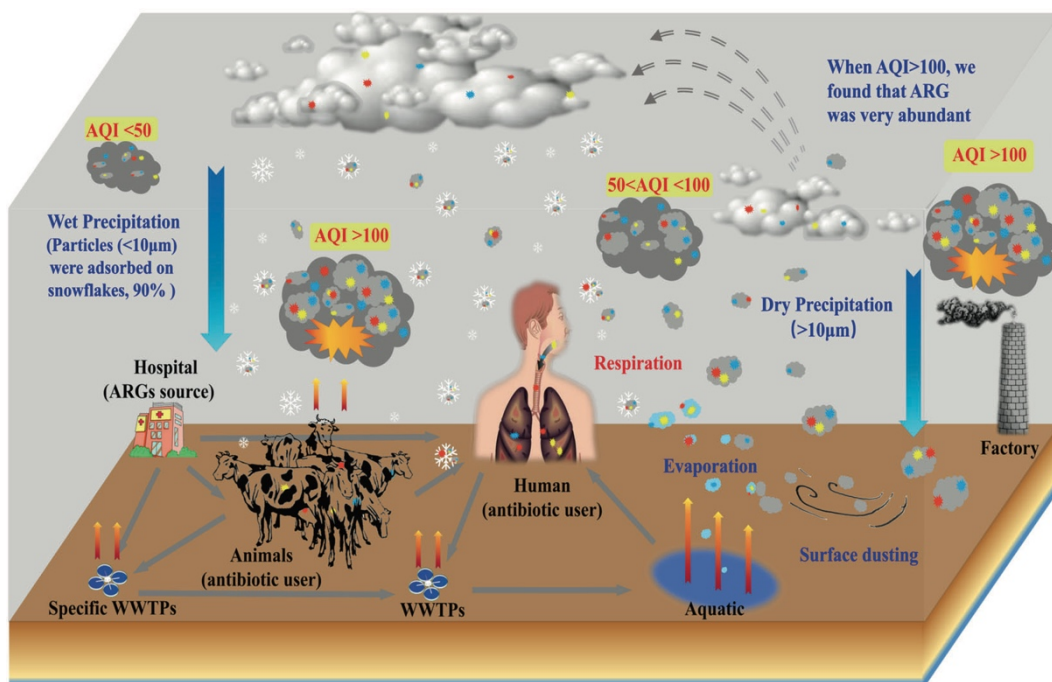


Figure 2.5: Schematic representation of the ARGs environmental loop (Zhu et al., 2021)

Only when resistance genes from commensal or animal pathogens can be transferred to human pathogens does resistance in those pathogens pose a risk to human health. This is true whether the transfer takes place in the environment, in the guts of farm animals, on or off farms, or in human guts. It has been extensively shown that human commensals and human infections can transfer genes horizontally (Huddlestone, 2014). Consequently, evidence of resistance gene transfer from animal-associated bacteria to

human commensals may be seen as a warning indication of a potential transmission to human pathogens and, as a result, as a cause for public health concern.

Regarding the problem of extra morbidity and mortality brought on by antibiotic resistance traits that arose on farms, at least three distinct methods by which antimicrobial resistance can negatively affect human health have been found (Barza, 2002). The following are some connections between these mechanisms and the resistance that arose in animal agriculture:

- a. Connection between resistance and virulence characteristics, resulting in drug-resistant strains with elevated pathogenicity;
- b. Therapy postponement due to ineffectiveness of early therapies;
- c. The requirement to select the less ideal course of treatment due to resistance to better antimicrobial medications.

## **2.5. One Health initiatives**

One Health as shown in Figure 2.6 is a cooperative, transdisciplinary, multisectoral strategy that, to achieve the best possible health outcomes at the local, regional, national, and international levels, acknowledges the linkages between people, animals, plants, and their shared environment. According to The European Consumer Organisation, (2013), it is committed to enhancing the quality of life for all animals and humans by fusing veterinary care, environmental research, and human medicine. The interdependence of ecosystems, animal health, and human health have been recognized by the World OIE, WHO, and the One Health concept. As a result, it is imperative to stop using antibiotics altogether because their usage in one habitat will influence microbial diversity and drive selection in another (The European Consumer Organisation, 2013). Animal surveillance has been a major topic of debate in One Health conferences in order to predict the possible appearance of new zoonotic diseases (Takashima and Day, 2014).

One Health Initiative at this level: The Global One Health Initiative states that there is a need for collaboration between veterinary personnel working in collaboration for example with pharmaceutical service providers in the livestock industry. Furthermore, farmers' inappropriate usage of antibiotics during growth promotion is exacerbated by

their ignorance of the fundamental pharmacological aspects of these antibiotics. Inappropriate use of antibiotics and non-sterile procedures in poultry increases the risk that resistant microorganisms will eventually spread from poultry to humans at various stages, contingent on the genes involved (Horel, 2019).

The implementation of effective collaboration between human and veterinary medicine, environmental protection, and public health is not a fully achieved goal, although several examples of integrated approaches have been documented in Europe. The concept of one world, one health has recently appeared, indicating that the world has suddenly woken up to the link between animal diseases, public health, and the environment. Collaboration in the fields of environmental, animal, and human health is now essential due to the rise in zoonotic human infectious diseases and microbial resistance to antimicrobial agents. Through the establishment of professional networks in the One Health domain, this innovative concept of collaboration has mostly been applied to the fields of vaccines, antimicrobial resistance, zoonosis, and zoo prophylaxis (Sikkema and Koopmans, 2016).

## **2.6. Conceptual framework**

A conceptual framework is an arrangement that the investigator feels best explains how the thing under study evolved naturally (Adom and Hussein, 2018). The current study will pursue a One Health approach as a conceptual framework. The One Health approach was developed as a holistic and interdisciplinary approach that seeks to maximize human health, animals, and the environment. Multi-disciplinary sectors involved in One Health strive to identify, assess, and fight any threat to the health of human beings, animals, and the environment. This approach is relevant to the study because it will guide the researcher to identify any threat to human beings, animals, and the environment due to the use of antibiotics during the development of chickens.

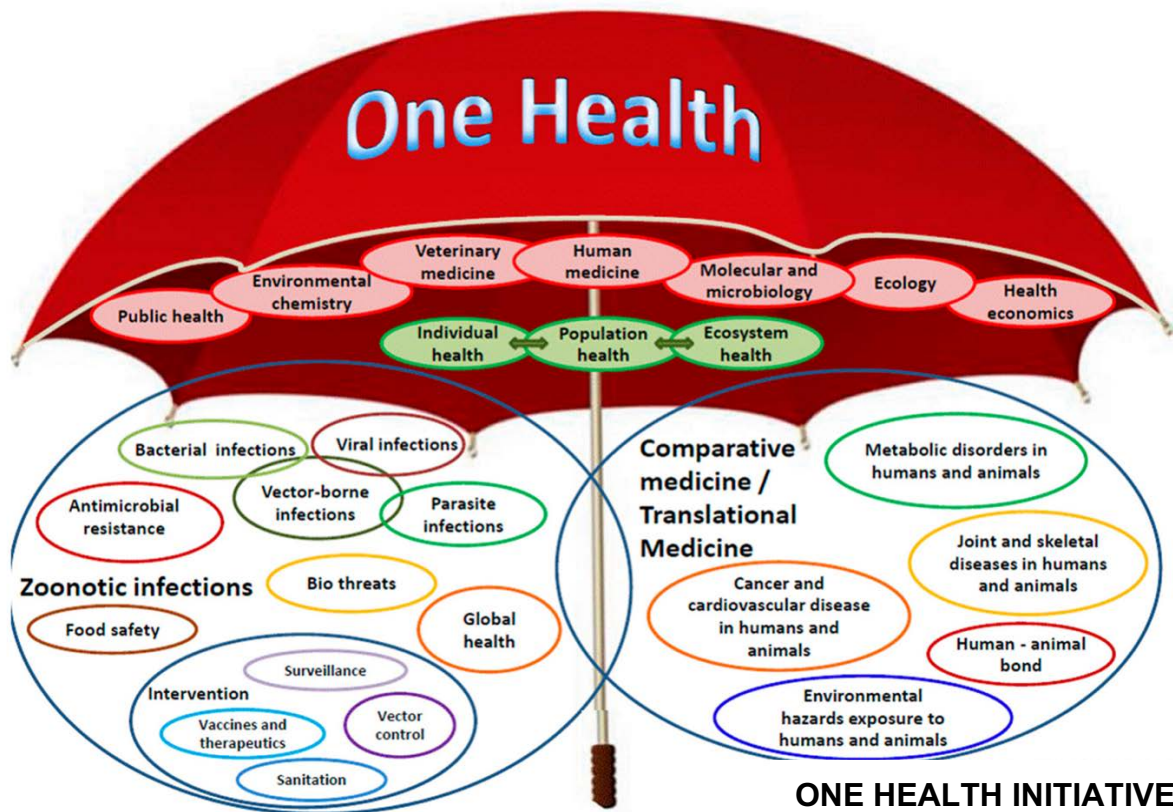


Figure 2.6: One Health flow chart (The European Consumer Organisation, 2013)

Figure 2.7 below illustrates the four different levels that serve as a guide on how to achieve One Health. The first level describes the different disciplines applied in One Health, the second level describes the health effects at three different health levels, the third level describes the research focus and the fourth level describes how the One Health approach could be applied for training purposes.

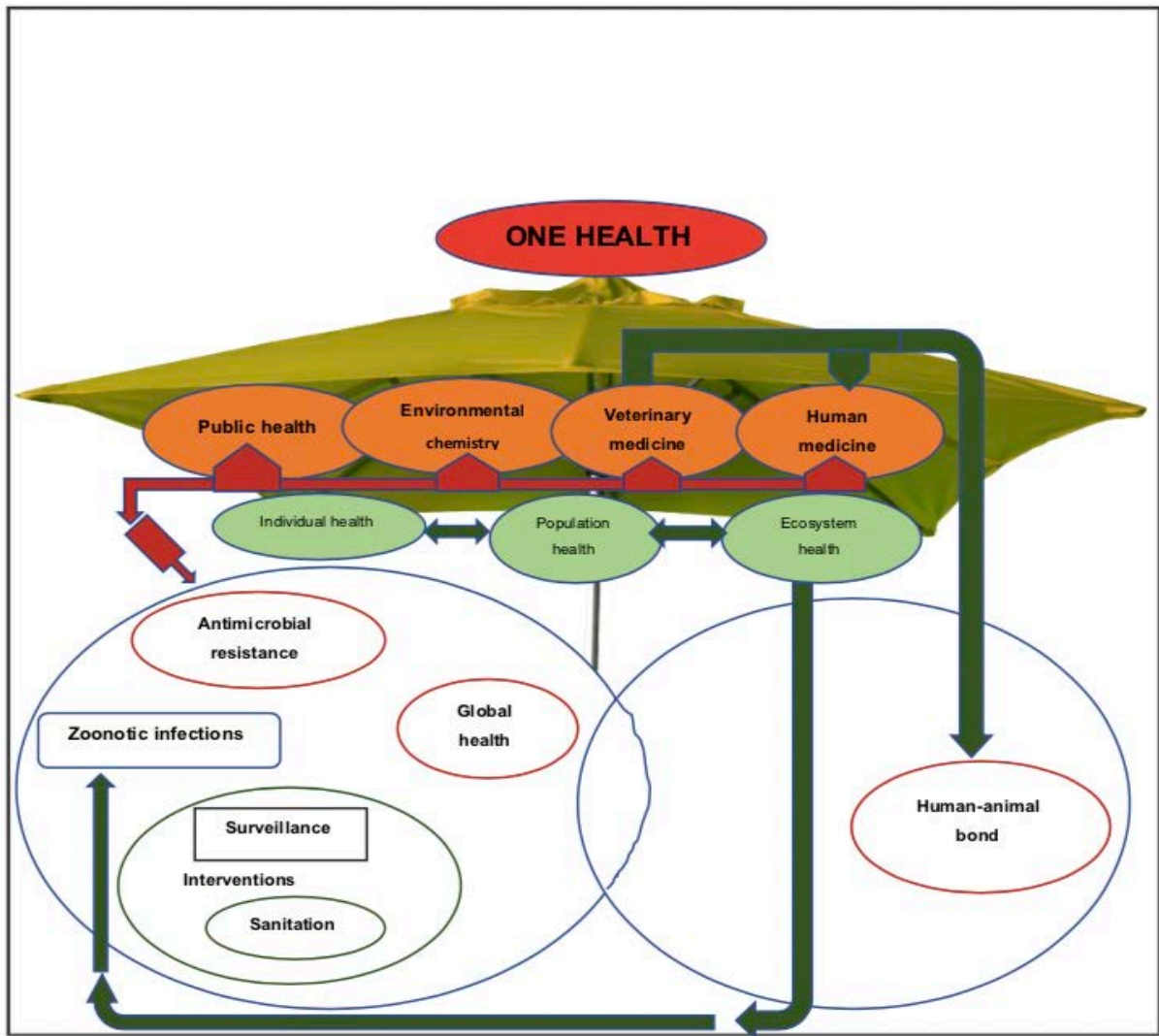


Figure 2.7: Conceptual framework on the interactions among veterinary medicine, human medicine, public health, and environmental chemistry under One Health (adapted and modified from The European Consumer Organisation, 2013).

### 2.6.1. Veterinary science and zoonosis

Veterinary medicine is a branch of medicine that focuses on treating, diagnosing, controlling, and preventing diseases that affect the health of both domestic and wild animals as well as preventing the spread of animal diseases to humans (Sikkema and Koopmans, 2016). The dimension of the zoonosis problem is a cause for alarm because zoonotic pathogens represent a clear threat to global health: these pathogens are globally present, accounting for about 75% of emerging infectious diseases affecting humans which will require a coordinated human medical and veterinary approach. The concept is used to identify and prevent zoonotic diseases that can be transferred from

animals to people and that are used to treat illnesses that impact both domestic and wild animals. The concept in the study will be used to focus on the judicious use of veterinary medicines essential for maximizing the state of health and productivity of animals on growth promotion without affecting human health through zoonotic diseases. Moreover, the ecosystem can happen when humans and animals mix and microorganisms exchange genetic material resulting in zoonosis.

#### **2.6.2. Human medical science**

Human medical science is a multidisciplinary field that studies the human body and its health conditions to appropriately treat and prevent diseases. It is frequently simply referred to as medicine (Takashima and Day, 2014). The concept is used in human health to identify human health conditions, causes, and treatment thereof affecting individual human health. In the study, the concept will be used to identify the level of antibiotic resistance in humans from poultry via gene transferase.

#### **2.6.3. Public health**

The science of preserving public safety and enhancing community health via illness and injury prevention research, policy development, and education is known as public health (Lushniak, 2014). The concept is mostly used to identify elements that are affecting the health of an individual ultimately to the public and also improving the individual health would improve the health of the public. In the study, the concept will be used mainly on the usage of antibiotics in poultry for growth promotion that results in gene transferase to a human at individual health that influences antibiotic resistance to a larger scale of the population health of humans that ultimately becomes a public health concern. The public health concern would then be the global health concern.

#### **2.6.4. Environmental chemistry**

The study of chemical and biological events that arise in nature is known as environmental chemistry (Gupta, Khoiyangbam and Jain, 2015). The concept will be used to identify the factors affecting human health. In the study, the concept will be used to identify factors to improve the rear of the flock through hygiene to improve human health.

In summary, the One Health approach has to start with examining the utilization of antibiotics in animals which has an impact on human medicine especially if there is a possibility of humans acquiring infection from animals as shown in Figure 2.7. Antibiotic resistance can also be acquired through the interaction between animals and humans especially if the microorganism acquired has been carrying an antibiotic resistance gene. The result is disaster which calls for an interdisciplinary and multidisciplinary approach to deal with the public health problem as recommended by the UN Inter-Agency Coordinating Group, IACG (UN-AICG, 2019).

## **2.7. PURPOSE OF THE STUDY**

### **2.7.1. Aim**

To explore what and how antimicrobial agents are used and to recommend smart regulations that can be put in place to promote judicious use in commercial poultry farms in Limpopo Province, according to one health approach.

### **2.7.2. Research questions (Interrogative objectives)**

Regarding the usage of antibiotics in commercial poultry farms in Limpopo Province:

- i. What is the level of knowledge, attitude, and practice toward AMR that governs their feeding practices following IPC recommendations?
- ii. What are the most prevalent pathogenic microorganisms that are zoonotic?
- iii. What is the resistance pattern of the most prevalent microorganisms and the specific genes involved?
- iv. Are there guidelines and policies followed on the usage of antibiotics in poultry farming?

### **2.7.3. Specific objectives (Declarative objectives):**

Regarding the usage of antibiotics in commercial poultry farms in Limpopo Province, the objectives were:

- i. To determine the level of knowledge about AMR and identify attitudes and practices toward the use of antibiotics during poultry production.
- ii. To identify, phenotype, and genotype where possible the most prevalent pathogenic microorganisms in poultry that are zoonotic.
- iii. To determine the resistance pattern of the most prevalent microorganisms.

- iv. To validate current guidelines and to make any amendments in collaboration with all stakeholders on the use of use of antibiotics in poultry farming applying the One Health Approach.

## **2.8. HYPOTHESIS**

### **2.8.1. Main hypothesis**

Greater adherence to the International Poultry Council (IPC) policy on antimicrobial use and enforcement of infection, prevention, and control practices through smart regulations in the poultry industry will lead to less injudicious use of antimicrobial agents that may lead to antibiotic resistance.

### **2.8.2. Ancillary hypotheses**

- i. The development and recommendation of smart regulations and greater adherence to antimicrobial policy in the poultry industry will lead to a reduction in the injudicious use of antimicrobial agents.
- ii. More effective infection, prevention, and control practices will reduce invisible costs in poultry farming and the emergence of antimicrobial-resistant zoonotic microorganisms.
- iii. Smart regulations that promote the One Health approach will contribute to the preservation of more antibiotics important to human health, which is an essential first step in creating risk management plans.

## **CHAPTER 3**

### **MATERIALS AND METHODS**

#### **3.1. Materials**

For human data collection, the following materials were used:

A questionnaire consisting of participants' demographics, knowledge, practice, and attitude.

For chicken droppings and gut contents, the following material was used for culture and sensitivity testing and sequencing: sterile swabs, ruler or caliper to measure zone sizes, incubator, forceps, McFarland 0.5 turbidity standard, Pasteur pipettes, sensitivity discs, sterile 0.85% saline, Löwenstein-Jensen agar plate.

#### **3.2. Study design**

The study was a combination of cross-sectional, longitudinal, and experimental designs. It therefore used mixed approaches of both qualitative and quantitative data collection.

#### **3.3. Study settings**

The study was conducted at three main commercial poultry farms in and around Polokwane. The commercial poultry farms were Lunds, Mikes Chicken, and Spif Chickens. Lunds is situated 16,7km via R101 road to Mokopane, Mikes Chicken is 13km from Polokwane along Dendron Road (R521), and Spif Chickens is situated 144,2km from Polokwane along R519 road in Mookgopong. All these farms are within reach of the Department of Agriculture and Rural Development, Veterinary Directorate, in Limpopo Province as shown in Figure 3.1, if and when veterinary services are required.

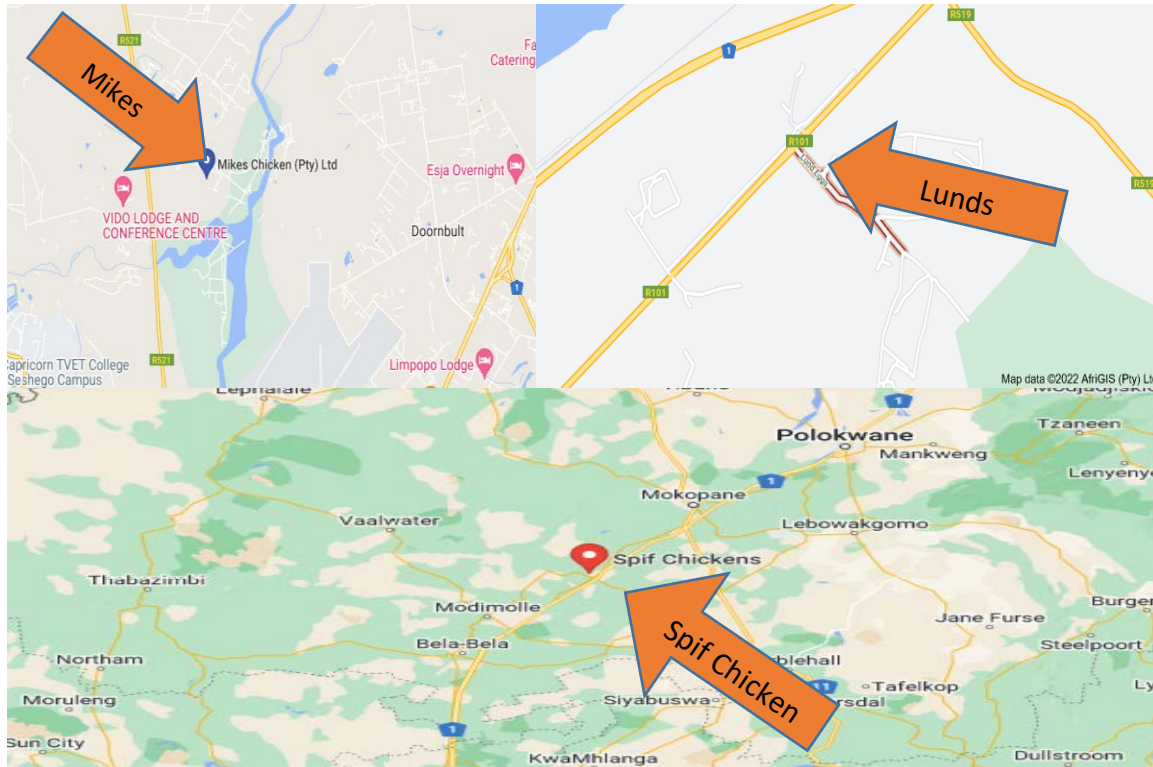


Figure 3.1: Study sites locations of Mikes Chicken, Lunds Chicken and Spif Chicken

#### 3.4. Study population

The study population consisted of 1) poultry farmers, 2) chicken feeders i.e. those solely responsible for feeding the chickens and chicken farm workers, ordinary general hands on the farms, and 3) veterinarians in the Department of Agriculture in Limpopo Province who were overseeing poultry farms, and chickens from which data was collected from their droppings and chicken gut contents.

