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**ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF *Escherichia coli*
ISOLATED FROM LOCAL CHICKEN IN BUMBOBI SUB
COUNTY, MBALE DISTRICT**

BY

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BVM V

**A SPECIAL RESEARCH PROJECT REPORT SUBMITTED TO THE COLLEGE
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DECLARATION AND APPROVAL

I, **Nakhumitsa jenipher**, hereby declare that the work presented in this project report is my original work and it has never been submitted to any University or institution of learning for any academic award.

Signature..........Date.....15th/10/2026.....

This research project was done under the supervision and approval of my supervisor.

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DEDICATION

This work is dedicated to my family, more so Shuyo Patrick and Dr Philip Wakimwere and also other people who supported me in research and completion of my studies

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Special thanks to the Almighty God for the gift of life, good health and knowledge. I acknowledge my parents Mr. Shuyo Patrick and Ms. Nafula Grace for the moral and financial support towards the completion of my education. Similarly, I would like to thank my supervisor, Dr. Ikwap kokas, Mbale laboratory team for the resources, guidance and knowledge they gave me during this research. May God bless you abundantly!

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ABBREVIATIONS AND ACRONYMS

%	Percentage
µg	Micrograms
AMP	Ampicillin
AmpC	AmpC β-lactamases
AMR	Antimicrobial resistance
APE	Avian pathogenic <i>E. coli</i>
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
<i>E. coli</i>	<i>Escherichia coli</i>
ESBL	Extended spectrum β-lactamases/carbapenemases
ExPEC	Extra intestinal pathogenic <i>E. coli</i>
FAO	Food and Agricultural Organization
GET	Gentamicin
IMVIC	Indole, methyl red, Voges-Proskauer and citrate utilization tests
MDR	Multidrug resistance/resistant
Spp	Species
TET	Tetracycline
WHO	World Health Organization

ABSTRACT

Antimicrobial resistance (AMR) is a growing global public health concern, with *Escherichia coli* serving as an indicator organism for monitoring resistance in livestock. These study aimed to establish antimicrobial susceptibility profiles of *Escherichia coli* isolates from local chicken Cloacal swabs in Bumbobi Sub County. One hundred fifty seven Cloacal swabs were randomly collected from local chickens however one hundred samples were cultured following standard protocols for a period of three months. Isolates of *E. coli* were subjected to antimicrobial susceptibility testing on different antibiotics using the Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute guidelines. The overall prevalence of *E. coli* in local chicken was 85.0%. Ampicillin showed highest resistance (89.4%) and least in ciprofloxacin (2.4%), with no statistical significant difference among the antibiotics used in the isolates of *E. coli* ($p > 0.585$). However, Multidrug resistance (MDR) of *E. coli* isolates (10.6%), showed slightly high significance different ($p < 0.05$).

These findings reveal a high burden of AMR in *E. coli* isolates from fecal samples of local chickens, which poses a risk to animal and public health. Therefore, strengthened antimicrobial stewardship, farmer education on prudent antibiotic use, and continued AMR surveillance are recommended to mitigate the spread of resistant *E. coli*.

CHAPTER ONE

INTRODUCTION

1.1 Back ground

Pathogenic bacteria have been incriminated to cause huge casualties in the poultry industry more than viral diseases (Byaruhanga *et al.* 2017). Bacterial pathogens including, *Escherichia coli*, *Staphylococci*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, and *Salmonella* have become the major threats to poultry health globally (Rafiq *et al.*, 2024). The use and misuse of antimicrobial agents has led to the wide development of resistance which is threatening their effectiveness in the treatment and prevention of bacterial infections (Kilari & Oroszi, 2024). The *E. coli* being part of the normal flora of humans and livestock, it's considered to be an excellent indicator of antimicrobial resistance which is a global concern in both human and veterinary medicine. The *E. coli* is one of the most important and frequently encountered bacterial avian pathogen causing a wide variety of disease in birds. Actually *E. coli* strains, designated as "avian pathogenic *Escherichia coli* (APEC) are causative agents of colibacillosis, a disease with up to 30% prevalence worldwide and affecting all age groups of local chicken (Guabiraba & Schouler, 2015).