#### 3.5. Sample size and sampling procedure

The quantity of participants or observations in a study is referred to as the sample size. The sample size has an impact on statistical properties concerning the accuracy of estimations and the power of the study to draw conclusions (Creswell, 2018). In order to determine the sample size, Yamane Formula was used.

##### 3.5.1. Yamane formula

The Yamane formula was used to calculate the sample size with a single proportion with a 95% confidence interval and a 5% margin sampling error:

$$n = \frac{N}{1+N(e)^2}$$

$N$  = population size

$n$  = sample size

$e$  = error of margin (0.05)

$$n = \frac{185}{1+185(0.05)^2} = 126$$

The sample size will be 126.

Based on the information provided, an adjusted sample size of 126 has been generated. 138 is the result of adding 126 and 10% of the non-response rate. The distribution of the sample followed the population's size in proportion as shown in Table 3.1.

Table 3.1: Sample Size distribution

<b>Farms</b>	<b>Total personnel known population</b>	<b>Sample Size</b>
Lunds	65	47
Mike's Chicken	50	38
Spif's Chicken	60	44
Poultry farmers and veterinarians	10	9
<b>Totals</b>	<b>185</b>	<b>138</b>

According to the calculation, Lunds, Mike's Chicken, and Spif's Chicken had a total number of 65, 50, and 60-foot print personnel on the ground respectively and according to the Yamane formula 47, 38, and 44 were the least number of participants that could participate in the study. This was to eliminate the margin error at a *p-value* of 0.05 percent, the findings are deemed to be trustworthy.

### 3.5.2. Farms

All three commercial poultry farms in and around Polokwane namely Lunds, Spif Chicken, and Mike's Chicken, each with an average of 3000 chicken trap capability, were included in the study. The poultry farms were sampled based on the large-scale production of commercial poultry farms within Limpopo Province. There are currently three large-scale poultry farms in Limpopo Province.

### **3.5.3. Farmers**

A total of 7 poultry farmers aged between 50 and 65 were recruited into the study and were all considered as the key informants. Two at Lunds, a male and female, two males at Mikes' Chicken, and 3 at Spif Chicken two males and a female.

### **3.5.4. Chicken farm workers**

A total number of 175 chicken farm workers 65 at Lunds, 50 at Mike's Chicken, and 60 at Spif Chicken farms were included in the study. Of these farm workers, 60% were male and 40% were female. Adding the total number of chicken farm workers, poultry farmers (7), and veterinarians (3) equals 185 for the total population.

### **3.5.5. Veterinarians**

All three veterinarians, two males and one female in Limpopo Province who are overseeing and supervising the three poultry farms were incorporated into the research. The average years of experience concerning poultry was 10 years plus.

### **3.5.6. Microbiological investigation with chickens' stools, and gut content of ileum, and caeca**

Plymouth Rock, *Gallus gallus domesticus* chickens as shown in Figure 3.2 were found to be the most commonly kept chickens at the farms included in the study. A large and enduring chicken is the Plymouth Rock. While some cultivars are primarily bred for meat, others make excellent layers. They have yellow skin and legs, a relatively deep and large breast, and a long, broad back. The hens' plump, deep abdomens are indicative of excellent layering and meat production.



Figure 3.2: Plymouth Rock *Gallus gallus domesticus*.

### 3.6. Inclusion criterion

Healthy broiler chickens six weeks old called Plymouth Rock in commercial farms were included in the study as most commercial poultry farmers rear broiler chickens for consumption. According to Chatterjee and Rajkumar (2015), large, long-lived hens are Plymouth Rocks. While some types are bred primarily for meat, others make good layers. They have yellow skin and legs, a long, broad back, and a breast that is large and rather deep. The hens' plump, deep abdomens indicate that they are excellent layers and producers of meat. A broiler chicken is a young, tender, meaty chicken of any gender that attains a weight of around 700g in approximately 6 weeks, having hatched at 38–40 g (Chatterjee and Rajkumar, 2015).

### 3.7. Exclusion criteria

The following chickens were excluded from the study: ISA Brown, Barnevelder, Frizzle, Belgian d'Uccle, Australorp, Naked Neck, Orpington, Silkie, New Hampshire Red, Rhode Island Red, Polish, Cochin, Leghorn, Sussex, Araucana, Wyandotte, Minorca, Faverolles, and Sebright. The study did not include these chicks as Plymouth Rock hens were raised on the commercial poultry farms that were part of the study.

### 3.8. Chicken specimen collection and treatment

According to the adopted National Health Laboratory Services (NHLS) SOP for human stool specimen processing, 30g of chicken droppings from healthy chickens of at least 6 weeks old were required per batch of ten chickens for culture and sensitivity testing. The stools were processed according to the previously described method by Olova et al. (2018).

Chicken droppings and gut contents of the ileum and caeca were collected from three farms, as shown in Table 3.2 and Table 3.3. For culture and sensitivity tests, up to 30g of chicken droppings were collected at different spots on each farm as shown in Table 3.2, and a total of 90g were taken to National Health Laboratory Services for culture and sensitivity testing.

Table 3.2: Number of chicken droppings per farm

<b>Lunds</b>	<b>Mikes Chicken</b>	<b>Spif Chickens</b>	<b>Totals per farm</b>
<b>30g</b>	30g	30g	90g
<b>Totals</b>			<b>90g</b>

Figure 3.3 shows pictures of normal chicken droppings that were collected. The chicken droppings collected appeared though to be a little watery. The stools were processed according to the previously described method by Olova et al. (2018).



Figure 3.3: Chicken droppings of broiler chickens

For gut contents, ten chickens, six weeks old, were procured from each farm and slaughtered using the dislocation of the neck method as per Animal Research Ethics Committee (AREC) recommendations. From the ileum and caeca, the gut contents were collected as illustrated in Figure 3.4 & Figure 3.5 from each chicken to make a total of 30g for culture and sensitivity testing.

Table 3.3: The amount of chicken ileum and caeca contents collected from each farm

<b>Farms</b>	<b>Guts content</b>	<b>Batches (chickens)</b>	<b>Totals</b>
Lunds	30g	10	300g
Mike's chicken	30g	10	300g
Spif's chicken	30g	10	300g
<b>Totals</b>		<b>30 chickens</b>	<b>900g</b>



Figure 3.4: Degutting and collection of the ileum and caeca contents from the chickens



Figure 3.5: Degutting and collection of ileum and caeca contents from the chicken contents

### 3.9. DATA COLLECTION

#### 3.9.1. Farm interviews

A pretested questionnaire was administered to poultry farmers, chicken farm workers, and veterinarians responsible for commercial poultry farms while at the same time carrying out a transect walk at each farm. Each participant had been given a consent form to sign in order to agree or disagree to take part in the study. A letter from the Department of Agriculture and Social Development. Appendix 7D also facilitated the obtaining of the consent. This was done during a transect walk on the farms. The information captured through the questionnaire included:

- a. Clients' demographics,
- b. Knowledge of chicken feeds,
- c. Knowledge about antibiotics usage in poultry production, and
- d. Availability of antibiotic guidelines and policies on antibiotic usage.

The questionnaires used are in Appendices 7A, 7B, and 7C.

### 3.10. Data collection tools

#### 3.10.1. Humans

The data collected as paper documents such as consent forms and questionnaires (adopted from the AU-One Health project) that contained personal identifying information were secured and locked when not in use. There were several ways to store the data, backed up, arranged well, accurate, completed, accessible, and handled only by the researcher and authorized personnel like supervisors when actively used during the project. The information was safeguarded in case it was needed later on to re-evaluate, establish priorities, or confirm the study conclusions.

#### 3.10.2. Specimen processing

##### a. Chicken droppings

The collected 30g of chicken droppings were collected and pulverized/meshed in accordance with the requirements of the NHLS susceptibility testing against antibiotics normally used in humans.

##### b. Chicken ileum and caeca specimens

From the poultry farms, ten chickens six weeks old per farm ready to be consumed were purchased and taken to the University of Limpopo for slaughtering following the Animal Research Ethics Committee of the University of Limpopo (UL) procedures. Chickens were slaughtered in the presence of a veterinarian using the cervical dislocation of the neck method and thereafter the chickens' gut contents were collected and taken to NHLS for further processing and analysis. The chicken gut contents of the ileum and ceca were collected from the carcass and were dried and pulverized. Ten grams (10g) of each batch was used for culture and sensitivity testing against antibiotics used in humans at NHLS as follows:

##### c. Procedure for culture, identification, and susceptibility testing

###### Preparation of stool samples for culture

A pea-sized portion of stool was emulsified in 0.5 ml sterile saline (DMP, Johannesburg) and vortexed for 3 minutes (Séparation Scientific, SA). The broth was then smeared with a loopful onto an appropriate total of 150 Petri dishes (Plastopro Scientific, SA).

The unformed stool was inoculated directly onto the plate using a swab dipped into the specimen and rolled swab over a small area of the agar media. For 24 hours, each inoculation plate was incubated at 37 degrees Celsius (Séparation Scientific, SA). All bacterial colonies from selective media were purified by subculturing onto blood agar.

d. *Salmonella* and *Shigella* (serotypes belonging to Group 1)

A loopful of emulsified stool was streaked on XLD (Difco, MI, USA) and Brilliant Green agar (Difco, MI, USA). Following incubation, the plates were checked for the presence of *Salmonella* colonies that were usual. For sub-culturing onto blood agar for purification, red colonies with a black center on XLD agar (Difco, MI, USA) and pink colonies on brilliant green agar (Difco, MI, USA) were used. According to the procedure followed *Shigella* colonies would remain red.

e. *Escherichia coli*

Sorbitol-MacConkey agar (DMP, Johannesburg) was aseptically inoculated with a loopful emulsified stool and then spread onto the agar. Following that, each plate's presumed *E. coli* colony would have large, pink colonies caused by lactose fermentation was checked for uniformity of colonies. The detected colonies were sub-cultivated onto blood agar to remove impurities.

f. *Enterococci*

Aseptic inoculation was performed using Oxoid, UK-manufactured Kanamycin Aesculin Azide agar (KAA) plates by spreading a loopful of emulsified stool onto the agar. The plates were examined in order to find any common colonies (small white colonies with a black halo due to aesculin fermentation).

g. *Clostridium perfringens*

Inoculation was done using *Clostridium* selective agar plates aseptically using a loopful of emulsified stool and then spread onto the agar. The agar plates were in turn incubated at 37°C. Black colonies, or presumed colonies, were selected and subsequently injected onto blood agar. Following 48 hours of incubation, the inoculated blood agar was re-incubated with the same conditions, and the formation of beta-haemolytic colonies was monitored.

h. *Campylobacter* agar

*Campylobacter* agar (DMP, Johannesburg) were aseptically inoculated and was examined for growth, every 48 hours for up to 144 hours. After incubation plates were scanned for grey, round, small and translucent colonies. Curved, S-shaped, or seagull-resembling wings will be observed on microscopic examination of *Campylobacter* positive colonies.

i. *Yersinia* agar

*Yersinia* selective agar plates were aseptically inoculated. After incubation colonies with a dark red centre and translucent margin resembling the "Bull's eye" will be observed. Colonies identified were purified by sub-culturing onto blood agar.

### 3.10.3. Testing for antimicrobial agents' susceptibility

Testing for antimicrobial agents' susceptibility of isolated pathogens to clinically relevant antimicrobials was carried out using the automated VITEK<sup>®</sup> 2 Compact system (Biome´rieux, Marcy l'E`toile, France) according to the guidelines published by the Clinical and Laboratory Standards Institute (CLSI) 2013. On Mueller-Hinton agar plates, pure bacterial colonies were streak-plated, cultured for 24 hours at 37°C, and then Gram-stained. Pure colonies were put in a tube with 3 mL of saline (BioM´erieux, USA), and the VITEK<sup>®</sup> 2 DensiCHEK™ was used to measure the density. Bacterial suspensions that were outside of the proper range were corrected to the McFarland standard of 0.5–0.63. After choosing the proper VITEK<sup>®</sup> 2 Identification Card, it was placed inside the tube holding the suspension. The antibiotics that were to be tested were Ampicillin, Baytril, Fosfomycin, Neomycin, Sulphamethoxazole/trimethoprim, Doxycycline, Lincospectin, Lincomycin, Gentamycin, and Vancomycin as they are most commonly applied antibiotics in poultry industry as shown in Table 1.1. All isolates were stored in Dorset slopes for sequencing which they were cultured on blood agar.

The percentage calculation of the resistant pattern will be calculated as follows:

$$PR = \frac{No\ RA}{No\ S + R} \times 100$$

PR: Percentage resistance

No RA: Number of isolated microorganism resistant to antibiotic

No S+R: Total number of isolates sensitive and resistant.

#### 3.10.4. **Sequencing DNA extraction**

The DNA extraction was performed using QIAamp Fast DNA Stool Mini Kit (06/2012) (QIAGEN®, Hilden, Germany) according to Palanee et al. (2022) method. Extracted genomic DNA was quantified using the Qubit™ dsDNA BR Assay kit (Life Technologies Corporation, Carlsbad, USA).

##### a. Sequencing

Sequencing was carried out using the MiSeq Platform (Illumina) in compliance with the manufacturer's guidelines. Library preparation and indexing were prepared using the Nextera XT Kit (250 bp). The Nextera XT DNA sample preparation was done in five steps:

- i. Fragmentation of genomic DNA: the input DNA was tagged and fragmented. In order to facilitate PCR amplification in later stages, the input DNA is concurrently fragmented and ends are appended with adaptor sequences by the Nextera XT transposome.
- ii. PCR amplification: using a limited-cycle PCR method, the tagmented DNA was amplified. In addition, the PCR step adds sequences necessary for cluster formation as well as index 1 (i7) and index 2 (i5). PCR was carried out in a thermal cycler with the following settings: 95<sup>0</sup>C for 30 seconds and 72<sup>0</sup>C for 3 minutes. 12 cycles of 10 seconds at 95<sup>0</sup>C, 30 seconds at 55<sup>0</sup>C, 30 seconds at 72<sup>0</sup>C, and 5 minutes at 72<sup>0</sup>C as the final phase.
- iii. PCR clean-up: The library DNA was purified using AMPure XP beads, which further provide a size selection phase to remove the population's very short library fragments.
- iv. Library normalization: in order to guarantee more equitable library representation in the pooled sample, this procedure normalizes the quantity of each library.
- v. Library pooling for MiSeq sequencing: to preparation for cluster construction and sequencing, equal parts of the normalized library are combined, diluted in hybridization buffer, and heat-denatured before MiSeq sequencing. Each lane had six libraries sequenced in total. Using FASTQC software in conjunction with MiSeq

Control software 2.0.5, the quality of every read was evaluated. The resulting reads was around 250 bp and the target average coverage for each line will be 100X. After quality checking, reads were filtered and trimmed using Qiagen CLC Genomics Workbench version 10 (Qiagen, Netherlands).

b. Bioinformatics of Whole Genome Sequencing (WGS)

FastQ Screen was then used to filter out non-viral reads from the resulting metagenomic sequence reads after they had been quality trimmed using Trim Galore (Olova et al., 2018; Wingett and Andrews, 2018). The consensus sequence was subsequently created by mapping the remaining viral reads to the isolates' whole genomes using CLC Bio Qiagen-2020. At the South African National Bioinformatics Institute (SANBI), South Africa, the consensus sequence was joined with a set of genomes obtained from GISAID and a multiple sequence alignment (MSA) conducted using Nextstrain (Hadfield et al., 2018).

c. Phylogenetic analysis

The phylogenetic analysis was done using the MEGA X (Zhang, Qunfu, and Zhang, 2020), applying the Maximum likelihood method (ML) and Tamura-Nei model (Maurya et al., 2020). Using 1000 repetitions and a General Time Reversible (GTR) substitution model with gamma distribution, the bootstrap consensus tree was determined (Maurya et al., 2020; Zhang, Qunfu, and Zhang, 2020).

The genotyping method cannot always be relied upon according to Wheeler et al. (2023) who reported that whole-genome sequencing of bacteria resistant to antibiotics is becoming more and more common for antimicrobial resistance (AMR) surveillance, especially in high-income nations. Although widespread adoption of these technologies is difficult, especially in low- and middle-income countries, they hold the promise of revolutionizing AMR surveillance and epidemiology through advances in genome sequencing and analytic technology.

### 3.10.5. Action research path

The action research path involved all the stakeholders in the poultry farming industry following a network sampling through the help of the One Health Programme at the

University of Limpopo, the African Farmers Association of South Africa (AFASA), and the South African Poultry Association (SAPA). This was done because the One Health approach to develop smart regulations for the judicious use of antimicrobial agents at the farms requires such a stakeholder participatory methodology.

As part of the path, an email outlining the purpose of the study and the need to share the results with commercial poultry farmers, at the seminar, was sent to the General Manager, SAPA. This was also followed by a similar invitation to the AFASA.

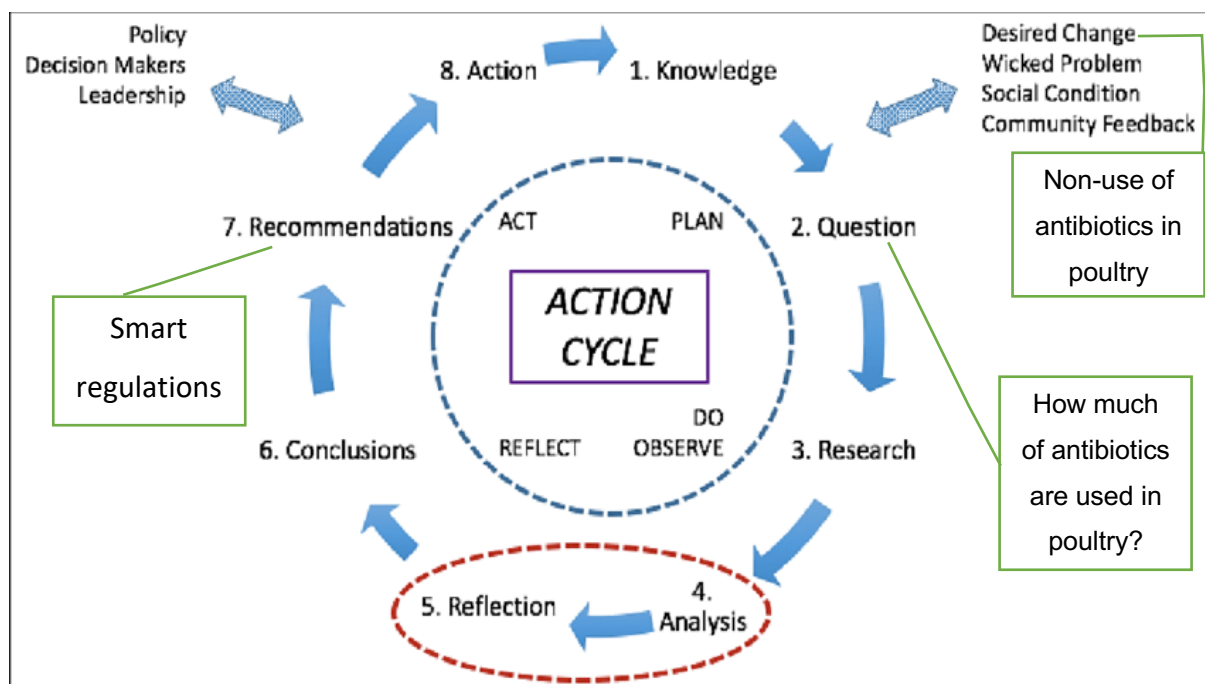


Figure 3.6. The action cycle followed in the development of guidelines and smart regulations for antibiotic use in chicken farming

The focus was on the 'action cycle' of the participatory action research path. There was interaction with stakeholders at the beginning of the study (steps 1-3) and the end of an action cycle (steps 7, 8) as indicated in Figure 3.6. Part of the summation was shared during the UN-World Antimicrobial Awareness Week and fed into new exploratory paths and new 'action cycles'. The red dashed lines highlight where personal reflection was incorporated. The inner circle (dashed blue line) presents how the 'action cycle' was related to additional approaches often applied to policy and project work, sometimes referred to as; 'Plan-Do-Check-Act' (Field, 2014).

### 3.10.6. **One Health Approach converts – veterinarians**

As part of steps 7-8 in the cycle, a PowerPoint presentation of interim results from the poultry farms was made to 30 members from South Africa, Bologna, Europe, and the Netherlands, Elephant project. This was considered as a network sampling of 10 participants, veterinarians, and animal production specialists, in particular. According to the Action Cycle, these were considered to be the leadership responsible for developing relevant guidelines, policies, and smart regulations regarding the use of antibiotics in veterinary services and the promotion of the One Health Approach.

### 3.11. **Reliability and validity**

In the study, the ability of the data gathering instruments and the technique to obtain the data, especially from the farmers and the farmworkers on the farm, was paid much attention to. It was important to do so to ascertain the quality, trustworthiness, credibility, and authenticity, of the data obtained when carrying out a study in a highly sensitive area of a business such as commercial poultry farming.

Reliability, in the study, was interpreted to mean the degree to which an instrument that was, adapted AU-One Health questionnaire could be relied upon to produce consistent results if applied frequently over a protracted duration to the same subject or if two researchers apply it, as per Brink et al. (2018). To ensure the reliability of the data collection tool, a pilot study was conducted among chicken feeders and veterinary doctors at the chicken farms who were not part of the study.

In spite of the fact that, by the very nature of the qualitative approach used in the study, not lend itself well to the statistical calculation of validity, extreme care was taken to maintain the quality of the data-collection technique. To improve the veracity or authenticity of the findings, multiple approaches were utilized during the process of gathering data. This included some non-traditional designs such as evaluation research, needs assessment, and action research. For culture and sensitivity testing and to obtain reliable microbiological results an automated calibrated VITEK<sup>®</sup> 2 Compact system machine was used.

### 3.12. Bias

In both qualitative and quantitative research, bias is an effect that results in a mistake or distortion that could compromise the quality of the evidence (Brink et al, 2018).

#### 3.12.1. Researcher bias

In both qualitative and quantitative research, bias is an effect that results in a mistake or distortion that could compromise the quality of the evidence (Brink et al, 2018). Typically, researcher bias manifests itself when a researcher attempts to sway participant responses or express an opinion about respondents (Creswell & Creswell, 2018). Even though the researcher was available to answer inquiries, the researcher did not in any way censor the participants' responses.

#### 3.12.2. Sampling bias

The over- or under-representation of a population segment in a sample is known as sampling bias, and it will have an effect on the study's validity and goal (Brink et al, 2018). The researcher made sure that the study population was precisely specified and that the sample size was computed correctly using tables and formulas in order to prevent sampling bias. During the network sampling, this obviously could not be an as the sampling procedure was non-probability sampling.

### 3.13. Data analysis

Statistical software was used to analyze the data that was gathered utilizing the quantitative approach (STATA 9.0; StataCorp; College Station, TX, developed by Computing Resource Centre in California). The data were interpreted using both inferential (Chi-square) and descriptive (frequency distribution) statistical techniques at a *p-value* of  $>0.5$ , which was deemed significant. Regression analysis was used to check correlations between dependent and independent variables. Regression analysis is a set of statistical methods used for the estimation of relationships between a dependent variable (e.g. the three farms) and one or more independent variables (e.g. the use of antibiotics).

### **3.14. Limitations**

- 3.14.1. Farmers refused the chicken feeds and water samples for culture and sensitivity testing. Farmers were reluctant to participate in the project. However, with the aid of the Section 21 letter from the Director of Veterinary Services in Limpopo, they reluctantly participated in the study.
- 3.14.2. On two farms, the farmers did not allow all their subordinates to fill in the questionnaires as they were afraid, they could be implicated even though it was clearly explained in the consent form.

### **3.15. Significance of the study**

Zoonotic infectious diseases and antimicrobial resistance are globally taking their toll and are difficult to treat with the current antibiotics. The study will contribute to the global One Health initiative objective of developing guidelines and policies, restricting antibiotic usage in poultry, and eradicating antibiotic resistance. This will enhance treatment options in treating zoonotic infectious diseases. Furthermore, policymakers in agriculture would benefit from the study when reviewing the policies on the management of antibiotic agents or establishing one, if not any. If policies are being reviewed or instituted in poultry, they will help poultry farmers to have proper Standard Operating Procedures (SOP) regarding biosafety, biosecurity, antibiotics stewardship, chicken environment, nutrition, and hygiene houses. The study will also help the veterinary department in monitoring the poultry farms' practices for farmers to adhere to the prescribed SOP. All this will ultimately help in curtailing the silent antibiotic resistance pandemic and protect the few agents that are currently in use in human beings.

### **3.16. Ethical considerations**

Ethical clearances were acquired from the Turfloop Research Ethics Committee (TREC) Ref No: TREC/540/2022: PG and University of Limpopo Animal Research Ethics Committee (AREC) Ref No: AREC/01/2023: PG and the Agricultural Research Council Animal Research Ethics Committee before initiating the project. With the letters of clearance, the Section 21 permission letter to carry out the study was acquired from the Department of Veterinary in Limpopo Province. Thus, the study was carried out following ethical standards for the welfare of animals.

To obtain consent from the participants, detailed written details of the investigation, which comprised the aim, objectives, and purpose of the study were provided. For those who could not understand English, everything was translated for them written consent was obtained and interviews were carried out with the participants before being anonymous and unlinked the details that the participants exchanged were kept confidential, and only accessible to authorized people at all times. Participation in this study was voluntary. There was no compensation given to participants." The chickens were slaughtered humanely as stipulated by the AREC of the University of Limpopo.

## CHAPTER 4

### RESULTS

#### Introduction

This chapter covers the various descriptions of the results obtained from the interviews with farmers and farm workers at the three farms that participated in the study. Microbiological results obtained from chicken gut and droppings are described. These include the most common microorganisms identified and their sensitivity to the most frequently used antimicrobial agents in human medicine. Finally, the results obtained from a participatory action method, and focus group discussion in the form of seminars are also described.

#### 4.1. Demographic characteristics of the participants

A total of 129 farm workers participated in the study, which assessed their knowledge, perceptions, and practices regarding antibiotic use in the presence of farm managers. Most of the participants were from Lunds (36.4%) and Spif's Chicken (34.1%), with fewer participants from Mike's Chicken (29.5%).

Table 4.1: Distribution of participants per farm

	No	%
Lunds	47	36.4
Mike's Chicken	38	29.5
Spif's Chicken	44	34.1

Table 4.2: Age and Gender distribution of participants

	No	%
Age		
<30	47	36.4
30-39	52	40.3
40-49	26	20.2
50+	4	3.1
Gender		
Male	79	61.2
Female	50	38.8

Overall, the majority of the participants (76.7%) were younger than 40 years old, and 61.2% were males (Table 4.2).

Table 4.3: Age and sex distribution of participants per farm

Age	Lunds		Mike's Chicken		Spif's Chicken	
	No	%	No	%	No	%
<30	17	36.2	10	26.3	20	45.5
30-39	20	42.6	15	39.5	17	38.6
40-49	10	21.3	10	26.3	6	13.6
50+	0	0.0	3	7.9	1	2.3
Gender						
Male	29	61.7	25	65.8	25	56.8
Female	18	38.3	13	34.2	19	43.2

The age and sex distribution of the participants per farm is presented in Table 4.3, indicating that the age and sex composition were comparable across farms. At Spif's Chicken, the largest proportion age group was 45.5%, followed by 36.2% at Lunds, and 26.3% at <30 years old. Similarly, the biggest percentage of the age group was 42.6% at Lunds, 39.5% at Mike's Chicken, and 38.6% in the 30- to 39-year-old range. Lastly, at Mike's Chicken, the largest proportion age group is 26.3%, followed by 21.3% at Lunds, and 13.6% at Spif's Chicken at 40-49 years of age.

#### 4.2. Knowledge and perception regarding antibiotic use

It seems that a significant portion of chicken feeders, 58%, were unaware of the specific ingredients in the chicken feed, while 42% had knowledge of what the feed contained, according to the results shown in Figure 4.1.

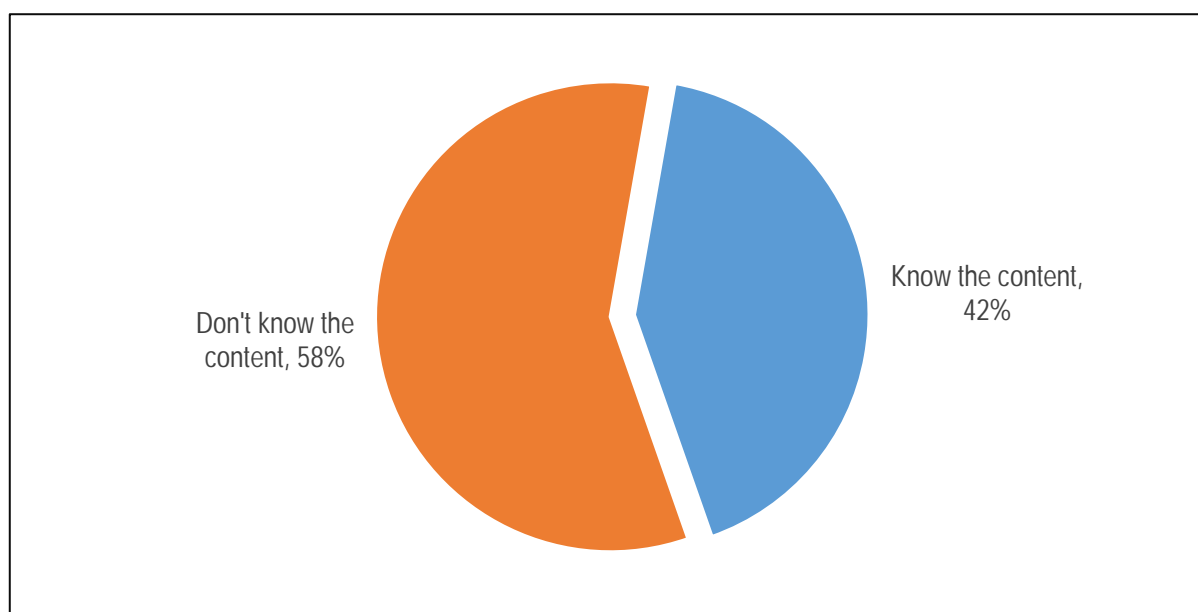


Figure 4.1: Knowledge of chicken feeders on chicken feeds

As shown in Table 4.2, there was no significant difference between the three farms regarding the level of knowledge of chicken feeds ( $p > 0.05$ ). However, the chicken feeders at Mike's Chicken were less likely to be knowledgeable as compared to the other chicken feeders at the two poultry farms.

Table 4.4: Level of knowledge of chicken feed farmworkers

Farms	n	Knowledgeable		p-value
		Yes, n (%)	No, n (%)	
Lunds	47	42.6	57.4	0.937
Mike's Chicken	38	39.5	60.5	
Spif's Chicken	44	43.2	56.8	

It was found out that chicken feed pellets were manufactured at the farms following Standard Operating Procedure, (SOP) based on ingredients purchased from chicken feed suppliers. The chicken feed formula was a guarded secret at each farm. One of the farmers indicated that over 80% of the costs associated with producing chickens are incurred in producing chicken feed.



Figure 4.2: Self-manufacturing chicken pellets

Poultry farmers' production of their own feed can reduce expenses and increase the farm's profit margin. Figure 4.2 illustrates the appearance of chicken feed pellets after manufacturing. However, producing pellets requires costly machinery.

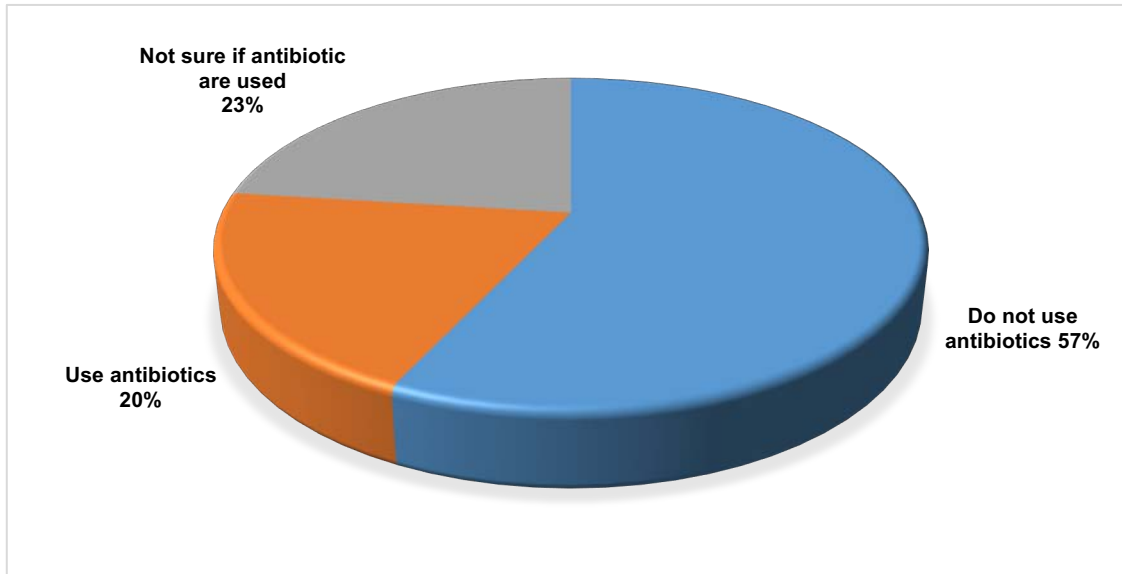


Figure 4.3: Chicken feeder's perception of antimicrobial agent use

According to Figure 4.3, among the 129 feeders interviewed, 57% stated that they do not use antimicrobial agents in poultry, 20% admitted to using these agents, and 23% were unsure whether antimicrobial agents were used or not.

### 4.3. Practice regarding antibiotic use

Table 4.5: Chicken feeders' perception of antimicrobial agents use

Farms	n	Perception			p-value
		Use antibiotic, n (%)	Don't Use antibiotic, n (%)	Not sure if antibiotic are used, n (%)	
Lunds	47	21	55	24	0.937
Mike's Chicken	38	19	58	23	
Spif's Chicken	44	21	57	22	

Table 4.3 articulates the distribution of the perception of antimicrobial agent use at the three farms.

Table 4.6: The most used antimicrobial agents

Farms	n	Antimicrobial agents used		p-value
		Penicillin, n (%)	Tetracycline, n (%)	
Lunds	47	67	33	0.937
Mike's Chicken	38	64	36	
Spif's Chicken	44	69	31	

The questionnaire conducted indicated that 67% of the feeders from Lunds, 64% from Mike's Chicken, and 69% from Spif's Chicken agreed that penicillin was the most used antibiotic while 33% from Lunds, 36% from Mike's Chicken, and 31% from Spif's Chicken maintained that tetracycline was the mostly used antibiotics as illustrated in Table 4.6.

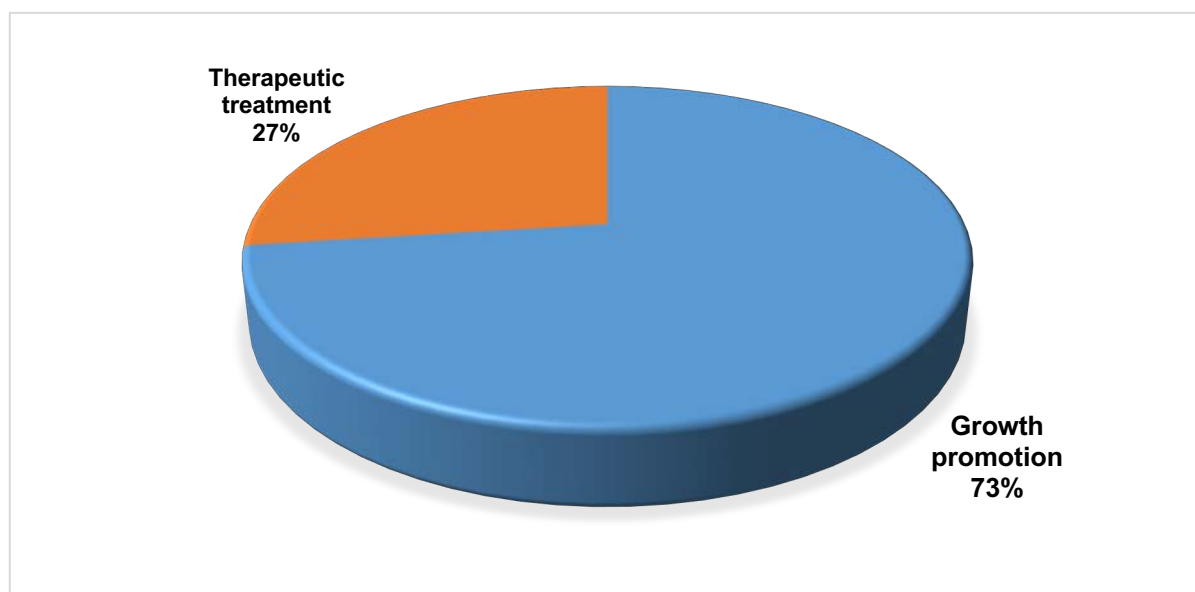


Figure 4.4: Antimicrobial agent usage in poultry

The pie chart above in Figure 4.4 indicates that the common indication of antimicrobial agents was for growth promotion 73%, while 27% maintained that they used antimicrobial agents for treatment of infectious diseases.

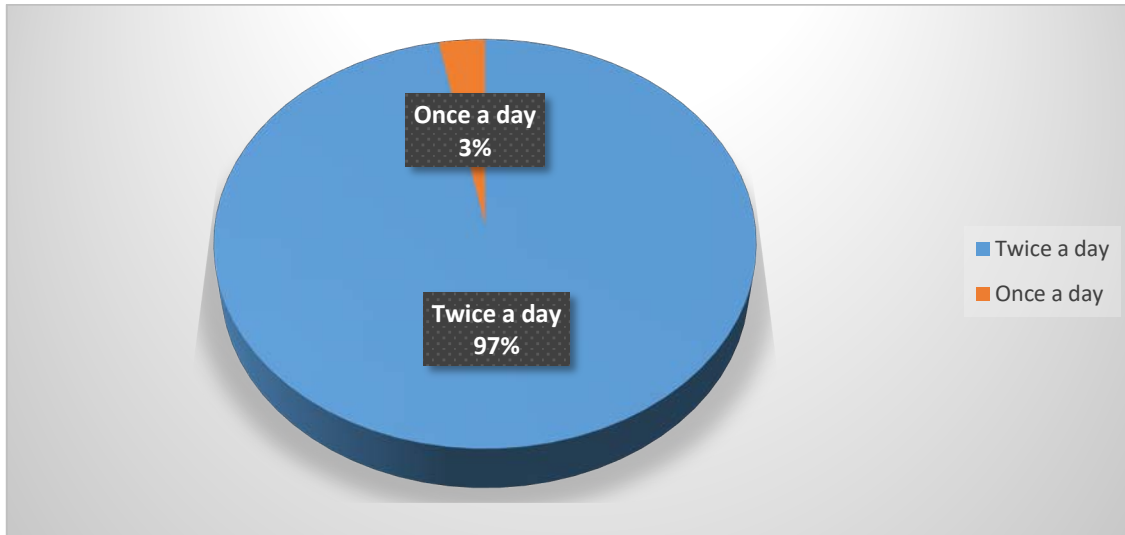


Figure 4.5: The administration frequency of antimicrobial agents

The pie chart in Figure 4.5 above indicates the administration frequency of antimicrobial agents was 97% twice a day and 3% once daily.

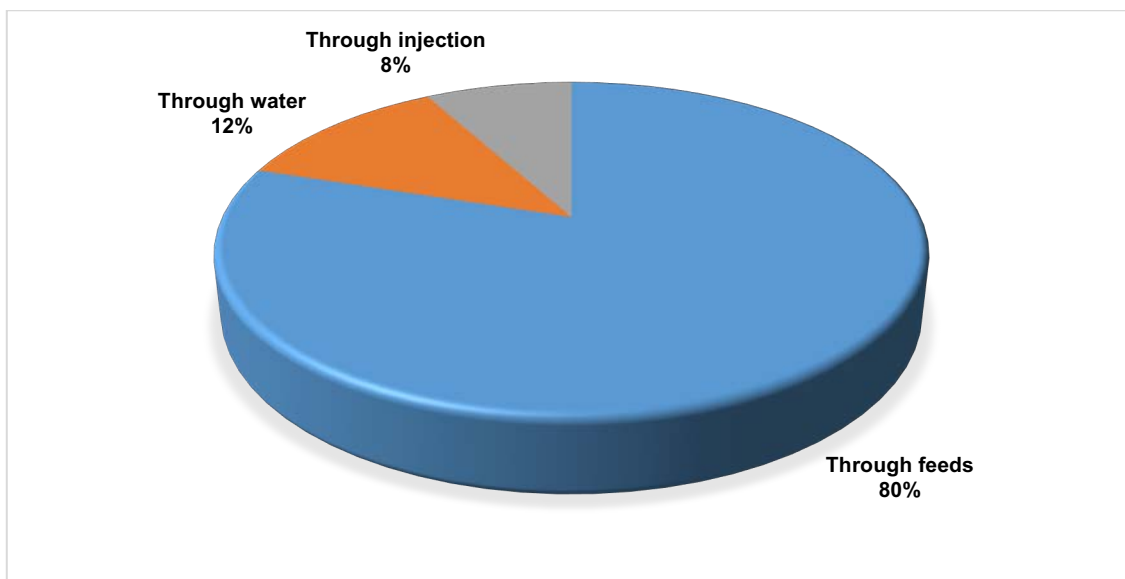


Figure 4.6: The route of administration of antimicrobial agents in poultry

The pie chart above in Figure 4.6 indicates the method of antimicrobial administration, of which 80% was through the feed, 12% was through water and 8% was through injection.

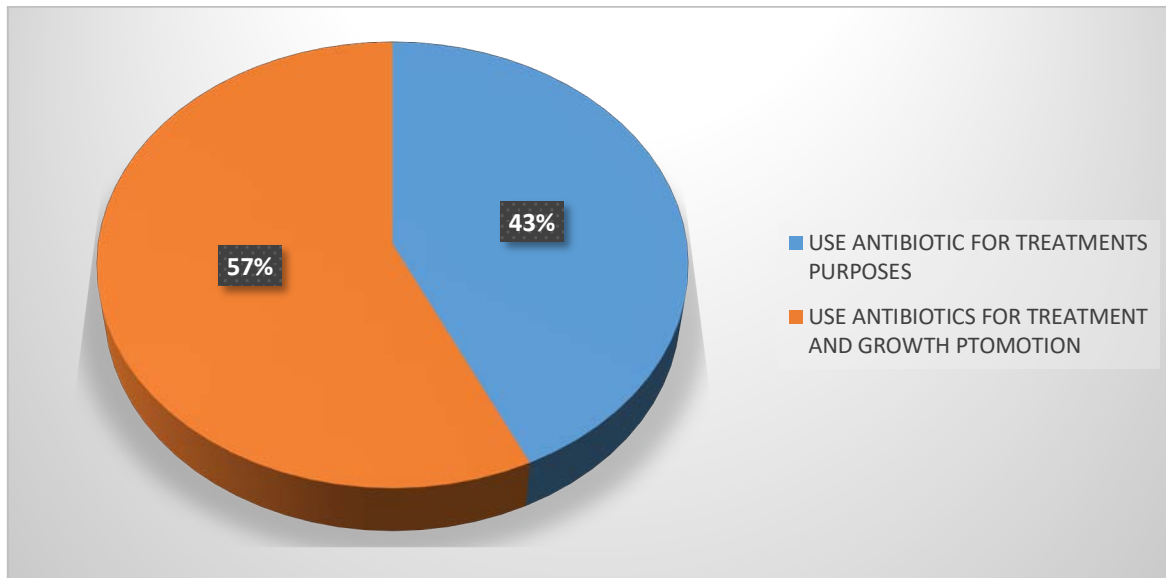


Figure 4.7: Illustration of the use of antibiotics by chicken feeders

The pie chart in Figure 4.7 indicates that 43% of the chicken feeders use antibiotics for therapeutic purposes whereas 57% use antibiotic for both therapeutic and growth promotion.