Globally, *E. coli* is responsible for many deaths in domestic birds (Byaruhanga *et al.*, 2017). Colibacillosis remains among the most commonly diagnosed poultry diseases in Uganda and is responsible for enormous economic losses, it has been reported in poultry that the most dangerous illness occurs at all ages and mortality rates reach up to 95% (Gedeno *et al.*, 2022). Colibacillosis often a secondary infection disseminated in feces and manifesting as acute enteritis, air– sacculitis, pericarditis, peritonitis, coli granuloma of liver, synovitis, swollen-head syndrome and osteomyelitis (Shehata & Hafez, 2024). In addition, *E. coli* has been extensively used to monitor antimicrobial resistance in food animals (including poultry). According to (Kabiswa *et al.*, 2018). 70% of bacterial isolates from chicken were resistant in central and eastern Uganda showing a high prevalence of antimicrobial resistant bacteria in Uganda. This was attributed to misuse of antimicrobials during rearing.

1.2 Statement of the problem

Antimicrobial resistance in *Escherichia coli* from local chickens poses a growing threat to animal and public health in Uganda, particularly in rural settings where free-range poultry systems dominate and antibiotic use is often poorly regulated. In Bumbobi Sub-County, local chickens are commonly kept under extensive management with frequent exposure to residual antimicrobials through informal treatment practices, yet systematic data on the susceptibility profiles of *E. coli* isolated from these birds remain limited. Existing interventions, including national antimicrobial stewardship guidelines and sporadic laboratory surveillance, have largely focused on commercial poultry or clinical isolates and have not adequately characterized resistance patterns in local chicken populations. This leaves a critical gap in understanding the prevalence of resistance and multidrug-resistant strains circulating in village poultry systems. The present study therefore sought to establish the antimicrobial susceptibility profiles of *Escherichia coli* isolates from local chicken Cloacal swabs in Bumbobi Sub-County, with particular attention to antibiograms and the extent of multidrug resistance.

1.3 General objectives of the study

To establish antimicrobial susceptibility profiles of *Escherichia coli* isolates from local chicken Cloacal swabs in Bumbobi Sub County.

1.4 Specific objective

- i. To determine the antibiograms of *Escherichia coli* isolates from local chicken cloacal swabs in Bumbobi sub county.
- ii. To assess the extent of multidrug resistance among the isolates.

1.5 Research question

1. What is the prevalence of *Escherichia coli* in local chicken cloacal swabs from Bumbobi Sub County?
2. What is the antibiotic susceptibility of *Escherichia coli* isolated from local chicken cloacal swabs from Bumbobi Sub County?

1.6 Justification and significance

Most studies on antimicrobial resistance in Uganda and worldwide have focused more on commercial layers and broiler birds excluding local chicken. This has been exacerbated with limited ongoing antimicrobial resistance surveillance in the local chicken industry in Uganda. It is against this background that this study was carried out purposely to investigate and establish antimicrobial susceptibility profiles of *E. coli* isolates from cloacal swabs of local chicken in Bumbobi sub county in order to address the knowledge gap. The generated data will provide the necessary baseline evidence to guide target One Health Policy interventions, improve local veterinary diagnostic protocols and safeguard both animal welfare and human health.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of *Escherichia coli*

First identified and described in 1885 by the German pediatrician Theodor Escherich (Altenbuchner & Mattes, 2004). The *E. coli* is a gram-negative, non-spore forming, non-acid fast, facultative anaerobic coccobacillus belonging to family Enterobacteriaceae. Motile strains do so by peritrichous flagella though some strains are exclusively non-motile. The fimbriae are mannose-sensitive and hem agglutinating of type 1 and are present in both motile and non-motile strains however some strains possess polysaccharide capsule that are isolated from extra intestinal infections. The genus *Escherichia* consists of five species *E. coli*, *E. fergusonii*, *E. hermannii*, *E. vulneris* and *E. blattae*. Of these *E. coli* is ranked to be the most important and most common species causing infections in humans (Parija et al., 2026). It's is ubiquitous bacteria which has a worldwide distribution and can be found in soil, water, and plants (Mohamudha et al., 2012). The *E. coli* has been used as an excellent indicator of fecal contamination easily disseminated through water and food chain in different ecosystems.