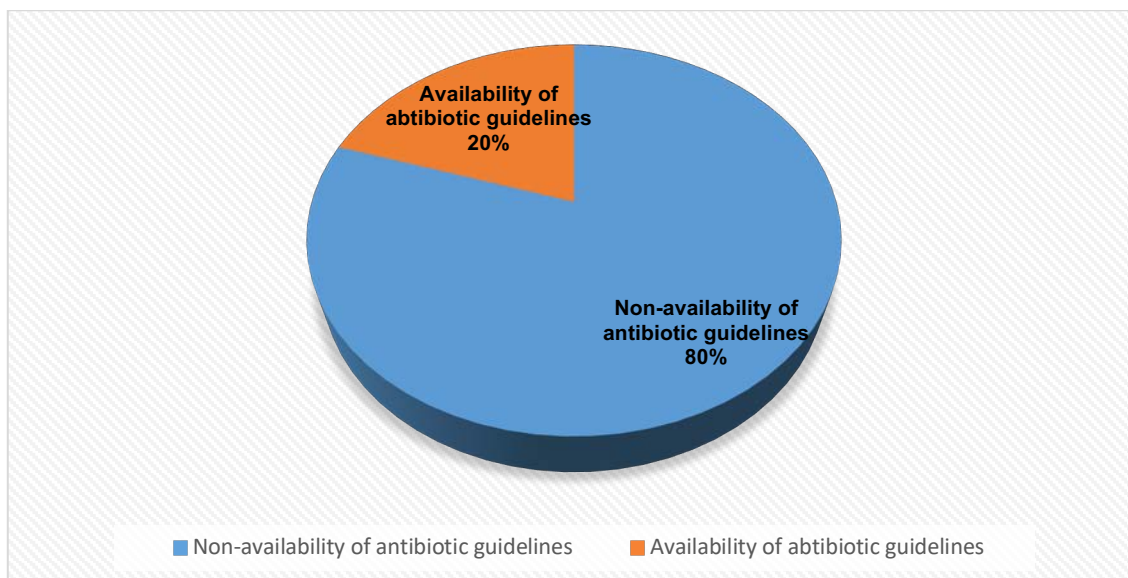


Figure 4.8: Availability of antimicrobial agents' guidelines perceived by chicken feeders

From the results, Figure 4.8 indicates that 80% of the feeders maintained that there are no antimicrobial guidelines whereas 20% said there are antimicrobial guidelines.

From the results, two of the veterinarians illustrated that there are antimicrobial agents' guidelines whereas one indicated that there are no guidelines.

Out of the three veterinarians overseeing the commercial poultry in and around Polokwane, two of the veterinarians maintained that they visit the poultry farms every quarter whereas one maintained that he visits the farms when there is a need once in a while.

#### 4.4. Culture and sensitivity testing

Table 4.7: Microorganisms at farms and their prevalence

FARM 1 (N=10)		FARM 2 (N=10)		FARM 3 (N=10)	
Microorganisms	%	Microorganisms	%	Microorganisms	%
<i>E. coli</i>	40%	<i>E. coli</i>	92%	<i>E. coli</i>	86%
<i>Citrobacter braakii</i>	15%	<i>Klebsiella rhinoscleromatis</i>	8%	<i>Enterobacter cloacae complex</i>	14%
<i>Pseudomonas fluorescens</i>	15%				
<i>Proteus mirabilis</i>	10%				
<i>Pseudomonas luteola</i>	10%				
<i>Salmonella typhumurium</i>	10%	<i>Salmonella typhumurium</i>	5%	<i>Salmonella typhumurium</i>	5%
<i>Enterobacter cloacae complex</i>	5%				
<i>Providencia rettgeri</i>	5%				
<i>Shigella</i>	5%	<i>Shigella</i>	5%	<i>Shigella</i>	5%

The most prevalent microorganisms in Farm 1 were *E. coli* at 40%, *Citrobacter braaki* and *Pseudomonas fluorescens* at 15%, *Proteus mirabilis* and *Pseudomonas luteola* at 10%, and *Enterobacter cloacae complex* and *Providencia rettgeri* at 5%. In Farm 2, *E. coli* and *Klebsiella rhinoscleromatis* were the most prevalent microorganisms at 92% and 8% respectively. Lastly in Farm 3, *E. coli* and *Enterobacter cloacae complex* were the most prevalent microorganisms at 86% and 14% respectively.

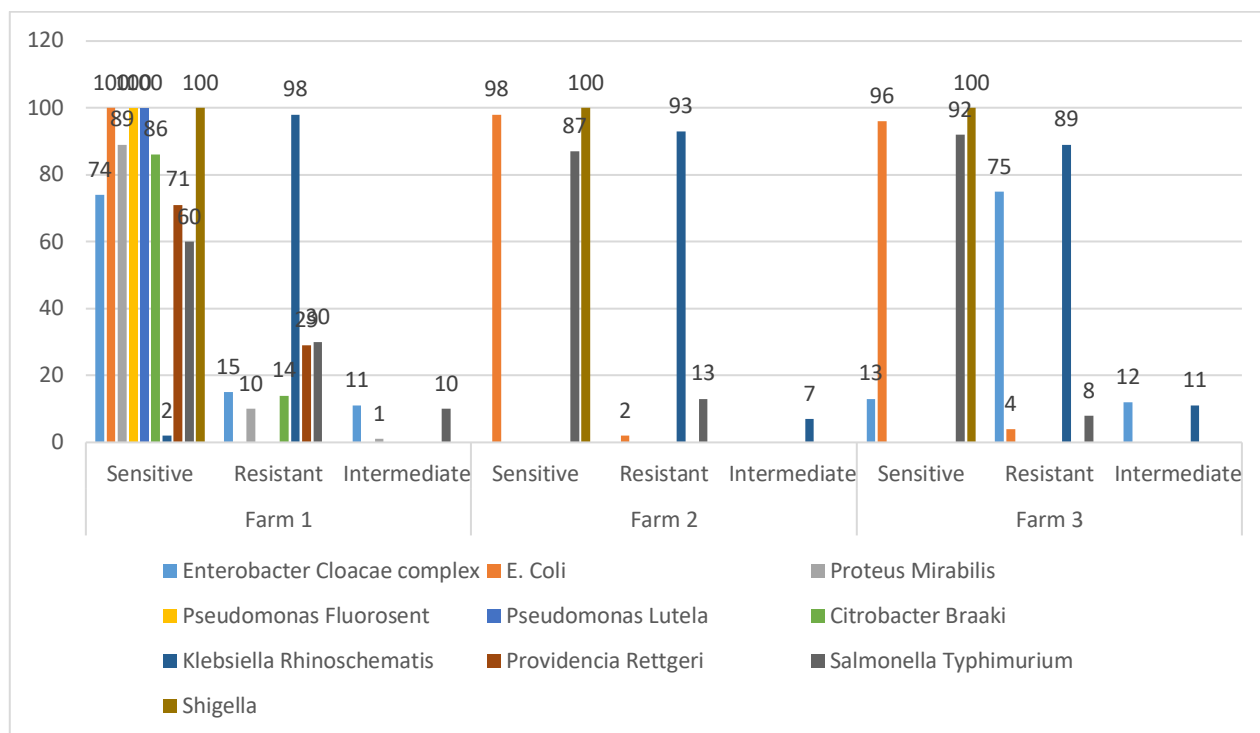


Figure 4.9: Microorganisms found from culture and sensitivity tests

The most prevalent microorganism from the farms was *Enterobacter cloacae complex* which was 15% multi-pharmacological phenotypic resistant to amoxycillin and cefoxitin in Farm 1 and 75% resistant to amoxicillin clavulanic, tazobactam, cefuroxime, cefuroxime, cefoxitin, cefotaxime, ceftazidime, cefepime, and ertapenem in Farm 3 as indicated in Figure 4.9. *Providencia rettgeri* was 29% resistant to ampicillin, cefuroxime, tigecycline, and nitrofurantoin. In Farm 1, *Salmonella Tyhimurium* was 30% resistant to augumentin and gentamycin and 60% sensitive to ampicillin. Furthermore, *Salmonella Tyhimurium* was 13% and 8% resistant to augumentin in Farm 2 and 3 respectively. In Farm 1, *Citrobacter braaki* was 14% resistant to amoxycillin, cefoxitin. In Farm 2, *Salmonella Tyhimurium* was 13% resistant to augumentin. In Farm 2, *E. coli* was 2% resistant to ampicillin, cefuroxime, and ciprofloxacin, whereas in Farm 3 it was 4% resistant to ampicillin and Trimethoprime/Sulphamethoxazole. *Proteus mirabilis* was 10% resistant to tigecycline and nitrofurantoin. *Klebsiella Rhinoschematis* was found to be 98 %, 93%, and 89% resistant to ampicillin and amoxycillin in Farm 1,2, and 3 respectively. *Shigella* trains were found to be 100% susceptible to ciprofloxacin and amikacin.

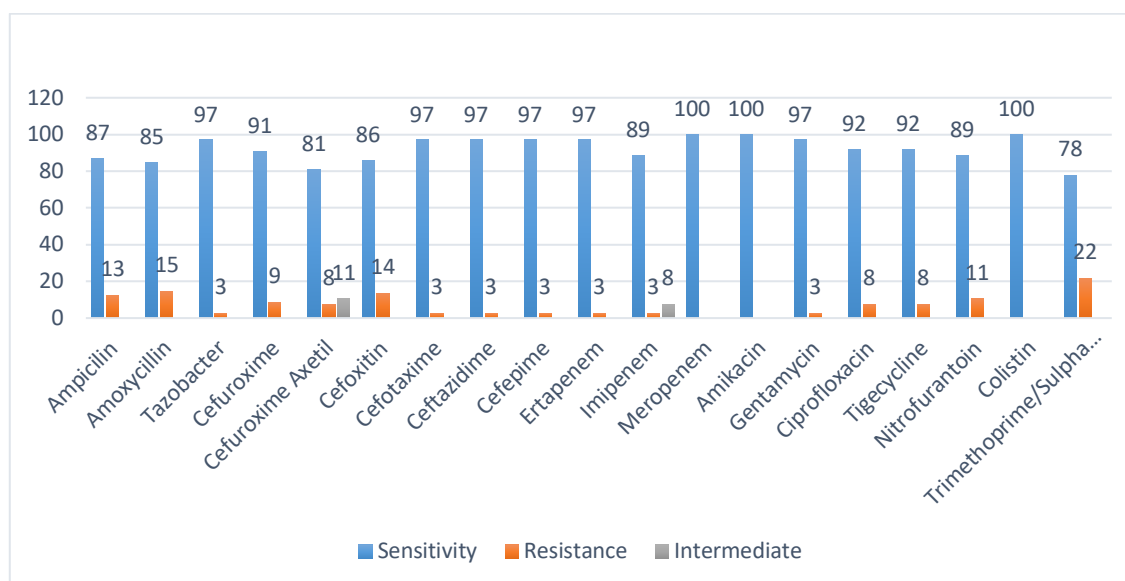


Figure 4.10: Antibiotics used and their culture and sensitivity tests

From the results in Figure 4.10, the most prevalent microorganisms such as *Citrobacter braaki*, *Enterobacter cloacae complex*, *Providencia rettgeri*, and *Proteus miralis* were found to be resistant to Amoxicillin, Cefuroxime, Cefoxitin, Ertapenem, Imipenem, Ciprofloxacin, Tigecycline, Nitrofurantoin, and Trimethoprim/Sulphamethoxazole.

Table 4.8: XLD agar plates for Farm 1

Agar plates	Growth/ no growth	Colour
F1C1	Growth	Black colonies
F1C2	Growth	Black colonies
F1C3	Growth	Black colonies
F1C4	Growth	Black colonies
F1C5	Growth	Black colonies
F1C6	Growth	Black colonies
F1C7	Growth	Black colonies
F1C8	Growth	Black colonies
F1C9	Growth	Black colonies
F1C10	Growth	Black colonies

From Farm 1 on the XLD agar plates, there were black colonies which indicated that there was *Clostridium perfringens* growth.

Table 4.9: TCBS agar plates for Farm 1

<b>Agar plates</b>	<b>Growth/ no growth</b>	<b>Colour</b>
F1C1	Growth	Yellow colonies
F1C2	Growth	Yellow colonies
F1C3	Growth	Yellow colonies
F1C4	Growth	Yellow colonies
F1C5	Growth	Yellow colonies
F1C6	Growth	Yellow colonies
F1C7	Growth	Yellow colonies
F1C8	Growth	Yellow colonies
F1C9	Growth	Yellow colonies
F1C10	Growth	Yellow colonies

From Farm 1 on the TCBS agar plates, there were yellow colonies which indicated that there was *E coli* growth.

Table 4.10: MacConkey and CV agar plates for Farm 1

<b>Agar plates</b>	<b>Growth/ no growth</b>	<b>Colour</b>
F1C1	Growth	Pink colonies
F1C2	Growth	Pink colonies
F1C3	Growth	Pink colonies
F1C4	Growth	Pink colonies
F1C5	Growth	Pink colonies
F1C6	Growth	Pink colonies
F1C7	Growth	Pink colonies
F1C8	Growth	Pink colonies
F1C9	Growth	Pink colonies
F1C10	Growth	Pink colonies

From Farm 1 on the MacConkey and CV agar plates, there were pink colonies which indicated that there was *Enterobacteriaceae* growth.

Table 4.11: MacConkey agar plates for Farm 1

<b>Agar plates</b>	<b>Growth/ No growth</b>	<b>Colour</b>
F1C1	Growth	Red/pink colonies
F1C2	Growth	Red/pink colonies
F1C3	Growth	Red/pink colonies
F1C4	Growth	Red/pink colonies
F1C5	Growth	Red/pink colonies
F1C6	Growth	Red/pink colonies
F1C7	Growth	Red/pink colonies
F1C8	Growth	Red/pink colonies
F1C9	Growth	Red/pink colonies
F1C10	Growth	Red/pink colonies

From Farm 1 on the MacConkey agar plates, there were red/pink colonies which indicated that there was *Enterobacteriaceae* growth.

Table 4.12: Culture and sensitivity testing on *Shigella* for Farm 1

<b>Plate number</b>	<b>Ampicillin</b>	<b>Tetracycline</b>	<b>Ceftriaxone</b>	<b>Ciprofloxacin</b>
F1C1	Resistant	Resistant	Susceptible	Susceptible
F1C2	Resistant	Resistant	Susceptible	Susceptible
F1C3	Intermediate resistant	Intermediate resistant	Susceptible	Susceptible
F1C4	Resistant	Intermediate resistant	Susceptible	Susceptible
F1C5	Resistant	Resistant	Susceptible	Susceptible
F1C6	Resistant	Resistant	Susceptible	Susceptible
F1C7	Resistant	Resistant	Susceptible	Susceptible
F1C8	Resistant	Intermediate resistant	Susceptible	Susceptible

F1C9	Intermediate resistant	Intermediate resistant	Susceptible	Susceptible
F1C10	Intermediate resistant	Intermediate resistant	Susceptible	Susceptible

From Farm 1 on the Culture and sensitivity testing on *Shigella*, it was resistant to ampicillin, and tetracycline and susceptible to ceftriaxone and ciprofloxacin.

Table 4.13: Culture and sensitivity testing *Salmonella Typhimurium* for Farm 1

<b>Plate number</b>	<b>Amikacin</b>	<b>Augmentin</b>	<b>Ceftriaxone</b>	<b>Gentamycin</b>
F1C1	Susceptible	Resistant	Intermediate resistant	Susceptible
F1C2	Intermediate resistant	Resistant	Resistant	Intermediate resistant
F1C3	Susceptible	Resistant	Susceptible	Susceptible
F1C4	Susceptible	Intermediate resistant	Resistant	Susceptible
F1C5	Susceptible	Susceptible	Intermediate resistant	Susceptible
F1C6	Susceptible	Resistant	Resistant	Susceptible
F1C7	Intermediate resistant	Intermediate resistant	Susceptible	Susceptible
F1C8	Susceptible	Resistant	Susceptible	Susceptible
F1C9	Susceptible	Intermediate resistant	Resistant	Intermediate resistant
F1C10	Intermediate resistant	Resistant	Intermediate resistant	Susceptible

From Farm 1 on the Culture and sensitivity testing *Salmonella tryphimurium*, was resistant to augumentin and ceftriaxone, on the other hand, susceptible to amikacin and gentamycin.

Table 4.14: Blood agar plates for Farm 2

<b>Agar plates</b>	<b>Growth/no growth</b>	<b>Colour</b>
F2C1	No growth	-
F2C2	No growth	-
F2C3	No growth	-
F2C4	No growth	-
F2C5	No growth	-
F2C6	No growth	-
F2C7	No growth	-
F2C8	No growth	-
F2C9	No growth	-
F2C10	No growth	-

From Farm 2 on blood agar plates, no growth was detected on the plates.

Table 4.15: XLD agar plates for Farm 2

<b>Agar</b>	<b>Growth/no growth</b>	<b>Colour</b>
F2C1	Growth	Black and pink colonies
F2C2	Growth	Black and pink colonies
F2C3	Growth	Black and pink colonies
F2C4	Growth	Black and pink colonies
F2C5	Growth	Black and pink colonies
F2C6	Growth	Black and pink colonies
F2C7	Growth	Black and pink colonies
F2C8	Growth	Black and pink colonies
F2C9	Growth	Black and pink colonies
F2C10	Growth	Black and pink colonies

From Farm 2 on the XLD agar plates, there were black and pink colonies which indicated that there was *Salmonella* and *Shigella* growth.

Table 4.16: Blood agar plates for Farm 3

<b>Agar plates</b>	<b>Growth/no growth</b>	<b>Colour</b>
F3C1	No growth	-
F3C2	No growth	-
F3C3	No growth	-
F3C4	No growth	-
F3C5	No growth	-
F3C6	No growth	-
F3C7	No growth	-
F3C8	No growth	-
F3C9	No growth	-
F3C10	No growth	-

From Farm 3 on blood agar plates, no growth was detected on the plates.

#### **4.5. Genome DNA sequencing**

The Whole Genome Sequencing was performed by the Agricultural Research Council; however, the outcomes were indeterminant and inconclusive due to the unserviceability of machinery.

#### **4.6. Participatory feedback method**

The following were some of the veterinarians' remarks regarding the usage of antimicrobial agents in poultry from a focus group that the One Health Organization conference convened with veterinarians at the Universities of Fort Hare and Limpopo and the veterinarians overseeing the poultry farms in Limpopo Province:

*'The farmers always use antibiotics rationally as they know they are beneficial for their chickens.'*

*'I do believe in prophylactic treatments because there are too many times where you try and not use antibiotics and then you end up with a bad mortality.'*

*'I think the one [prescribing practice] that we as poultry veterinarians are weak on are the habitual repeat users. It's the repeated in feed prescription that's the issue, isn't it? I'm as guilty as the next man of that.'*

*'Antimicrobial resistance is undoubtedly a problem in human medicine, and I believe they are scapegoating us for it. I believe that for now, all we need to do to follow the party line is appear to be conforming or cutting back on our usage.'*

*'My opinion, personally, is that if the doctors and the human health control was more under control, we would probably get less resistance.'*

*'I think there's a greater danger when they're dished out like Smarties in GP practices for somebody with a common cold.'*

*'Should we be saying we shouldn't be letting humans have antibiotics? In terms of why are we so hell-bent on stopping animals when it's the humans themselves in some respects that are causing all their problems?'*

*'What is the role of veterinarians in Antimicrobial Stewardship committees?'*

The chairperson of the South African Poultry Association (SAPA) iterated that *"poultry farmers are voluntarily joining SAPA"*, and after some engagement and intent to take it up with International Poultry Council (IPC), he then confirmed that *'SAPA is a member of IPC'*.

During data collection, it was observed that IPC has clear guidelines to be followed in poultry farming by farmers and SAPA is a member of the IPC. However, at the three farms, the guidelines were not followed, in particular, at Farm 3, the food disinfectant trays were not used and bypassed.

## CHAPTER 5

### DISCUSSION, CONCLUSION AND RECOMMENDATIONS

#### 5.1. DISCUSSION

From the results, it was observed that the most prevalent microorganism from the farms was *Enterobacter cloacae complex* which was 15% multi-pharmacological phenotypic resistant to amoxicillin and ceftazidime in Farm 1 and 75% resistant to amoxicillin clavulanic, tazobactam, cefuroxime, cefuroxime, ceftazidime, ceftazidime, cefepime, and ertapenem in Farm 3 as indicated in Figure 4.9. *Providencia rettgeri* was 29% resistant to ampicillin, cefuroxime, tigecycline, and nitrofurantoin. In Farm 1, *Salmonella Tyhimurium* was 30% resistant to augumentin and gentamycin and 60% sensitive to ampicillin. Furthermore, *Salmonella Tyhimurium* was 13% and 8% resistant to augumentin in Farm 2 and 3 respectively. In Farm 1, *Citrobacter braaki* was 14% resistant to amoxicillin, ceftazidime. In Farm 2, *Salmonella Tyhimurium* was 13% resistant to augumentin. In Farm 2, *E. coli* was 2% resistant to ampicillin, cefuroxime, and ciprofloxacin, whereas in Farm 3 it was 4% resistant to ampicillin and Trimethoprim/Sulphamethoxazole. *Proteus mirabilis* was 10% resistant to tigecycline and nitrofurantoin. *Klebsiella Rhinoschematis* was found to be 98 %, 93%, and 89% resistant to ampicillin and amoxicillin in Farm 1,2, and 3 respectively. *Shigella* strains were found to be 100% susceptible to ciprofloxacin and amikacin.

Before going into interpretation and analysis of the results obtained from the study, it is important to put into context some aspects of commercial poultry farms. The aim is to shed light on some observations made during transacting walks at the farms.

When it comes to each chicken while growing, within the gut, there are numerous interactions between the bird, microorganisms, and digestion (Zoetendal et al., 2004). That is why gut contents were used in the microbiota analysis. Through a process known as competitive exclusion, the gut's microbiota forms a barrier that prevents harmful germs from attaching themselves to the host cells (Mogotlane et al., 2023). The poultry business is concerned about enteric infections because they can lead to production losses, decreased bird welfare, higher bird mortality, and contaminated goods intended for human consumption (Petrovska et al., 2016). Zoonosis, as clearly articulated earlier,

is the spread of contagious illnesses from animals to people. It must also be borne in mind that the transmission can be the other way around. Zoonotic bacterial infections can negatively impact animal husbandry, food safety, and most importantly public health. Hence the promotion of the One Health approach whose aim is to see to it that AMAs are used judiciously. This is clearly spelled out as strategic objective 5 of the South African AMR National Strategy Framework which speaks to the promotion of appropriate usage of antimicrobials in humans and animals (Shabangu et al., 2023).

#### 5.1.1. **Chicken gut microbiota and the need to preserve it**

From the study, it was found that each farm slaughtered broiler chicken whilst juvenile and immature containing very few microorganisms in their gut, which the immune system is not fully developed. This was important because gut microorganisms can be separated into species that have either beneficial or negative (pathogenic) effects such as nutrient absorption. For example, in the gut, harmful microorganisms are constantly present, but the ratio of pathogenic to non-pathogenic bacteria will have a significant impact on the disease and the chicken's study performance (Mokgotlane et al., 2023). Numerous investigations have been conducted to create and give freshly hatched chicks from mature hens either single species or complicated mixes of bacterial species. In a study by Zhu et al. (2021), it was discovered that *Salmonella* could only be suppressed by a complicated mixture of bacteria. They were unable to determine which species provided the inhibition, though.

It has been shown that when a pathogenic species colonizes an area, it can create favourable conditions for other infections, for example, those that lead to necrotic enteritis. *Clostridium perfringens* Type A and C are said to be responsible for the disease in young broilers which is critical to avoid, and hence the secret use of antibiotics. It is reported that the levels of necrotic enteritis dramatically increased after the banning of antibiotic growth promoters, showing that antimicrobials had a prophylactic effect in controlling the disease (Dugget, 2016). The spores of *C. perfringens* are ubiquitous in the environment and are therefore ingested via poultry feed regularly which is why poultry farmers were keen to prepare their feeds. From the present study, it was likely that this was missed by the farm workers and therefore would need their involvement in coming up with regulations that would control antibiotic use in chicken feed. That way it

would also prevent them from acquiring any form of infection resistant to the antibiotics used in human medicine.

It was observed that the majority of the chicken farm workers did not know the specific ingredients as shown in Tables 5.1 and 5.2 of the chicken feeds which were kept as secret by the farm management. Another disease that poultry farmers would be keen to prevent by secretly adding antimicrobial agents in the feed is coccidiosis without the supervision of veterinarians. Coccidiosis, a protozoal disease that causes diarrhoea, weight loss, and decreased production in poultry has been studied in domesticated animals for over a century. These studies have demonstrated mortality, interruption of digestive processes and nutrient absorption, increased susceptibility to other diseases (such as necrotic enteritis), and reduced weight gain caused by the multiplication of these protozoan parasites. The severity of the lesions caused by the disease is dependent on the number of oocysts ingested.

Chickens are natural reservoirs for *Campylobacter* species as an *Enterobacter cloacae* complex, especially in slaughter-age broiler flocks, and can reach as high as 100% on some farms and no farmer would like that despite the species being a commensal. Even though *Campylobacter* is insignificant for poultry health, it is however a leading cause of food-borne gastroenteritis in humans worldwide (Hakeem and Lu, 2021). The transmission of *Campylobacter* is primarily through broiler flocks due to faecal shedding and coprophagia. However, it can also occur through feed and water which the farmer would be keen to avoid if it affects the bottom line of his business. Poultry farmers have to adopt supervised veterinary services and on-farm intervention strategies that do not require the use of antibiotics against *Campylobacter*. This will ultimately help reduce the AMR spread and still maintain essential microbiota in the chicken intestines needed for feed conversion.

The microbiota of high feed-conversion ratio (FCR) and low FCR birds have been compared in an attempt to identify the species that are more abundant in the high FCR birds (Dugget, 2016). FCR is calculated by dividing the average feed intake by the average weight gain as shown below:

$$FCR \left( \frac{DMF}{LWG} \right) = \frac{AFI}{AWG}$$

DMF: Dry Matter Feed

LWG: Live Weight Gain

AFI: Average Feed Intake

AWG: Average Weight Gain

Feed conversion ratio (Dry Matter feed/live weight gain) = Average feed intake/Average weight gain (McDonald et al., 2010).

This type of calculation would only be done at the management level at factory farms in order to monitor how the chicken production was going which also required close supervision or advice from a veterinarian. This would include a decision on what type of chicken feed content to be added in chicken feed which contributed almost 80% of the production leading to an obviously a big concern for the poultry industry business.

#### 5.1.2. **Chicken feeding formulations**

One of the main factors influencing success in the chicken farming industry is the feed quality and formulation systems since about 80% accounts for poultry farming production. To achieve the intended growth rate, the farmer must buy and supply extremely nutritious chicken feed, especially for broiler chickens which are bred mainly for their meat. Generally, the hybrid breeds used to produce broiler chickens are meant to grow rapidly and are widespread within factory farms around the world. Regrettably, fast-growing chickens not only have inferior welfare outcomes but also yield meat of lower quality (Liu et al., 2020). As a result, poultry farming is an ever-growing business and high demand for meat possibly contributes to shortcuts if there is no monitoring which was the case observed in the present study. The hybrid bred in farms studied were Plymouth selectively bred to possess certain desirable traits, such as growing larger breast muscles, which are later sold as “white meat” or “chicken breasts” in most chicken outlets and restaurants in South Africa.

According to Eze (2016), broiler chickens are, generally, claimed to need various feeds according to the calorie, protein, and mineral requirements at different phases of their growth as shown in Tables 5.1 and 5.2. It was not surprising, therefore that in order to maximize output, farmers would modify their feed regimens accordingly, in other words, chickens that grow fast. In the poultry industry, the lives of broiler chickens are cut drastically short once they have reached their target weight for slaughter. In the EU the average slaughter age is 42 days, as compared to 47 days in the US according to the Global Animal Partnership organization (Chan et al., 2022), there is now a global push to promote slow-growing chickens and concerns about their welfare on farm factories. This can be argued, indeed, is an aspect of the One Health approach whose definition has expanded over the years and may result in less use of antibiotics if appropriate smart regulations are developed.

Table 5.1: Formula for broiler chicken feeds for 1-4 weeks (Jones et al., 1995)

<b>Formula for 100kg of Broiler starter feed (1-4 weeks)</b>
57.2kg of whole maize
17.2kg of fishmeal
20kg of soya bean meal
5.8kg of lime
140g of premix (e.g. Vitamins and minerals, toxin binder, and growth enhancers)
Amino acids to add
70g of lysine
70g of threonine

It can be seen from the formulation that in the first 4 weeks as shown in Table 5.1, after the chicks have hatched there is no antibiotic in their feed. However, one cannot be sure if there is no antibiotic egg injection to eliminate diseases such as mycoplasma. Vertical transmission to both egg and shell from parent hen to broiler chick has been reported (Jansen et al., 2020). It would be reasonable to assume that this is a piece of information that might be known to poultry farmers. If the following statement made by a veterinarian at a feedback meeting is anything to go by. Be that as it may, assuming that there are indeed no chances of injudicious use of antibiotics earlier, as can be seen in Table 5.2, it appears there is a general recommendation to include zinc-bacitracin in the feed after 4 weeks irrespective of the feed formulation followed.

This is an internet recommendation from virtual animal nutritionists which poultry factory farm managers would be aware of and cannot be supervised by veterinarians or veterinary technicians. Given that this might be going on since according to the results obtained from the study, the farmers kept secret how they formulated their feed, command or control regulations would not be effective. According to Selden (2010), with regard to the Pharmacy Law Compendium, registration of farm foods, fertilizers, and agricultural medicines:

- a. Applications for agricultural remedies, farm feed, and fertilizers registration must be submitted and must be submitted to the registrar using the approved form and must include the approved application fee.
- b. Anyone submitting an application for registration under paragraph (a) must provide or make available to the registrar the samples and information he needs, in the way, at the time, and at the location he designates.

After reviewing any such application and conducting any research and inquiries he sees fit, the registrar will only proceed if he is confident that:

- a. If it is found that a fertilizer, farm feed, or agricultural remedy is appropriate and sufficiently effective for the intended applications and that registering it is not against the public interest, the proposed registration will be handled, and if the manufacturing facility is judged suitable for producing such products.

The only way to achieve this objective would be to emphasize compliance with the existing laws and/or develop smart regulations by involving, animal nutritionist organizations, feed producer companies, chicken buyers such as fast-food companies, and animal welfare advocates.

Table 5.2: Formula for broiler chicken feeds of >4 weeks (Jones et al., 1995)

<b>Formula for 100kg of Broiler finisher feed (&gt;4weeks)</b>	
Option 1	Option 2
Maize: 44Kg	Whole maize: 14.5kg
Soybean Full Fat: 34Kg	Maize germ: 24kg
Groundnut Cake: 2kg	Wheat pollard: 19kg
Brewer Dry Grain: 12Kg	Wheat bran: 14.4kg
Maize Offal: 4kg	Beniseed meal, groundnut cake, linseed meal, or cotton seed meal:16kg

Bone Meal: 3Kg	Fishmeal: 2.2kg
Methionine: 0.25Kg or 250 grams	Lime: 2.8kg
Lysine: 0.25Kg or 250 grams	Soya meal: 5kg
Vitamin/Mineral Premix: 0.25kg or 250 grams	Bone meal: 90g
Salt: 0.25Kg or 250 grams	Grower premix: 20g
You should add 10-20grams each of toxin binder, coccidiostat and growth enhancers like zinc bacitracin.	Salt: 30g
	Coccidiostat: 10g
	Zinc bacitracin:10g

It was unfortunate that the majority of the chicken farm workers did not know the specific ingredients of the chicken feeds which was kept as secret by the farm management. Moreover, this is supported by the inferences for chicken feed farmworkers that outline their knowledge regarding the usage of antibiotics in Table 4.4. It is worrisome that the highest percentage was their lack of knowledge. Their perception as stipulated in Table 4.5 in line with not knowing whether antibiotics are used or not, the majority concurred that antibiotics are not used. This meant that whatever smart regulation has been developed would have to take into consideration the level of knowledge about antibiotics use and feed ingredients among chicken feeders working at poultry farm factories. Moreover, SAPA should through veterinarians make sure the guidelines tailor-made for poultry farms are being adhered to in as far as feed ingredients are concerned.

It is likely that the chicken feed contained antibiotics if the results shown in Figures 4.3 to 4.7 are anything to go by. Animal nutritionists recommend bacitracin for growth promotion which is a polypeptide antibiotic, a mixture of related cyclic peptides produced by *Bacillus licheniformis*. The peptides are bactericidal to gram-positive bacteria such as *Staphylococcus* which can cause infection in human beings. Because the inclusion of this antibiotic is recommended through the internet, it, therefore, makes it difficult to monitor and quantify antibiotic use since the suppliers of the antibiotic are known only to whoever purchases the ingredients for the feed at the factory. According to a study by Jones et al. (1995) these suppliers are seldom known in general which makes it difficult for them to effectively police the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act, 1947 (Act 36 of 1947). The Act intends to authorize and:

- a) To allow for the registration of specific treatments, farm feeds, fertilizers, and plant sterilization;

- b) To provide for the availability, lapse, and return of a certificate of registration, the furnishing of reasons for the refusal, imposition of conditions or cancellation of a certificate of registration, and an appeal against decisions of the said Registrar to a board;
- c) To control the sale, purchase, importation, and disposal of seeds, fertilizers, farm feeds, and certain medications; and
- d) To take care of matters related thereto (Jones et al., 1995).

It is further supposed to govern the usage of antimicrobials for growth promotion and prophylaxis/metaphylaxis and the purchase of over-the-counter (OTC) antimicrobials by lay public (chiefly farmers) according to the act, the person to whom a certificate of registration has been issued under section 3(3) about any fertilizer, farm feed, agricultural remedy, or stock remedy shall, in the event of fertilizer, farm feed, agricultural remedy, or stock remedy, because of the said certificate of registration, or a copy of it, to be kept on file at the location where the fertilizer, farm feed, agricultural remedy, or stock remedy is manufactured at all times for inspection by the registrar.

This simply means that farmers who are manufacturing their feeds must have applied for and be in the position of the certificate at all times even during inspections. The Act is administered by the South Africa Health Products Regulatory Authority (SAHPRA). From the study at the poultry factory farms, the Act appeared not to be obeyed.

According to the study done by Caudell et al. (2020) demonstrated that livestock farmers in five African countries make decisions on the usage of antimicrobial agents with little to no input from animal health experts, who are frequently unavailable owing to distance or budgetary constraints. Given these facts, if short-term interventions to encourage responsible use practices are to be effective, they must concentrate on the situations in which farm-level decisions regarding animal healthcare are made and must be implemented through channels that have been shown to have an impact. Community members and agroveter shop employees will continue to take the lead in providing veterinary advice and care until the livestock sectors within these countries support a sufficient number of veterinarians offering high-quality and easily accessible services, including accurate information on responsible drug use practices.

As a result, any interventions meant to maximize the use of antibiotics must take into account the contributions made by agrovets and community members to changing farming methods. The basis of these therapies ought to be a bottom-up strategy that pinpoints the beliefs, behaviours, and knowledge that correlate with drug use. Therefore, if any smart regulations have to be developed SAHPRA should take the lead in collaboration with the UN Quadripartite group, namely the Food and Agricultural Organization (FAO), United Nations Environment Programme (UNEP), World Health Organization (WHO) and the World Organization for Animal Health (WOAH). These UN organizations established the Quadripartite Group on Integrated Surveillance on antimicrobial use and resistance. The strategic partner in the case poultry industry would be the Department of Agriculture, the Veterinary Directorate in particular. From the study it would appear that the staff from the Directorate did not regularly visit the farms, leading one to conclude that they did not see the need. Due to financial difficulties faced by farmers and feed manufacturers, a lack of state-specific veterinary healthcare, regulatory, and supervisory services, and an increase in unlicensed poultry, as well as pharmaceutical salespeople, to sway the decisions made by animal farm owners. This is supported by the following statement made in a focus group discussion with veterinarians

*'The farmers always use antibiotics rationally as they know they are beneficial for their chickens.'*

Basically, this meant that poultry factory farming had to be left alone in spite of the possible spread of antimicrobial resistance, the silent pandemic.

In any case, the IPC is very clear on what needs to be done, namely, that on-farm feed preparations require recordings that have to be inspected from time to time (Horel, 2019). The key principles are to replace, minimize, and improve the use of antimicrobial agents.

The Council's principles regarding antimicrobials are very clear. They are as follows:

- a. Antimicrobials will only be used in compliance with national authorization. In the case of South Africa Act 36, 1947 would preside.

- b. Those antimicrobials were critically important for human medicine and should be used for therapeutic purposes only and under a supervising veterinarian's diagnosis and oversight.

The statement goes on to state that IPC and its members will actively engage with inter-government organizations such as the South African Poultry Farmers Association (SAPA) which SAPA is a member of the IPC, governments, and stakeholders to help shape public policy to address AMR. We will work to advance the 'One Health' approach leading to healthy people, healthy animals, and a healthy planet (Horel, 2017). It would be reasonable to assume, therefore, that in the poultry farm factories that took part in the study, it was clear that regulations with respect to antimicrobial agents' use are minimally implemented or absent.

From the results obtained from the chicken farm workers who are ordinary farm hands, it appeared that there was a lack of coordination among the poultry farm. It was not surprising, therefore that the mean average of 57% of the chicken farm workers in the three farms maintained that they did not use antimicrobial agents in poultry, the mean average of 20% argued that they used these agents and the mean average of 23% were not sure whether the antimicrobial agents were used or not as shown in Table 4.5. On the contrary, Figure 4.4 illustrated that 73% of the chicken feeders stipulated that antimicrobial agents are being used for growth promotion as they feed the chickens with antimicrobial agents through feeds as indicated in Figure 4.6. What this meant was there was confusion between antimicrobial agents' use for preventative, therapeutic, and growth-promoting purposes which meant that the chicken farm workers did not really know much about antibiotic use. All they did perhaps, was to follow Standard Operating Procedures (SOPs) at the poultry farm as each had its SOPs though they don't have standardized or harmonized SOPs. This made one wonder what the biosafety implications were at these farms if the perception among the farm workers deducted that there were no antibiotics used. As was previously mentioned, the feed formula was a factory secret for chicken farms, so they wouldn't even know if they wanted to. This to a certain extent confirmed what the SAPA, claimed, that it had no control over poultry farms which were voluntary members and left to their own devices. This meant that SAPA did not have any influence on adherence to IPC principles which worries some.

In such a situation, if it is a common practice there really would be no room for command and control regulations but flexible, imaginative, and innovative control, in other words, smart regulations. Such regulations would aim to harness not only governments but also the poultry business and third parties such as the pharmaceutical industry to curb the spread of AMR, the silent pandemic, and to aggressively promote the One Health approach.

According to Poole and Sheffield (2013) in veterinary medicine antibiotics are mainly used for growth promotion which is very common in the poultry industry, it is common knowledge that this would be common practice for larger production in the commercial poultry industry. According to Wang et al. (2020), there is intense pressure to raise animal productivity, reproductivity, and economic output. At this time, the use of antimicrobial agents as growth promoters is one of the inevitable tools for animal husbandry farmers/producers to tune up with the current situation demanding a heavy rise in productivity. As far as they are concerned the use of antimicrobial drugs has several significant advantages which include:

- A. Enhances the efficiency of nutrient utilization,
- B. Less feed intake,
- C. Provides a stable fermentation process,
- D. Reduce bacterial load and hence immune reactivity,  
    Reduces variation in size of carcasses, helps to achieve quality standards in slaughter process and product, and
- E. Suppresses pathogenic bacteria and thereby reduces incidences of enteric disease.

The above, to a certain extent, was confirmed by the study conducted at Ogun State with 58 poultry farmers who openly admitted to the administration of antibiotics for different purposes. For example, 36.2% said they use them for treatment therapy, whereas 29.3% were using them for prophylaxis, while 32.8% said they were using them for both purposes. In this study 50% of the poultry farmers had the antibiotics prescribed by the veterinarian and the rest had other sources (Oluwasile et al., 2014). It can be concluded that in this state in Nigeria, veterinarians worked very closely with the farmers and their workers who, it can be assumed, followed some feeding Standard Operating Procedures (SOP). Whatever they were, they would obviously follow antibiotic

guidelines to prevent unnecessary use of antibiotics that would inevitably lead to AMR. There was, what would appear, close cooperation between farmers and veterinarians. The 3 veterinarians who took part in the present study admitted that they visited the farms every 3 months only or when necessary. Otherwise most of the time the farmers were left alone with no specific guidelines supervised by the veterinarians who acknowledged that they were not aware of any specific feeding guidelines. Hence as mentioned earlier each farm could manufacture its own chicken feed which calls for smart regulations regarding the One Health approach that can be applied across the industry.

Other veterinarians in the feedback group discussion had this to say about AMR and human medical practice.

*It's [antimicrobial resistance] obviously an issue in human medicine, which I think they're probably using us as the scapegoats for. At the moment I think we've just got to be seen to conform or to reduce our usages to take the party line.'*

Such attitude among veterinarians does not augur well for the One Health approach to curb the spread of AMR. Sadly, one veterinarian at the farm results feedback session had this to say,

*'What is the role of veterinarians in Antimicrobial Stewardship committees?'*

It is indeed such sentiments that will hamper the effective implementation of the One Health approach in order to refine, reduce, and replace antibiotic usage by employing smart regulations in the poultry industry in South Africa. There is a need for continued dialogue among all healthcare professionals to clarify the role each profession can play in curbing the spread of AMR.

### **5.1.3. Antibiotic use in poultry**

What was interesting from the results of the questionnaire administered was that the majority of farm workers were aware that penicillin was commonly used as shown in Table 4.6. The rest of the workers felt it was, instead, tetracycline, that was commonly used for growth promotion. What this means is that, indeed, the chicken feeders despite other issues raised earlier, had knowledge about which antibiotic was used at the farms,

namely either penicillin or tetracycline. Furthermore, the inferential comparison between different farms regarding the usage of penicillin and tetracycline, appears to be a common standard in the three farms.

Whatever the case may be, these two classes of antibiotics are on the WHO's critically important antimicrobials for human medicine list (Collignon et al., 2016). The WHO list is a ranking list of medically important antimicrobials for risk management of antimicrobial resistance due to non-human uses as part of the One Health approach. This is because antimicrobial resistance due to resistant organisms resulting from non-human use of antimicrobials, for example in the poultry industry, has now been recognized as a silent pandemic (Nukala and Tripathi, 2022). From the interview results, it would not be possible, for example, to categorize which group of penicillin was being referred to. The WHO list has the following six penicillin categories, 1) antipseudomonal penicillin e.g. piperacillin, 2) aminopenicillins e.g. ampicillin, 3) aminopenicillin with beta-lactamase inhibitor e.g. amoxicillin and clavulanic acid, 4) amidinopenicillins e.g. mecillinam, 5) anti-staphylococcal penicillin e.g. flucloxacillin, and 6) narrow spectrum penicillin e.g. benzylpenicillin.

It would be reasonable to conclude that, even if the chicken feeders had access to an antibiotic use guideline, they would not know much about the penicillin categories. The farm manager would be expected to have an idea of these categories through, hopefully, the veterinarian which is difficult to believe given the lack of adequate supervision. Additionally, their use of antibiotics appears to be driven by their commercial objective rather than the welfare of the animals as stated by one veterinarian. He said;

*'The farmers always use antibiotics rationally as they know they are beneficial for their chickens.'* Additionally, he said:

*'I do believe in prophylactic treatments because there are too many times where you try and not use antibiotics and then you end up with a bad mortality.'*

What is important to note is that the World Animal Health Organization (WAHO) has its list of important for veterinary medicine (Aidara-Kane et al., 2018). It is, therefore critical, that the two lists, namely the WHO list and OIE list, be considered when developing AMR risk management strategies, if there is an overlap between them which is true

when considering penicillin, for example. This is crucial because there has to be a balance between animal health and welfare, and public health. Therefore, during the development of any guidelines or smart regulation by all concerned, namely, human and animal healthcare providers, the balance ought to be taken into consideration. This is assuming that all things are equal.