2.2 *Escherichia coli* occurrence and pathogenicity

Escherichia coli is a gram- negative rod shaped bacterium, a strain that causes colibacillosis in poultry and other vertebrates. Though many *E. coli* are not pathogenic, some have acquired virulence factors by acquiring specialized packages of DNA from other bacteria through horizontal gene transfer, which gives them the necessary genetic tools to stick to host tissues, use vital iron and survive the bird's immunity. Avian colibacillosis has been noticed to be a major infectious disease in birds of all ages, colibacillosis results in localized or systemic infection caused by Avian Pathogenic *Escherichia coli* (APEC). The portal of entry into birds varies but can include the respiratory tract, areas of skin trauma, cloaca, damaged intestinal mucosa, and navel in chicks. From the entry sites, *E. coli* can extend locally or gain access to blood stream to cause coli septicemia to death.

The disease can manifest in diverse ways, acute fatal septicemia, sub-acute pericarditis, airsacculitis, salpingitis and peritonitis (Singh et al., 2013). The infection results into

diarrhea and septicemia in poultry and other avian species as well as in animals like lambs foal and calves.

Poultry and livestock are ranked to be the excellent reservoirs for pathogenic *E. coli* and indiscriminate antimicrobial use in animal farming is among the factors promoting the emergence, selection and dissemination of antimicrobial-resistant microorganisms (Yassin et al., 2017).

2.3 Epidemiology of *E. coli* infections in poultry

Escherichia coli infection is considered as a zoonosis and a major poultry bacterial disease world-wide (Khoo et al., 2010). Poultry are potential carriers of pathogenic bacteria which are capable of transmitting zoonotic diseases to humans as a result of the various interactions with man. Poultry droppings may contaminate the environment with antimicrobial-resistant bacteria (Olonitola et al., 2015). Avian pathogenic *E. coli* results into systemic or local infection outside the gut known as extra intestinal pathogenic *E. coli* (ExPEC). Its characterized by fatal septicemia or sub-acute fibrinous pericarditis, air sacculitis, peritonitis in poultry (Ibrahim et al., 2019). The main clinical signs manifested are depression, loss of appetite, tendency to huddle, respiratory distress, closed eyes, dropped wings, cyanosis and labored breathing (Shehata & Hafez, 2024). Stress and inappropriate husbandry might be the major predisposing factors for systemic APEC infections. The natural route of infection for APEC is not clearly defined, although oral and respiratory routes seem to be significant modes of entry (Dziva and Stevens, 2008). An estimated 10% to 15% of avian alimentary tract coliforms have been reported to belong to potentially pathogenic APEC serotypes (Harry and Hemsley, 1965). Consistently, both virulent and a virulent *E. coli* have been shown to colonize and persist efficiently in the intestinal tract, with extra intestinal translocation occurring only in the presence of stressors (Dominick and Jensen, 1984; Leitner and Heller, 1992). Avian pathogenic *E. coli* has been reported in dust in poultry units and dry environment harboring up to 10⁶ colony-forming units per gram. Intestinal nature of APEC provides an excellent opportunity for transmission and dissemination via feces where it has been reported to persist in the environment. A systemic APEC infection results from inhalation.

2.4 Prevalence of *E. coli* in poultry

The prevalence of *Escherichia coli* (*E. coli*) in the fecal samples of birds varies extensively across global populations, ranging from less than 5% to over 90%. Unlike in mammals, where *E. coli* serves as a universal intestinal commensal, its presence in the avian gut micro biota is highly conditional upon host, species, diet and habitat (Gordon & Cowling, 2003). Wild birds occupying pristine, isolated ecosystems exhibit remarkably low colonization rates, often maintaining a baseline prevalence below 15%. This baseline suggests that *E. coli* may not be permanent or obligate resident of the natural gut micro biota of all avian species, but rather a transient organism acquired through environmental interactions.