From the results obtained, it is more likely that all this is not appreciated in the poultry farming industry. This can be confirmed, if it is indeed true, that tetracycline oxytetracycline in particular was commonly used. It is commonly recognized that when bacteria are exposed to low dosages of oxytetracycline for a lengthy period, the bacteria develop resistant genes. Through direct contact, as is the situation in poultry farm factories, one person exhibiting this resistance can quickly infect other individuals in the same species as well as others who reside and work there. Oxytetracycline, can however, be used for both therapy and prophylaxis (Wegener, 2003). As a matter of fact, tetracycline has been used as a growth promoter in animals since the 1940s (Habiba et al., 2023). Given the level of education of the chicken farm factory workers, it would be reasonable to assume they would have a poor understating of the IPC statement concerning the use of antibiotics in general. Implementation of the statement, in other words, refining, reducing, and replacing the use of any antibiotic can only be done by designing smart regulations (Porter et al., 2020) that promote the One Health approach. The development of the regulations would require basic knowledge about microorganisms, antibiotics, and zoonosis.

Be that as it may, it was not clear how the chicken feeders knew how the farmer acquired antibiotics for the poultry farm factory. Currently in South Africa, when it comes to the over-the-counter sales of antibiotics at retail pharmacies, the current regulations are fairly clear. and at agricultural retail stores for both human and animal use. However, from the results obtained, it would appear that compliance with regulations was poor and enforcement was patchy. This was hardly surprising from an antimicrobial agents regulatory and chicken feed supply point of view because there were multiple stakeholders involved since the antibiotics were said to be used, mainly for growth promotion. For instance, if the chicken feed formula were kept a secret it would not be possible to know where and how the farmer obtained antibiotics he used in the feed or

even for therapy. On top of that, no one knew the disposal procedures followed for leftover antibiotics especially, if there were irregular visits by the veterinarian. It would be reasonable to conclude that veterinarians had no influence on how antibiotics were used at poultry farm factories. It would not be surprising that, in general, veterinarians who were supposed to supervise antibiotic usage at farms would not link the spread of AMR and the One Health approach. In a study in Brazil among veterinarians responsible for supervising chick egg production, it was found that there was very little knowledge about AMR among humans (Torres et al., 2022).

The stakeholders mentioned above would include licensed and unlicensed pharmaceutical suppliers (Eagar et al., 2012) nutrient suppliers, animal scientists, veterinarians, and poultry farmers. All of them are important in designing smart regulations to refine, reduce, and replace with possible alternatives such as probiotics and bacteriophages. In fact, according to a study by Mogotlane et al. (2023) replacing antimicrobials with probiotics (*Lactobacillus planetarium* species *Candida valida* species which is good in producing amino acids), actinomycetes *Streptomyces albus* species and fermenting fungi *Aspergillus oryzae* species did not have much effect on production parameters, carcass characteristics and meat quality of Ross 308 broiler chickens. Obviously when developing the guidelines and designing the smart regulations issues regarding chicken genetics and probiotics mixture would have to be taken into consideration. The broiler chicken used in the study done by Mogotlane et al. (2023) was not the same as the Plymouth gallus-gallus which is commonly bred in the poultry factory farms that took part in the present study.

#### 5.1.4. Feeding practice at the farms

From the study, it can be assumed that secret administration of the antibiotic for growth promotion would have been done in groups through feeds to ensure that all the chickens were being fed the same amount of antibiotics at the same frequency. This would be in line with what Gautier & Page (2012) recommended in their paper. In fact, Wang et al. (2020) found that these antimicrobial compounds may considerably reduce bacterial contamination in animal products when added to drinking water or animal feed. This illustrates why the most common route of administration especially for growth promotion and prophylaxis as shown in Figure 4.6 was through the feeds and water that the poultry

were fed collectively as indicated in Figure 4.6. It can be argued that such a practice makes it difficult to measure the dosage being administered to each chicken because it is possible that, some will be over-dosed, and others under-dosed. In the worst-case situation, if they are receiving antibiotics twice a day as shown in Figure 4.5 at 97%, it would seem the antibiotics are included in daily meals for growth promotion rather than for therapeutic purposes as shown in Figure 4.4. The wrong administration and frequency might lead to resistance in humans who end up eating such chicken products as there would be residues from particularly overdosed chicken. In addition, consumption by humans of chicken products containing antibiotic residues could lead to anaphylactic shock in allergic patients, and in some cases, this could even lead to death. This is a point that was missed by one veterinarian during the discussion on antimicrobial stewardship who made the following statement;

*‘Should we be saying we shouldn’t be letting humans have antibiotics? In terms of why are we so hell-bent on stopping animals when it’s the humans themselves in some respects that are causing all their problems?’*

This really was an unfortunate statement which sounded like a blame game. If veterinarians kept themselves abreast with what happens at the factories such sentiments would not be expressed. This calls for an urgent local dialogue between the different professions which is why the UN Quadripartite group was set up.

Figure 4.5 shows how often chickens in the factory were exposed to antibiotics whose amount could not be disclosed by the feeders since feeds were prepared in-house using an undisclosed formula. As mentioned before the formula was supposed to be an intellectual property of the farm as indicated by a farm manager at one of the study sites. Unfortunately, also, not much, on the volumes of antibiotic consumption, is easily available from poultry industry pharmaceutical suppliers who may include illegal importers (Eagar et al., 2012). Authorized antimicrobial agents available in South Africa are registered under the Stock Remedies Act 36 1947 which the veterinary profession is supposed to see to it that it is obeyed. The different veterinary domains that speak to the One Health approach are so clear as shown in Figure 5.1.

During One Health organization held at the University of Fort Hare, one of the veterinarians said *“The farmers always use antibiotics rationally as they know they are beneficial for their chickens”*. From the results, one of the veterinarians indicated that he visits the poultry farms once in a while when there is a need. Furthermore, given the fact that poultry farmers manufacture their chicken feeds within the house, it would appear that poultry farmers are given the liberty to put in any ingredients they think are fit for the chicken to grow faster which would be good for the market. In the study done by Torres et al. (2022), the custom of acquiring and keeping antibiotics was declared that antibiotics are purchased from pharmaceutical industry suppliers, and the administration doses are set according to the manufacturer’s recommendation and by the veterinarians; however, the administration itself is done by the farmers either in feed or water. From what the veterinarian from the OH organization said, although the doses are set according to the manufacturer’s recommendations and by veterinarians, the administration is done by farmers. If the visitation to the farms was every quarter as it was said by the two veterinarians who were included in the study, it can be arguable that the doses could be increased for the best outcomes. One of the farmers who was visited during the international One Health Organization symposium made a disclaimer that *“I don’t want veterinarians’ visiting in my farm because I do my own things within the farm e.g. post-mortems of his carcasses”*. This appears to be the feeling of most of the farmers as they are manufacturing their feeds, and performing post-mortem of their carcasses. In Bangladesh, every district, sub-district (Upazila), or metro veterinary hospital has one to two registered veterinarians on staff in addition to additional supporting sub-technical staff members (Amin et al., 2020). In terms of the production of feeds, this would eliminate anarchy in farms. Furthermore, as one of the farmers stated above, farmers would not perform post-mortems on their own.

Padda et al. (2021) reported that gentamicin, amoxicillin, bacitracin, and quinolone are the used antibiotics in poultry. The results of the study demonstrated that the usage of antibiotics in the poultry sector is directly influenced by veterinarians. In this case, veterinarians must contribute to the development of AMR knowledge and the hunt for remedies (Bordier et al., 2020). In this sense, understanding the variables that contribute to the prescription of antibiotics and veterinarians' assessments of the drives toward resistance have been connected to successful attempts to minimize the use of

antibiotics in animal production (Padda et al., 2021). The veterinarian stated at the One Health organization conference that *"it's [antimicrobial resistance] certainly an issue in human health, which I think they're probably using us as the scapegoats for."* This is in light of the rising degree of antimicrobial resistance coming from poultry. *At the moment I think we've just got to be seen to conform or to reduce our usages to take the party line'* This would imply that veterinarians can be held accountable for all consequences associated with the use of antibiotics in poultry farming.

It is true that the kinds and amounts of antimicrobial agents used vary from nation to nation depending on factors such as the economy, degree of development, animal husbandry, and species of animals (Roth et al., 2019). According to Rosengren et al. (2010) depending on the disease risk and the stage of production, different antibiotics are administered in different ways. Unfortunately, from the present study, this cannot be said about the poultry industry in South Africa if the interaction with the South African Poultry Association is anything to go by. Internationally what has been observed, in general, is that there are different methods of monitoring antimicrobial agent use which are as follows:

- A. One approach is to track antibiotic sales, yet long-term data for specific animal species are not available (with the Exception of France),
- B. The identification of antimicrobial drugs prescribed for each species of animal, and
- C. The identification of antimicrobial agents in farm animals, or the number of active components in milligrams per kilogram of produced chicken (Roth et al., 2019).

There is no reason why these methods cannot be followed in the poultry industry in South Africa wherein smart regulations are developed with the cooperation of every stakeholder to promote the One Health approach to deal with AMR. There would be a need to make sure that methods are harmonized to provide useful data.



- d. Agricultural Product Standards Act, 1990 (Act No. 119 of 1990),
- e. National Environmental Management Act, 1998 (Act No. 107 of 1998) and the
- f. National Environmental Management Amendment Act, 2008 (Act No. 62 of 2008).

Having said that about the laws and regulations, it appeared from the study that the veterinarians were taking a back seat regarding overseeing what was happening in the poultry factory farms especially when it came to issues concerning chicken feeds. If there was active oversight, farmers would not have the liberty of injudicious inclusion of antimicrobial agents in the chicken feeds.

From the results of the present study everything about antimicrobial use in the poultry industry seemed to be shrouded in secrecy, perhaps the regulatory authority South African Health Products Regulatory Authorities (SAHPRA) on the Veterinary Products Committee would be better informed through the South Africa Veterinary Association (SAVA). It is hoped that information on actual amounts of antimicrobial agents consumed particularly in the poultry industry would be available to the association which would have an idea of the consumption pattern from the antibiotic dosage schedule which would have an idea of the consumption pattern from the antibiotic dosage schedule.

What could be deduced from the frequency of administration was that perhaps the doses used were not that high especially if the antibiotics were in the feed as shown in Figure 4.6. If indeed that was the case, that small doses of the antibiotic were employed, one would wonder whether the consequence of using small doses was appreciated by the chicken feeders. It would also be reasonable to assume that the chances of a withdrawal period were slim, thereby creating an opportunity for the emergence of antibiotic resistance even among the feeders once in contact with resistant microorganisms. But still, it must be emphasized that this would not indicate the amount of antibiotics consumed in at the poultry factory farms.

Generally, the exact estimates of antibiotic consumption in the food animal industry in both high-income and low-income countries are not known. In North America, for example it had been regarded as a secret for a long time (Webster, 2009). Figure 4.6

gives an idea of the in-feed dosage form for growth promotion in chickens that the feeders were most familiar with, neither were the veterinarians. What is important to note about this is, unlike in humans where exposure to antimicrobial agents is directed at one person, in chickens the entire groups of chickens are exposed when medicated feed is administered and water. In the case of growth promotion, the dosages are typically present for long periods at low quantities (Eagar et al., 2012). When combined, these two methods could hasten the rise of bacteria that are resistant to treatment, such as *Salmonella* in chickens. *Salmonella* can then infect humans, through either direct contact or via the food chain (Hoeler et al., 2017). One would then expect *Salmonellosis* data to be easily accessible to veterinarians if indeed veterinarians believed in the domains as stipulated by the Department of Agriculture and Forestry. This would require them to have a close collaboration working relationship with SAPA which, unfortunately, appears to be more concerned about poultry industry economics. If SAPA were to join hands with the SAVA harmonized biosafety guidelines would be effectively followed and the One Health approach implemented.

The result obtained, as shown in Figure 4.9 regarding knowledge about the availability of antibiotic guidelines was indeed interesting given that the feeders or the poultry farm factories seemed to compile with expected antibiotic administration in 1-3 days (Habiba et al., 2023). The feeding schedule was indeed part of the farm factory SOPs which would, one assumed, include matters related to biosafety/biosecurity measures at the poultry factory farm.

The South African AMR National Strategy Framework: A One Health Approach 2017-2024 calls for biosecurity and hygiene from farm to retail levels. Biosecurity in, for example, chickens, is a core component of the prevention and control of the spread of resistant microorganisms in animal husbandry (Shabangu et al., 2023). This is more relevant for poultry farm factories. The strategy framework goes on to state that there are guidelines already available through farmer interest groups and veterinary societies. Unfortunately, the guidelines were never revealed during the study. This was not surprising because the strategy framework acknowledges that whatever guidelines that are there are not standardized and therefore poorly implemented. This means any surveillance of microorganisms would not be reliable. Sub-objective 1.2 of the national

strategy plan calls for governance structures at operational levels to be established to see to it that there are standardized and harmonized biosecurity guidelines to enforce proper warning systems of sentinel organisms. The enforcement of anything regarding, for example, a warning system for sentinel organisms at the farms would be possible if smart regulations are developed and effectively implemented. To get a good buy-in would require evidence of the prevalence of microorganisms at the farms.

From the results, the foot disinfectants were bypassed and ignored, according to Horel (2019) IPC guidelines, whenever entering and leaving the hatchery, the foot disinfectant must be used to prevent cross-contamination. It is therefore assumed that the process is being avoided due to a lack of supervision by veterinarians.

#### **5.1.6. Microorganisms identified at the farms**

There were different profiles of the most prevalent microorganisms at the farms as shown in Table 5.3. This could mean that each farm factory had its SOPs, a clear indication of the need for smart regulations developed with the collaboration of all stakeholders, SAPA, SAVA, SAHPRA, and the pharmaceutical industry.

In general, microorganisms found and their frequency in humans, animals, and the environmental ecosystem are shown in Figure 5.2. The present study results as shown in Table 4.9, did confirm the results from the review carried out by Abayneh et al. (2023).

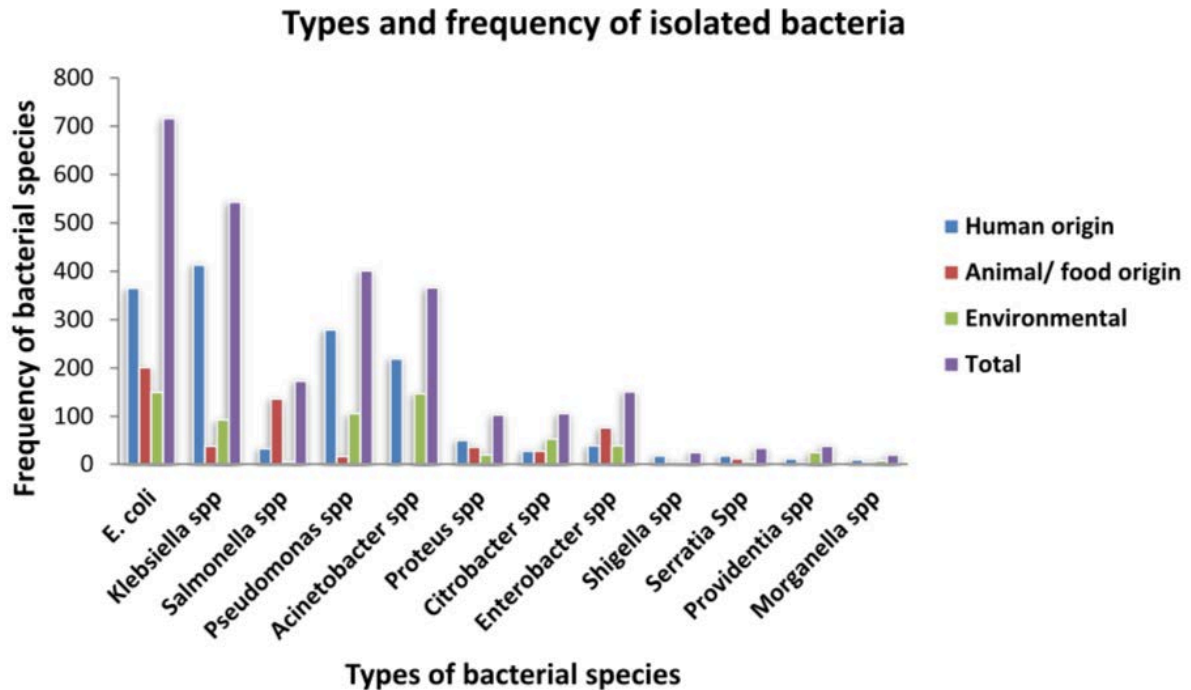


Figure 5.2: Bacteria isolated from humans, environment, and from animals or food of animal origin (Abayneh et al., 2023)

The most common microorganism found in all three poultry farms in the chicken gut contents was *E. coli* which is regarded as the sentinel microorganism, in other words, an indicator organism of AR in a variety of bacterial species (Roth et al., 2019).

Gram-negative, rod-shaped bacteria called *Escherichia* organisms are often found in the large intestine. The observation from the present study was not of much concern as it has been noted that this microorganism can be found in the chicken intestinal flora. However, the substantial amounts found in the chicken guts and droppings at the farms were worrisome because of the possibility of transfer of *E. coli* with antibiotic resistant genes to the farm workers. The laboratory results confirmed that some *E. coli* found at the farms were capable of producing ESBL and AmpC enzymes responsible for resistance to some antibiotics shown in Table 5.3 if they were to be used for the diseases in humans as indicated.

Table 5.3: Microorganisms found in chickens and their effects on chickens and humans and their sensitivity to commonly used antibiotics in human medicine (Ghodousi et al., 2015)

Microorganisms	In chicken	In humans	Antimicrobial resistance	ESBL/AMPC
<i>E. coli</i>	Diarrhoea, decreased appetite, lethargy, and in severe cases, death.	Diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome, mild diarrhoea, severe bloody diarrhoea, stomach cramps, occasionally fever, urinary tract infection, kidney failure, and even death.	Ampicillin, cefuroxime, and ciprofloxacin, Trimethoprim/Sulphamethoxazole.	ESBL
<i>Citrobacter braaki</i>	Non-pathogenic microorganism.	Lethargy, poor feeding, vomiting, irritability, bulging fontanelle, seizures, jaundice, urinary tract infections, and nosocomial infections.	Amoxicillin and cefoxitin.	AMPC
<i>Proteus mirabilis</i>	Cellulitis, caseous plaques and haemorrhages.	Urinary tract infections and formation of kidney stones.	Nitrofurantoin and tigecycline.	ESBL
<i>Providencia rettgeri</i>	Respiratory infections such as coughing, sneezing, and difficulty breathing, swollen eyes and nasal discharge.	Urinary tract infections, pneumonia, and sepsis.	Ampicillin, cefuroxime, tigecycline, and nitrofurantoin.	AMPC
<i>Salmonella typhimurium</i>	Non-pathogenic.	Watery diarrhoea, fever and fatigue, may develop dysentery, abdominal cramps, and nausea.	Augmentin and gentamycin.	ESBL
<i>Enterobacter cloacae complex</i>	Avian colibacillosis, which can lead to respiratory, digestive, and genitourinary infections.	Respiratory infections, urinary tract infections, and bloodstream infections.	Amoxicillin/clavulanic, tazobactam, cefoxitin, cefotaxime, ceftazidime, cefepime, and ertapenem.	AMPC

In addition, *Escherichia coli* and other bacteria found in the sentinel flora have the ability to store AR genes, which can then be shared by different bacterial species and even human and animal pathogens. *E. coli* can also be transferred to humans either by direct

contact between animals and humans or indirectly via the food production chain; or as a result of the spread of animal waste on land (Marshall and Levy, 2011). *E. coli* isolates may be non-pathogenic commensals or pathogens that affect humans or animals. Depending on the presence of particular virulence factors, the pathogenic ones are categorized as enterotoxigenic, enteropathogenic, entero-invasive, or enterohaemorrhagic. Some isolates (i.e. *E. coli* shiga-like toxin producers) are zoonotic and may cause serious intra- and extra-intestinal diseases like diarrhoea, haemorrhagic colitis, and haemolytic uraemic syndrome, mild diarrhoea, severe bloody diarrhoea, stomach cramps, occasionally fever, urinary tract infection, kidney failure, other complications which can be of high concern, especially to the immunocompromised and infants, and even death in rare cases. The severity of the illness depends on the strain of *E. coli* and the health of the individual infected. *E. coli* infections are typically contracted from contaminated food or water, or through contact with infected animals or people (Guerra et al., 2003). In the present study, 2% of *E. coli* isolates were found to be resistant to ampicillin, cefuroxime, and ciprofloxacin in Farm 2, and 4% resistant to ampicillin and trimethoprim/sulphamethoxazole as indicated in Figure 4.11. According to Diarra and Malouin (2014), it has been demonstrated that *E. Coli* strains resistant to streptomycin and trimethoprim can survive for several weeks in a chicken farm without the use of antimicrobial drugs.

Results confirmed the conclusion made by Muloi et al. (2022) when they stepped outside of 'the blame game' of livestock and human health applying the One Health approach in their study on *E. coli* AMR genes. Additionally, 94% (98/104) of the ESBL-producing *E. Coli* isolates were found to be phenotypically resistant to colistin, and 13.5% of the isolates tested positive for *mcr-1* in LBMs, RPFs, and HBPs, according to a study conducted by Amin et al. (2020). Despite side effects and resistance, colistin is used increasingly to treat patients with infections caused by multidrug-resistant organisms against which colistin is still active. They demonstrated that AMR genes that conferred resistance to critically important antimicrobials for both human and veterinary medicine were widespread. The present study in a way contributed to what little is known about the role of keeping livestock in the emergence and transmission of AMR to the human population.

The laboratory investigations were clear about the identity of *Citrobacter braaki* a gram-negative, AmpC lactamase-producing enzyme, facultatively anaerobic, enzyme-producing bacillus from the chicken samples. Claudińska-Czarnak (2021) notes that *Salmonella spp.*, which was also detected in the chicken samples, is frequently mistakenly labelled as *Citrobacter* bacteria. Although it is thought to be a non-pathogenic bacterium, it has been reported to induce urinary tract infections in humans and to be a source of nosocomial infections in immune-compromised patients.