Anthropogenic encroachment and environmental pollution drastically elevate the shading rates of *E. coli* in avian feces. Migratory waterfowl and synanthropic species such as gulls and sparrows living near urban areas act as environmental sentinels, frequently picking up bacteria from contaminated water sources, agricultural runoffs, and landfills (Reed et al., 2003). Waterfowl often yield detection rates between 40% and 75% due to their aquatic foraging behaviors. Conversely, commercial poultry operations represent high extreme of avian prevalence: intensive farming conditions and high density housing facilities continuous fecal oral cycles, leading to widespread herd colonization that regularly exceeds 80% (Barnes et al., 2008).

Avian fecal *E. coli* isolates are subject to intense epidemiological surveillance due to their capacity to harbor virulence and antibiotic resistance genes. A significant subset of these isolates is classified as avian pathogenic *E. coli* (APEC), which possesses specialized plasmid-born genes responsible for systemic infections like colibacillosis in flocks. Furthermore, wild and migratory birds frequently serves as long distance vectors for multidrug resistance and extended spectrum Beta-lactamase (ESBL)–producing strains. By ingesting human or veterinary waste, these avian vectors transport high-consequence resistance profile across intercontinental migratory flyways, bypassing localized biosecurity boundaries (Guenther et al., 2011).

2.5 Susceptibility and resistance profiles of *E. coli* from poultry

The global prevalence of antimicrobial resistance in *Escherichia coli* isolated from poultry is defined by an alarming trend of multidrug resistance (MDR), largely driven by

the pervasive use of antibiotics for growth promotion and prophylaxis in intensive farming systems (Landers et al., 2012). Globally these avian *E. coli* isolates consistently demonstrate profound resistance to fundamental therapeutic agents, including Penicillins, Tetracyclines and sulfonamides (World Health Organization, 2017). Widespread use of these agents in industrialized agriculture exerts continuous selective pressure, which facilitates the world wide spread of resistant bacterial strains (Marshall & Levy, 2011). Because these poultry based reservoirs contribute directly to global dissemination of resistant genetic elements, they are recognized as a primary risk factor for international public health stability (Holmes et al., 2016).

In many developing nations, the resistance profile of *E. coli* in poultry is significantly aggravated by the systemic availability of antibiotics as over the counter products, often purchased without a professional veterinary prescription (Laxminarayan et al., 2013). Research indicates that poultry in these regions frequently carry *E. coli* strains showing low level of susceptibility to antibiotics classified as critically important for human healthcare (Van Boeckel et al., 2015). Due to minimal regulatory enforcement of pharmaceutical standards, poor farm biosecurity practices, and inadequate water waste management practices, these resistant pathogens readily migrate from livestock environments into the general human community (Prestinaci et al., 2015). Consequently, the high prevalence of these resistant strains in communal and informal market setting represents a failure in the containment of bacterial pathogens at the animal-human interface (Manyi-Loh et al., 2018).

Across the African continent, antimicrobial resistance in the poultry industry is sustained by a combination of high infectious disease burdens and over reliance on affordable, broad spectrum antibiotic therapies (Manyi-Loh et al., 2018). Surveillance efforts have documented elevated resistance levels to common therapeutic classes in *E. coli* recovered from raw poultry meat and environmental farm samples (Djegbale et al., 2021). Because many African regions experience fragmented veterinary policy enforcement and limited surveillance infrastructure, resistance determinants persist at high frequencies in poultry agriculture eco system (Anyanwu et al., 2020).

The lack of continuous environmental monitoring ensures that poultry meat often serves as a major vehicle for transmitting resistant bacteria into the rural diets, thereby exacerbating the Africa's public health challenge (Omoya et al., 2020).

In Uganda, poultry derived *E. coli* is characterized by exceptionally high rates of multidrug resistance, with some agricultural surveillance reporting farm level colonization rates as high as 62.7% (Bazira et al., 2023). Investigations of isolates from Ugandan poultry industry frequently reveal profound non susceptibility to first line antibiotics, such as tetracyclines, penicillins, with resistance occurrences in over 79% of tested samples (Mugo et al., 2020).