It has also been shown to cause mild infections in healthy individuals. *Citrobacter braaki* is part of the *Citrobacter* complex which can lead to septicaemia in patients who exhibit several risk factors. In newborns and early children, *Citrobacter spp.* has also been linked to pulmonary infections, septicaemia, and meningitis. *Citrobacter spp.* Common signs and symptoms of *Citrobacter braaki* infection are fever, lethargy, poor eating, vomiting, irritability, bulging fontanelle, convulsions, jaundice, and gastrointestinal, urinary, or hepatobiliary tract infections that enter the abdomen. All this kind of information would be important to share if the One Health approach was adopted and effectively implemented by all stakeholders in poultry. Much more so if all stakeholders were made aware that *Citrobacter braaki* was found to be resistant to amoxicillin and cefoxitin both with a 14% resistance rate.

These are two medications that are most frequently used in human medicine when patients are hospitalized. The result of the present agreed with what Witaningrum et al. (2021) obtained in broiler chicken samples where they found that *Citrobacter freundii* is multidrug resistant. From their study, they recommended evaluation management plans at commercial poultry farms in Indonesia to stop the spread of bacteria resistant to many drugs from chickens to humans and the surrounding environment. This obviously meant a One Health approach with smart regulations in place and respected by all stakeholders.

*Proteus mirabilis* was the third most common prevalent in the chicken specimen from the factory farms and was found to be resistant to nitrofurantoin and tigecycline resistance. This is a bacterium that is frequently present in water and soil. It has also been linked to human urinary tract infections (UTIs). Its flagella enable it to move across

surfaces, and *P. mirabilis* is able to move quickly through a process known as swarming. This bacterium is also known for its ability to produce urease, an enzyme that breaks down urea into ammonia, which can lead to the formation of kidney stones. Fortunately, in human medicine, cephalosporins or fluoroquinolones are typically used to treat *P. mirabilis* infection (Armbruster et al., 2018). Although some patients with UTI infections are prescribed nitrofurantoin and tigecyclines. The ability of the bacterium to create urease, an enzyme that breaks down urea into ammonia and can cause kidney stones, can exacerbate the problem if a farm worker comes into contact with it.

Infection caused by *P. mirabilis* at the broiler chicken farm is certainly a sign of poor foot hygiene, biosafety measures in particular which are supposed to be regularly monitored and prevalence surveillance data available. In the study carried out in Bangladesh (Nahar et al., 2014) MDR *P. mirabilis* was found around poultry farms, and the data obtained was used to assist the veterinarian in biosafety planning and adherence to IPC principles.

From the present study, it would be very difficult for any veterinarian to do so except to leave biosafety issues to the farm management. There is an urgent need to train more veterinary technicians to assist farmers who should be glad for their assistance in promoting antimicrobial stewardship as part of the One Health approach (Redding et al., 2023). The idea of training more veterinarian technicians should be noble, but the crisis looms as South Africa bleeds for veterinarians. A potential crisis in animal health, generally, and food safety and security are looming because of a shortage of vets in the country, with the South African Veterinary Council (SAVC) concerned that the situation had been exacerbated by the removal of veterinarians from South Africa's critical skills list in 2022. Reinstating vets on the list would help the country retain qualified vets and aid foreign veterinarians who want to practice here (Maruve and Essack, 2022).

*Providencia rettgeri* is a ubiquitous organism but seldom associated with human disease according to Sagar et al. (2017) but now its infection is reported to be an emerging nosocomial uropathogen. Its identification at one of the poultry factory farms during the present study does not board well with the infection prevention and control program in Limpopo Province. It has been linked to sepsis, pneumonia, and infections of the urinary

tract in humans. Antibiotics are typically used throughout treatment; however, the type of therapy and length of treatment may differ based on the nature and severity of the illness as well as the bacterial resistance pattern (Adam et al., 2020). According to the present study it was found to be resistant to the second-line treatment for UTI infections, nitrofurantoin. If a patient is admitted with UTI infections and fed chicken that has a gene resistant to *P. rettgeri*, this could make the problem worse. There wouldn't be many options for treatment in this case. *P. rettgeri* infections can be avoided with the use of good hygiene practices like hand washing and sensible food handling.

According to IPC, proper hygiene must always be upheld in hatcheries, including the provision of fresh, clean drinking water. The results obtained from the study would seem to indicate that there was poor hygiene practice.

*P. rettgeri* is said to produce AmpC lactamase which mediates resistance to cephalothin, cefazolin, cefoxitin, most penicillin, and  $\beta$ -lactamase inhibitor- $\beta$ -lactam combinations. *P. rettgeri* was found to be resistant to ampicillin, cefuroxime, tigecycline, and nitrofurantoin which then confirms the activity of the AmpC lactamase gene which would then narrow the treatment options more especially with antibiotics like nitrofurantoin in human medicine. *P. rettgeri* yet again illustrates the need for transdisciplinary and multidisciplinary need to promote the One Health approach to prevent AMR.

*Enterobacter cloacae* is a member of the normal gut flora of many humans and is not usually a primary pathogen. In a study in Nigeria, all isolates of environmental *Enterobacteriaceae* and resistant bovines possessed resistance genes.  $\beta$ -lactamase genes, aminoglycoside modifying enzymes, *qnr* genes, sulphonamide, tetracycline, and trimethoprim resistance genes were among the resistance determinants, in that order (Jesumirhewe et al., 2022).

Some strains have been associated with urinary tract and respiratory tract infections in immunocompromised individuals. In other words, it can be an opportunistic pathogen in humans, which means that it can infect individuals who are already weak or vulnerable, such as those who are hospitalized. These infections can include, among others, bloodstream infections, urinary tract infections, and respiratory infections. In addition to

leukocytosis, which is seen in many different bloodstream presentations, *Enterobacteriaceae* can cause systemic inflammatory response, hypotension, shock, and fever, which is the most prevalent presentation in this syndrome. Chest x-ray consolidations, coughing, and shortness of breath are typical symptoms of *Enterobacter pneumonia*.

Potential therapies for *Enterobacter pneumonia* include carbapenems, aminoglycosides, fluoroquinolones, beta-lactams, beta-lactamase inhibitors, and sulfamethoxazole/trimethoprim. Infections can occasionally be challenging to treat because the bacteria may be multidrug resistant. Given that it was resistant to the majority of antimicrobial treatments, this fits with what was discovered. Figure 4.10 showed that the *Enterobacter cloacae* complex was 75% multi-pharmacological phenotypic resistant to amoxicillin-clavulanic, tazobactam, cefuroxime, cefoxitin, cefotaxime, ceftazidime, cefepime, and ertapenem at one of the farms and 15% multi-pharmacological phenotypic resistant to amoxycillin and cefoxitin at another. From this, it can be concluded that it was one of the pieces of evidence to prove the lack of harmonized guidelines for biosafety and antimicrobial use in poultry farming.

*Enterobacter cloacae* complex has AmpC-lactamase which mediates resistance to cephalothin, cefazolin, cefoxitin, most penicillin, and  $\beta$ -lactamase inhibitor- $\beta$ -lactam combinations. Even for infections brought on by initially susceptible isolates, exposure to AmpC-lactams like *Enterobacter cloacae*, which was isolated, could set off a chain of events that resulted in significant AmpC-lactam resistance production. It would be challenging to treat patients with infections brought on by the bacterium because of its resistance to the majority of broad-spectrum, some of the cephalosporin (cefoxitin in particular), and carbapenem (ertapenem) antibiotics. *Enterobacter pneumonia* cannot be treated with cephalosporins of either first or second generation, but according to the findings, third-generation cephalosporins such cefazolin were equally ineffective. Additionally, some carbapenems, such as ertapenem, which are used as a last option when other antimicrobial agents fail to work, are also impacted.

This demonstrates that, should there be gene transfer from chicken to human and a patient develops the aforementioned symptoms, the patient's treatment options will be

severely constrained leaving prescribers with no choice but to use drugs covered by Section 21 such as colistin and death may ultimately result. Having said the results from the farms should serve as a warning about the prevalence *Enterobacteriaceae*. The spread of carbapenemase-producing *Enterobacteriaceae* no matter the source, is a threat to healthcare delivery.

*Salmonella Typhimurium* was identified at Farms 1, 2, and 3, with a prevalence of resistance to the broad-spectrum antibiotic augumentin of 30%, 13%, and 8%, respectively. The bacteria are a family of bacteria that live in the intestines of warm-blooded animals and people. Nevertheless, the possibility of bacterial transmission between reservoirs and between humans and other species is well demonstrated when looking at non-typhoidal *Salmonella*, regardless of its resistance. *Salmonella spp.* are facultative anaerobic gram-negative rods and members of the *Enterobacteriaceae* family, according to the Food Safety Authority of Ireland (Duggan et al., 2012). *Salmonella* can enter the food chain through contaminated hatcheries, persistently contaminated livestock settings, contaminated animal feed facilities, vertical transmission, and faecal-oral contact with humans.

Salmonellosis, which can result in a variety of symptoms in people, including diarrhoea, abdominal pain, fever, nausea, and vomiting, can manifest in humans 12 to 72 hours after consuming food contaminated with the *Salmonella bacterium*. In severe situations, it can cause dehydration and even death, especially in populations that are more susceptible to it, like small children, the elderly, and people with compromised immune systems. Dehydration can happen to vulnerable groups, such as infants, old people, and people with impaired immune systems. Long-term health issues like reactive arthritis and irritable bowel syndrome are also linked to *Salmonella* infection (Ehuwa et al., 2021). These are facts that would need to be appreciated by animal health and environmental specialists, for example, as they go about their work at poultry farm factories as possible transmission of *Salmonella* as indicated on Figure 5.3.

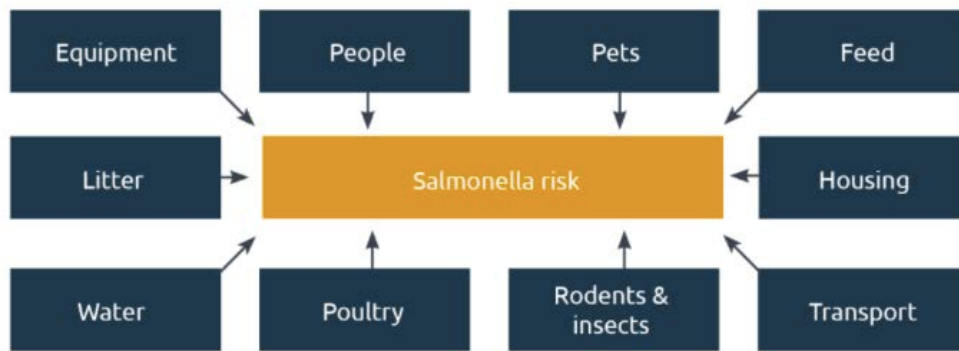


Figure 5.3: Main reasons for horizontal and vertical transmission of *Salmonella* within a flock but also, in consecutive flocks (Vervloesem, 2023)

According to a study done by Cox et al. (2021) in South Africa revealed the presence of *Salmonella* species in the eggs of broiler eggs. A total number of 13 different egg brands and 468 egg samples were analyzed. The results showed that 73% of the egg samples had bacterial contamination distributed on the shell, albumin, and yolk as follows: *Escherichia coli* 159 (34%), *Enterococcus faecalis* 66 (14%), *Proteus mirabilis* 42 (9%), *Klebsiella pneumoniae* 33 (7%), *Salmonella* serotype *Typhimurium* 28 (6%), *Enterobacter cloacae* 5 (1%), *Stenotrophomonas maltophilia* 3 (0.6%), *Salmonella* serotype *Dublin* 3 (0.6%) and *Salmonella* serotype *Braenderup* 1 (0.2%).

The conducted by (Salehi et al., 2005), indicated that out of 192 samples, 30 *Salmonella* strains were susceptible to antimicrobial agents such as colistin ciprofloxacin, gentamycin, and ceftriaxone which the test of the *Salmonella* strains was resistant to tetracycline, streptomycin, amikacin, and nalidixic acid. This study revealed that from the *Salmonella* strains from the faecal dropping that 80% of the *Salmonella* strain were susceptible to amikacin, hence rendering amikacin the most effective agent, and was 30% resistant to both gentamycin and amoxicillin/clavulanic acid as shown in Figure 4.11.

From Figure 4.3, the most common antimicrobial agents used were penicillin and tetracycline; hence presence of *Salmonella* in all farms may be due to the fact that they use penicillin, which is shown to be rendered ineffective against *Salmonella* strains which can be transferred to humans via gene transferase after consumption. In comparison to the study conducted by (Salehi et al., 2005), it can be deduced that there

is a mutation in the *Salmonella spp.* strain which leads to resistance from poultry to humans through consumption. According to Khan and Rahman (2022), the majority of cases of salmonellosis are treated with antibiotics. Regrettably, the overuse of antibiotics contributes to the development and spread of multidrug resistant *Salmonella species*. MDR strain illnesses will be harder to treat since medicines are losing their effectiveness.

The pink colonies on the plates represent *Shigella spp.* which are gram-negative, non-spore-forming rod-shaped bacteria and are members of the family *Enterobacteriaceae* (Lampel and Maurelli, 2003). Shigellosis can cause anything from moderate diarrhoea to severe dysentery as clinical signs. Fever, exhaustion, and watery diarrhoea are some of the initial symptoms. Abdominal cramps, nausea, and dysentery which is a condition marked by frequent, unpleasant stools that contain blood and mucus can occur in patients. From the above noticeable outcomes, taking into consideration the outcomes of the study carried out in Tshwane, it can be concluded that the antimicrobial agents used in the farms are only active against gram-negative species. This might be why gram-positive species such as *Salmonella* and *Shigella* are still thriving regardless of the continuous use of antimicrobial agents in the poultry and this further contributes heavily to the development of resistant patterns and strains in poultry which can thus be passed on to humans as consumers.

*E. coli*, *Salmonella*, *Enterobacter*, and *Campylobacter species* are among the multidrug-resistant (MDR) bacteria that pose a direct risk to human health as zoonotic diseases. The risks of AMR spreading to humans are increased by unsanitary techniques used in animal husbandry, such as letting farm waste outside, sharing dwellings and water sources with animals, and having unclean hands among farm personnel and animal occupants. Furthermore, the presence of MDR bacteria in milk, frozen chicken meat, broiler meat, bovine meat, and other animal-derived food products exposes consumers to AMR risks.

The mere fact that MDR pathogens were found in the food production system was enough to raise alarm not only in the poultry industry but also in the national AMR program. According to Zhou et al. (2022), global antimicrobial use in food animals in

2013 stood at 130,000 tons which data was possible to obtain. It is estimated that by 2030 it shall be 200 235 tons. From the results obtained from the present study, one can argue that the tonnage will be more than that given the misuse observed and reported by others before. It cannot be overemphasized that this has to be curbed to stop the possible transfer of resistant gene pool, particularly of the pathogens identified, into the surrounding environment at the poultry farm factories and ultimately into hospitals.

From the results obtained it was clear that, currently, there are problems in trying to address AMR by applying a multi-sectoral or multi-system approach as required by the One Health Concept. It also appears that assuming what was found is what is true nationally in South Africa, the contribution to the Global Antimicrobial Resistance and Use Surveillance System (GLASS) and data for the Global Health Security Index would not be meaningful. In any case, it has been suggested that none of them provide a comprehensive analysis of the global AMR situation under the One Health concept.

In summary, it is important to revisit what is meant by 'smart regulation' in order to put into context the implication of the findings from the present study. The term smart regulation was first used by Gunningham et al. (1998) in order to deal with problems encountered when applying traditional regulations in issues related to the environment. They found that there were pitfalls of deregulation and regulation on the one hand. Clearly, this appears to be the case in the poultry industry regarding the use of antimicrobials. Gunningham et al. (1998) concluded that smart regulation was an effective way of delivering policy objectives and that it also increases efficiency at least cost to the government, business, and the community. They also found that regulating complex areas such as, for example, the poultry industry which has multiple stakeholders with converging and diverging interest could be done using the smart regulation approach.

The approach uses five core principles that can be used in the poultry industry given the results obtained from the study, and also the fact that the desired AMR policy objectives are clearly stated in the South African AMR National Strategy Framework. In this case, AMR is the problem with its unique characteristics that have been identified and need solutions applying the five core principles in designing the smart regulation as follows:

- f. The incorporation of broad range complementary legal instruments and actors:
  - i. Legal instruments that would need to be considered,
  - ii. The Constitution of South Africa,
  - iii. The National Health Act,
  - iv. The Medicine and Related Substances Act (Act 101 of 1965),
  - v. Pharmacy Act (Act 53 of 174),
  - vi. The Fertilizers Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act 36 of 1947),
  - vii. The Public Finance Management Act (Act 1 of 1999),
  - viii. Foodstuff, Cosmetics and Disinfectant Act (Act of 1972),
  - ix. Meat Safety Act 2000,
  - x. Health Professions Act (Act 56 of 1974),
  - xi. Veterinary and Para-Veterinary Profession Act (Act 19 of 1988),
  - xii. National Environment: Waste Act (Act 59 of 2008),
  - xiii. National Environmental Health Norms and Standards for Premises and Acceptable Monitoring Standard for Environment,
  - xiv. Health Practitioners as part of National Health Act 2003 (Act 61 of 2003),
  - xv. Animal Disease Act (Act No 35 of 1984),
  - xvi. Animal Protection Act (Act No 71 Of 1962).

All these instruments must eventually speak to each other when promoting the One Health approach

- g. Actors (Stakeholders) identified during the study and also supported by literature:
  - i. Poultry farmers,
  - ii. Veterinary profession,
  - iii. Human health profession,
  - iv. Consumers,
  - v. SAHPRA, and
  - vi. Pharmaceutical industry.
- h. The second design principle to be followed when developing smart regulation is really about continuing education and awareness campaign about AMR of all stakeholders.

- i. The third design principles are made up of identified enforcers of the regulations. From the study results they would include:
  - i. SAHPRA,
  - ii. The South African Pharmacy Council,
  - iii. The Veterinary Council,
  - iv. Consumer Association.

The enforcers have to agree on the form of penalties to be enforced.

- j. The fourth design principle is about coming up with surrogate regulators made up of the actors identified in the First design Principle when designing the regulations. It would be necessary to include all but effort must be made to make sure that the actors are aware of the AMR smart regulation.
- k. The fifth principle would involve all stakeholders in the poultry industry to explore and optimize opportunities for win-win opportunities by satisfying the following:
  - i. Public health interest – preventing overuse or misuse of antimicrobial agents,
  - ii. Poultry farmers' business interests,
  - iii. Food industry interest.

If these principles are followed to the letter in the poultry industry it might be possible to avoid or mitigate problems arising from the unintended consequences of AMR regulation.

#### **5.1.7. Antimicrobial resistance**

From Figure 4.5, with the 97% feeding of chickens twice a day with antimicrobial agents through chicken feeds (80%) as indicated, it would be easy for antimicrobial resistance to develop which will ultimately be transferred to humans via gene transferase. Figure 4.4 showed that were mostly used twice a day as indicated in Figure 4.5, penicillin in human are mostly given three times a day due to their half-life, whereas in chicken is twice a day. This could contribute to antimicrobial resistance due to irrational use of antimicrobial agents. Hence, in the study conducted, there were *Salmonella* strains seen despite the use of antimicrobial agents. It would appear that problems could be emanating from inadequate frequency dose which differs from animals to humans, for example in these chickens are given twice daily (which is supposed to be mg/kg) while

in humans it is known to be three times daily. This would put the end consumers at risk of food-borne pathogens as the concentration may not be adequate to eradicate the bacteria and this could ultimately lead to antimicrobial resistance. Furthermore, the need for protein derived from animals and the lack of information regarding medications, animal illnesses, and the prevalence of antimicrobial resistance have prompted farmers to either abuse or overuse antibiotics. Animal AMR infections have emerged as a result of multifaceted irregularities in the use of antibiotics in veterinary practices and lax regulatory regimes for antimicrobial stewardship initiatives.

The genotyping results seemed to corroborate the observations made by Galhano et al. (2021), who interpreted the results by proposing that Whole Genome Sequencing (WGS) applications for AMR surveillance also exhibited limitations. The latest study identified the reasons why the experimentally determined AMR phenotype and the WGS genotype disagreed. Five cases of hetero-resistance in *S. enterica* were reported in the study, along with the observation that WGS was unable to predict phenotypic resistance since there were insufficient genotypic resistance determinants. The WGS failures were linked to the potential for unstable genetic traits such as transient gene amplification. As a result, culture and sensitivity test results are considered adequate in the literature (Galhano et al., 2021). Unfortunately, this is what has happened in the study. Gaps in the genome during genome assembly obscure resistance, and even the greatest and most appropriate bioinformatics software and sequencing technology cannot prevent them.

It would therefore be assumed that the result from the traditional methods of culture and sensitivity test be considered for policy review and implementation and even to take care of IPC principles in the poultry industry.

## 5.2. CONCLUSION

The results from the study have contributed more information in support of the fact that antimicrobial resistance (AMR) remains a threat to human health and that improper antimicrobial use in poultry farming is one of the possible drivers of resistance. Everyone involved in the industry needs to accept that AMR can develop in the poultry industry and that resistant microbes and the genes that encode this resistance can spread.

There is no denying that over the years, antibiotics have played a critical role in both preventing infectious diseases and promoting animal health, particularly chickens resulting in the growth of the poultry industry which is now such a big business. From the results obtained and attempts to interact with the South African Poultry Association (SAPA), it can be concluded that those involved in the industry closely and jealously guard the enterprise. Despite scientific data that indicates the widespread use of antibiotics in food-producing animals which has led to antibiotic resistance due to residues in food and the spread of the resistance in the environment resulting in a human health risk, there seems to be no sense of urgency to reduce antibiotic use in poultry in particular. This is borne by the way the poultry industry keeps ignoring results from numerous tests of prospective antibiotic substitutes given the fact that chicken feed accounts for 80% of chicken production. These substitutes provide comparable or superior effects to antibiotics, lower mortality rates, save the environment, and have no concerns about human health.

Poultry farmers should be encouraged to really take, seriously, some of the recommendations from these animal and veterinary scientific studies to reduce AMR spread. There certainly would be no need to be secretive about chicken feed formulation if chicken feed companies take the idea on board. At the same time, there is a need to review the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act (Act 36 of 1947) which from the results obtained was not effectively policed. From the results obtained, there is certainly no time to waste in developing guidelines that can be interpreted in different ways by all and sundry in poultry. What is needed are smart regulations starting from chicken feed formulation.

Going forward in-house chicken feed manufacturing by poultry farmers may need to be regulated and be subjected to tests to regulate and prevent the secret practice of using antimicrobial agents. That way everyone involved in the industry will appreciate the need to safeguard human health, the environment, and the promotion of the One Health approach. The standard chicken feed formulas must be adhered to and monitored by veterinarians at all times. The smart regulations on chicken feed formulations must be developed involving animal nutritionist organizations, feed producer companies, chicken buyers such as fast food companies, and animal welfare advocates.

Chicken feeders and poultry farmers lacked knowledge of antimicrobial agent use, antimicrobial resistance in poultry, and how this had a negative impact on humans. Hence emphasis on the need for their involvement in coming up with smart regulations to control the utilization of antimicrobial agents in chicken feeds. The smart regulations must be developed taking into consideration the level of basic education about chicken gut health and how this has an impact on food conversion ratio and chicken growth. Chicken feeders must be made to understand in a simple way what is meant by the chicken performance efficiency factor which is based on feed conversion ratio, life expectancy, live weight, and slaughter age. Basic knowledge about antibiotics and feed ingredients needs to be imparted to chicken feeders working at poultry farms instead of following blindly the farm factory standard operating procedures where currently most poultry farms are following the economics of fast-growing broilers.

Numerous tests of prospective antibiotic substitutes have produced results that are highly pertinent as far as antibiotic substitution is concerned. These substitutes provide comparable or superior effects to antibiotics (excellent cattle performance), lower mortality rates, and save the environment and the health of consumers. It would be very tempting to apply the findings of these studies to the feed industry, animal farmers, and veterinary practice.

The development of smart regulations would also require basic knowledge about microorganisms, antimicrobial agents in general, and zoonotic diseases. From the results obtained it can be concluded that most farm workers did not appreciate that humans can directly be exposed to resistant bacteria of nosocomial and/or community

origin. In nosocomial infections, antimicrobial resistance threatens the success of controlling the bacteria and causes hospital admission to the intensive care unit (ICU), pneumonia, and bloodstream infections. The link between chickens and humans, during production, in terms of the One Health approach has to be emphasized in the poultry industry. Chicken feeders need to be educated about the acquisition of infection along the chicken production value from the factory farm to the abattoir. They need to understand that it is also likely that consumers, even without direct contact, can acquire these infections through the consumption of chickens from poultry farm factories which in turn can lead to a larger number of hospitalizations, clinical resistance, and an increase in the number of deaths as a consequence of infections caused by resistant bacteria identified at the farms.

There were a number of bacteria identified from the three farms whose genotypes could not be definitively determined in order to conclude how the resistance to the antibiotics that they were exposed to, came about. What could, however, be concluded from the study was that there was a possibility of a link between AMU at the poultry farm factories and antimicrobial resistant infections ultimately in humans. The study did not look into the emergence of resistance but could only speculate the following:

- a. Emergence through point mutation,
- b. Horizontal gene transfer occurs through several mechanisms,
- c. Plasmids carrying resistant genes can be shared among bacterial strains,
- d. Bacteriophages can transfer resistant genes from one bacterium to another,
- e. Bacteria can take up naked DNA for example genes originating from dead bacteria.

What can be concluded from all this is that there may be many aspects of the emergence, ecology, and evolution of AMR in poultry factory farming that still need to be explored. It might, therefore, be difficult to extrapolate the findings of the present study to other settings, and even attempt to evaluate all direct and indirect impacts of AMU, generally, on the emergence of AMR. Given that situation, it was therefore not possible to develop any other guidelines that could be evaluated.

A conclusion from the behaviour of poultry farmers linked to a reluctance to open up about antibiotic use in chicken feed is the worry about economic losses. This is in

addition to possible market constraints caused by bacterial diseases such as colibacillosis due to avian pathogenic *E. coli* which were found at every poultry farm. This is despite there being substantial efforts in biosafety/biosecurity measures for prevention and control. Veterinarians are becoming more and more involved in addressing the threats that antibiotic resistance poses to public health and biosecurity in other nations, such as Bangladesh (Bourelly et al., 2018). From the results on the microorganism prevalence, it could be reasonably concluded that the biosafety measures seemed not to be standardized in the poultry farming community. This certainly would have encouraged the use of antibiotics not only for the treatment and prevention of avian diseases but not for growth promotion. Antibiotics were used in three factory farms but unfortunately, the exact package of practice was difficult to determine

Both gram-negative and gram-positive bacteria harbouring multiple AMR determinants were found at the farms in this review, we discussed major poultry production systems (conventional, ABF, and organic) and their impacts on AMR in some potential pathogenic Gram-negative and Gram-positive bacteria which could allow identifying issues and opportunities to develop efficient and safe production practices in controlling pathogens.

The study demonstrated the importance of prioritizing AMA that are critically important for humans which is a valuable and strategic risk management tool. This risk management approach has to be applied in the whole poultry industry focusing attention and resources on the highest-priority agents, unfortunately from the study, it was difficult to know the highest-priority classes. AMR can be reduced when antimicrobials are used only as a treatment, rarely for prophylaxis, and never as growth promoters. Success will require strict and efficient control of the types and amounts of antimicrobials used in both animals and medical practice; monitoring and controlling the proliferation of resistant bacteria that spread to the environment.

Unhygienic chicken husbandry practices, disposal of poultry farm factory wastes directly in the environment, along with possible poor personal hygiene of farm workers can exacerbate the risks of AMR if veterinarians do not effectively monitor what happens at the farms. However, for effective monitoring, surveillance, AMR stewardship activities,

and awareness building on AMU and AMR, qualified manpower along with adequate funding is a prerequisite. Capacity building of veterinary hospitals and laboratories with adequate manpower, training, logistics, and fund mobilization are crucial for the successful containment of AMR in the veterinary sector of South Africa. The following measures if the One Health approach is to succeed, are recommended for the effective mitigation of AMR problems in veterinary practices in South Africa:

- a. Cross-sectorial policy commitment and coordination for the implementation of NAP,
- b. Development of the annual action plan, including national surveillance of AMR, awareness campaigns, and assessment of knowledge about AMR,
- c. Increasing regulatory role or law enforcement capacities of implementing organizations involved in the curtailment of AMR, and
- d. Research on understanding the risk factors for the emergence and spread of AMR.

To truly understand the state of AMR in veterinary practices, in general, and specifically poultry in South Africa, a comprehensive and practical surveillance program that must be supervised by veterinarians must be developed and implemented. There is a need for timeous inspections and monitoring by veterinarians to poultry farms to make sure that regulations, policies, and guidelines are followed as far as AMU and AMR resistance are concerned. Moreover, the surveillance program should include a survey on the type and amount of AMA used at different poultry factory farms in South Africa. Scientific research to improve understanding of the risk factors for the emergence and spread of AMR is required to design evidence-based appropriate interventions for poultry factory farms.

The study makes it abundantly evident that there is a strong need for instructional models and targeted extension efforts to educate poultry farmers about the value of antibiotics and the negative effects of their indiscriminate use on human and animal health. It is also necessary to raise awareness among farmers about the necessity to use the appropriate services at the appropriate times from the appropriate source. Thus, the serious effects of antibiotic resistance which is a complex phenomenon, and their negative effects on human health will be lessened.

To prevent antimicrobial resistance and other zoonotic infections, South African Health Products Regulatory Authorities and the South African Pharmacy Council together with different stakeholders such as the South African Veterinary Association, and South African Poultry Association need to collaboratively identify areas that need effective adherence to the International Poultry Council AMR principles. In order to achieve this, it is necessary to strengthen global governance and accountability, accelerate global progress, innovate to ensure the future, collaborate for more effective action, and invest in sustainable solutions.

Given the current regulations, it would be reasonable to suggest that perhaps punitive measures against poultry factory farmers who do not follow regulations as stipulated in PSSA Pharmacy Law Compendium on chicken feeds formulations, utilization of antimicrobial agents for growth promotion must be brought to book. Unfortunately, this would not be possible unless smart regulations are developed.

There is a need to strengthen the national strategy framework on the promotion of judicious use of antimicrobial agents in humans and animals. The present study has clearly contributed scientific evidence to support each step in the causal pathway, from AMU on poultry factory farms to a possible public health burden that can be caused by infection with resistant pathogens such as *E. coli*, *Enterobacter*, and *Salmonella*.

The IPC has guidelines to be followed in poultry farming which SAPA is a member of the IPC, therefore there is no need to develop guidelines, but rather IPC through SAPA must make it a point that guidelines are being followed at all times in poultry farming.

### 5.3. RECOMMENDATIONS

A bigger study with more poultry farms would need to be carried out to have a completely bigger picture of the exact package of practice in the poultry industry. There is also a need for further studies to investigate the effects of Antimicrobial Free practices before they are taken on board in the poultry industry, on how such practices will influence chicken microbiota.

Research should address veterinary and human antimicrobial agent use, social, and economic influences on prescribing and drug dispensing practices, traditional beliefs and local cultures, and environmental factors that promote the development of drug-resistant pathogens. The SAHPRA should consider the categorization of antimicrobial agents and preserve some items as prescription-only medicines. This will avoid misuse of antimicrobial agents because pre-authorization or their use under the supervision of infection experts will be required. AMR is a “One Health” problem, and its rational containment warrants the inclusion of the veterinary sector.

Secondly, a campaign should be run among livestock farmers for awareness and to raise knowledge on the advantages and disadvantages of their prudent use of antimicrobial agents, the drawbacks of their indiscriminate use on animal health, and the subsequent repercussions on human health. A minimum of once per year, a village-level "antibiotics awareness week" should be held to assist in spreading the word and serve as a frequent warning to livestock farmers.

After treating the animal, veterinarians need to educate the livestock farmers on withdrawal periods and the effect of livestock products that contain antibiotic traces on human health. Additionally, veterinarians should inform livestock owners of the requirement for follow-up services to finish the proper antibiotic dosing regimen. As a whole, a multi-sectorial and multi-dimensional approach is needed to address the challenge of the AMR issue and a single organization or discipline cannot solely address its immediate threat. Poultry farmers must report their antimicrobial agent use for therapeutic purposes. Restrictions on antimicrobial agents' use as growth promotion should be implemented and monitored by veterinarians overseeing the poultry farms. More stakeholders should be held accountable for high antimicrobial agent usage. Laws

must be tightened and fines should be implemented for those highly using antimicrobial agents. This current situation also demands an imminent convergence of different state animal husbandry departments along with various NGOs, animal welfare associations, and veterinary universities as a prerequisite mount to fight against AMR effectively. The results of this study are expected to aid policymakers and authorities in planning how to battle this silent epidemic and reduce antibiotic resistance.

To reduce the use of antibiotics and minimize the risk of AMR, alternative measures should be implemented, such as improving hygiene, implementing good animal husbandry practices, maintaining appropriate living conditions, and implementing vaccinations that reduce the risk of infectious diseases in poultry. It is always important to seek advice from veterinarians or poultry experts in South Africa for the most accurate and up-to-date information and guidance on the use of antibiotics in poultry production.

In response to this issue, many countries, including South Africa, have implemented regulations and guidelines to restrict the use of antibiotics in animal agriculture. In South Africa, the use of antibiotics in livestock production should adhere to the Medicines and Related Substances Act, controlled by the Department of Agriculture, Forestry, and Fisheries. This act regulates the sale, distribution, and use of veterinary antibiotics, including those administered to poultry. In 2013, the World Health Organization (WHO) issued guidelines recommending that antibiotics, vital to human medicine, should not be used as growth promoters in food-producing animals. The European Union has also banned the use of antibiotics for growth promotion in livestock. Therefore, it is recommended that the Department of Agriculture, Forestry, and Fisheries conform to the recommendations made by WHO in safeguarding the use of antibiotics in poultry.

To effectively monitor and limit antimicrobial resistance (AMR) and reduce infections, these actors must reach a consensus on important action priorities and policies governing the use of antibiotics. The following are key tactics for tackling AMR using the One Health approach:

- a. Launch a regional, provincial, national, and global public awareness effort to inform people about the dangers of using antibiotics excessively and

inappropriately. Reducing the amount of antibiotic prescriptions can be achieved by implementing successful public campaigns.

- b. To stop the spread of illnesses, bolster and improve hygiene practices. Enhancing living conditions and healthcare systems will greatly lower the need for antibiotics, lowering the likelihood that a new resistant strain will emerge.
- c. Minimize the needless application of antibiotics in poultry farming and the environmental contamination they cause.
- d. Boost medication resistance monitoring worldwide. To comprehend the new processes of resistance acquisition, to identify existing cases with certainty, and to anticipate future risks, the scientific and medical community needs a thorough understanding of both historical and current data on AMR. To do this, a deeper comprehension of three areas is necessary: the molecular underpinnings of antimicrobial resistance (AMR), the usage of antibiotics by humans and animals, and the present rates of antibiotic resistance.
- e. Acknowledge and expand the pool of individuals handling infectious diseases. Experts in infectious disease, infection control, nursing, microbiology, pharmacy, veterinary medicine, and epidemiology are needed to address antimicrobial resistance (AMR). Countries must invest heavily in this human resource training in order to do this.
- f. Establish an international alliance to combat AMR with genuine action. For the fight against AMR to make meaningful progress, global action is required. To bring about change, it is critical to place AMR on the global political agenda and tackle it with a One Health approach (Velazquez-Meza et al., 2022).

A Global Action Plan (GAP) based on the "One Health" strategy was approved by the WHO in 2015 to address the growing global danger posed by antimicrobial resistance. Additionally, for the same goal, the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) created comparable plans and strategies (Hoque et al., 2020). A National Action Plan (NAP) for the containment of AMR in the human, animal, and environmental sectors in South Africa must be developed and approved in accordance with the WHO GAP recommendations. Together, all sectors must take coordinated and coordinated action for the NAP to be

implemented successfully. To do this, comprehensive knowledge of the existing AMR scenario in South Africa's animal, human, and environmental sectors is required.

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**7. Appendix A: Chicken farm workers** (Adopted and modified from Sikder et al., 2019).

1. Farm	Lunds <input type="checkbox"/>		Spif Chicken <input type="checkbox"/>		Mike's Chicken <input type="checkbox"/>
2. Population parameters	Age	18-30	30-40	40-50	50+
	Gender	Male <input type="checkbox"/>	Female <input type="checkbox"/>		
3. How often are chickens fed?	a. 1 time a day <input type="checkbox"/> b. 2 times a day <input type="checkbox"/> c. 3 times a day <input type="checkbox"/>				
	d. Throughout the whole day <input type="checkbox"/>				
4. The name(s) of the chicken feeds	1.				
	2.				
	3.				
	4.				
5. To your knowledge, do chicken feeds contain any antibiotics?	Yes <input type="checkbox"/>		No <input type="checkbox"/>		
6. If yes in question 5, which antibiotics?	1..				
	2.				
	3.				
	4.				
	5.				
7. Are there a standard working procedure for sterility maintenance?	Yes <input type="checkbox"/> No <input type="checkbox"/>				
8. If yes in question 7, where were they developed?	1.				
	2.				
	3.				
	4.				

**Appendix B: Poultry farmers questionnaire.**

1. Are there a standard working procedure for sterility maintenance?	Yes <input type="checkbox"/>	No <input type="checkbox"/>
2. If yes in question 1, where were they developed?	1. .... 2. .... 3. .... 4. .... 5. ....	
3. Are national or international policies regulating the farm?	Yes <input type="checkbox"/>	No <input type="checkbox"/>
4. If yes in question 3, where were they developed?	Justified <input type="checkbox"/>	unjustified <input type="checkbox"/>
Elaborate on both answers	..... .....	
5. What are the chicken feeds used?	1. .... 2. .... 3. .... 4. .... 5. ....	

**Appendix C: Veterinarians questionnaire.**

1. Are there any antibiotic guidelines or policies?	Yes <input type="checkbox"/>	No <input type="checkbox"/>
2. If <u>Yes</u> in question 1, where were they developed?	South African guidelines.  <input type="checkbox"/>	Other countries' guidelines.  Specify..... .....
3. If <u>NO</u> , how are poultry farms governed?	..... ..... .....	
4. To the best of your knowledge, are antibiotics used in poultry?	Yes <input type="checkbox"/>	No <input type="checkbox"/>
5. If <u>Yes</u> in question 4, which antibiotics are being used in poultry and why?	1. .... 2. .... 3. .... 4. .... 5. .... 6. ....	
6. Which chicken feeds are being used?	1. .... 2. .... 3. .... 4. ....	
7. How often do you visit the poultry farms?	1. .... 2. .... 3. ....	

**Appendix D: Consent form.**

<b>TITLE: DEVELOPMENT OF GUIDELINES ON THE USAGE OF ANTIBIOTICS IN POULTRY FARMS: TOWARDS ONE HEALTH</b>		
INVESTIGATOR: MATHOBELA CASWELL KWENA (Pharmacologist)		
<p>In response to the proper use of antibiotics in poultry farms and further learning, I am herewith inviting you to participate in a research study on the use of antibiotics in commercial poultry farms in Limpopo Province: towards one health initiative. The research will benefit the consumers in eradicating increased levels of antibiotic resistance emanating from the use of antibiotics in poultry for growth promotion.</p> <p>Your participation in this research has no risk attached and the participants will be done on the farm with other farm workers.</p> <p>You will be requested to fill in the questionnaire given and the data collected will be captured and no one except the research group will have access to it. Your identification will not be revealed when the research will be published.</p> <p>Should you have any questions about the study or about participating in the study, feel free to ask me (Kwena Mathobela). You can contact me on 0822822701 or 015 577 2049 (work).</p> <p>Your participation in this study is voluntary; you are under no obligation to participate. You have the right to withdraw at any time if you care to, without any repercussions or penalty, even in the middle of the process.</p> <p>The study and its procedures have been approved by the University of Limpopo Animal Research Ethics Committee (AREC) and Turfloop Research Ethics Committee (TREC).</p> <p>I have discussed the above points with the participant. It is my opinion that the participant understands the risk, benefits, and obligations involved in participating in this research.</p>		
..... Researcher	..... Date	
I understand that my participation is voluntary and that I may refuse to participate or can withdraw my consent and stop taking part at any time without penalty.		
..... Signature of witness	..... Signature of participant	..... Date

## Appendix E: Turfloop Research Ethics Committee clearance certificate.



**University of Limpopo**  
Department of Research Administration and Development  
Private Bag X1106, Sovenga, 0727, South Africa  
Tel: (015) 268 3935, Fax: (015) 268 2306, Email: anastasia.ngobe@ul.ac.za

**TURFLOOP RESEARCH ETHICS COMMITTEE**  
**ETHICS CLEARANCE CERTIFICATE**

**MEETING:** 31 October 2022  
**PROJECT NUMBER:** TREC/540/2022: PG  
**PROJECT:**

**Title:** Development of guidelines on the usage of antibiotics in poultry farms: Towards one health.  
**Researcher:** CKK Mathobela  
**Supervisor:** Prof NZ Nyazema  
**Co-Supervisor/s:** Prof M Nlooto  
Prof JW Ng'ambi  
Prof MR Lekalakala  
**School:** Health Care Sciences  
**Degree:** Doctor of Pharmacy in Pharmacology

**PROF D MAPOSA**  
**CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE**

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: **REC-0310111-031**

**Note:**

- i) This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.
- ii) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.
- iii) PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

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## Appendix F: Animal Research Ethics Committee clearance certificate



**University of Limpopo**  
Department of Research Administration and Development  
Private Bag X1106, Sovenga, 0727, South Africa  
Tel: (015) 268 4713/3766, Fax: (015) 268 2306, Email: arec@ul.ac.za

**ANIMAL RESEARCH ETHICS  
COMMITTEE CLEARANCE CERTIFICATE**

**MEETING:** 09 JANUARY 2023

**PROJECT NUMBER:** AREC/01/2023: PG

**PROJECT:**

**Title:** Development of guidelines on the usage of antibiotics in poultry farms:  
Towards one health.  
**Researcher:** CKK Mathobela  
**Supervisor:** Prof NZ Nyazema  
**Co-Supervisor:** Prof M Nloto  
Prof JW Ng'ambi  
Prof MR Lekalakala  
**School:** Health sciences  
**Degree:** Doctor of Pharmacy in Pharmacology

  
**PROF JW NG'AMBI**  
**CHAIRPERSON: ANIMAL RESEARCH ETHICS COMMITTEE**

The Animal Research Ethics Committee (AREC) is registered with the National Health Research Ethics Council,  
Registration Number: AREC-290914-017

**Note:**

- i) i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.
- ii) ii) The budget for the research will be considered separately from the protocol.
- iii) PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

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## Appendix G: Permission from Department of Agriculture and Rural Development



**LIMPOPO**  
PROVINCIAL GOVERNMENT  
REPUBLIC OF SOUTH AFRICA

### DEPARTMENT OF AGRICULTURE AND RURAL DEVELOPMENT

Director Animal Health  
Department of Agriculture, Forestry and Fisheries  
Private Bag X250  
PRETORIA  
0001

Veterinary Services  
Limpopo Department of Agriculture and Rural Development  
Private Bag X 9847  
POLOKWANE  
0700  
22 November 2022

**Dear Dr Maja**

#### SUPPORT FOR APPLICATION FOR PERMISSION UNDER SECTION 20 OF THE ANIMAL DISEASES ACT (ACT 35 OF 1984) TO PERFORM RESEARCH

I, Dr MK Mabunda (Director Veterinary Services Limpopo Province), hereby declare that I take note and have no objection for the research to be undertaken by Mr CKK Mathobela in Capricorn and Waterberg districts of Limpopo Province:

The title of the study is "**Development of guidelines on the usage of antibiotics in poultry farms: towards One Health**".

The Research Project is supported.

Regards

A handwritten signature in blue ink, appearing to read 'M. Mabunda'.

Dr. M.K. Mabunda  
Director of Veterinary Services  
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