Clinical data focusing on symptomatic poultry population in Uganda has confirmed multidrug resistance rates reaching as high as 88.4%, underscoring the severe impact of unregulated drug use on avian health (Olila et al., 2021). These susceptibility profiles confirm that the poultry sector in Uganda functions as an acute reservoir for resistant *E. coli* strains (Bazira et al., 2023).

2.6 Diagnostic features and biochemical characteristics of *E. coli*

Biochemically, *E. coli* ferments maltose, glucose, mannitol, lactose and many other sugars with production of acid and gas. Some *E. coli* strains are non-lactose and sucrose fermenters, they do not utilize urea, liquefy gelatin or produce hydrogen sulfide (Jakaria et al., 2012). The widely used important four biochemical tests are generally: indole, voges-proskauer, methyl red and citrate utilization tests (IMVIC tests) in classification of enterobacteria (Parija, 2012). When indole, methyl red, voges-proskauer and citrate utilization tests are performed on previously incubated colonies showing growth characteristics of *E. coli*. After incubation for 24 hours at 37°C positive isolates, the medium develops red color on the surface after addition of 2-3 drops of Kovac's reagent for indole, a red color and yellow in 15 minutes or more after addition of 0.6 ml of 5% α -Naphthol followed by 0.2 ml of 40% KOH for methyl red and voges-proskauer respectively, for citrate utilization test the media remains green since the organisms cannot use citrate as its only source of carbon.

2.7 Culture characteristics of *E. coli*

The *E. coli* grows on simple laboratory media in temperature range of (10-40), optimally at 37°C and a media pH of 7.2. They grow on a wide range of media such as MacConkey agar, nutrient agar, Mueller-Hinton agar and blood agar. Mostly primary isolation is made on nutrient agar, after 18-24 hours of incubation at 37°C the colonies appear as large, circular, low convex, grayish, white, moist, smooth, opaque or translucent colonies. On

sub culturing on MacConkey agar, bright pink colonies due to fermentation of lactose are seen, produce beta-hemolytic colonies on blood agar especially with strains isolated from pathogenic conditions (Mandal et al., 2012; Mohamudha et al., 2012).

2.8 Antigenic types of *E. coli*

Antigenic types of *E. coli* include: O1, O2, O8 and O78 cause colibacillosis and range of virulence factors have been described (Kika et al., 2023). The severe disease situation in avian species is potentiated by virulence factors, including adhesins, iron acquisition systems, hemolysins, and antibactericidal factors (Yamamoto and Echeverria, 1996, Schubert et al., 1998, 2000; Janßen et al., 2001, Ewers et al., 2004, 2005, 2007). The Shiga toxin *E. coli* is among the novel serotypes.

2.9 Antibiotic Sensitivity measurement for different antibiotic drugs

The bacteria strains are classified into three levels: Susceptible (S) means standard doses will infectively inhibit the bacteria, Intermediate (I) means the drug can work but requires higher doses, Resistant (R) means standard doses will fail to treat the infection (Table 1).

Table 1: Interpretation guide for the resistance profiles of *E. coli* to different antibiotics

Antimicrobial class	Drug	Concentration	Resistance	Intermediate	Susceptible
Fluoroquinolones	Ciprofloxacin	5µg	≤ 21	22-25	≥ 26
Tetracyclines	Tetracycline	30µg	≤ 11	12-14	≥ 15
Macrolides	Erythromycin	15µg	≤ 13	14-22	≥ 23
Penicillin	Ampicillin	10µg	≤ 13	14-16	≥ 17

Sourced from Mbale Veterinary Diagnostic Laboratory

2.10 Mechanism of resistance of *E. coli* isolates

Escherichia coli are among the members of Enterobacteriaceae family that pose the greatest threat to humans because of the growing resistance to antibiotics. So far these bacteria are more diverse than those in other families as they show resistance to different antibiotic groups and could this partially explain why they are the most common causes of antibiotic resistant bacterial infection in humans. The past two decades have shown a drastic increase in the emergence and spread of *E. coli* resistant strains to major classes of

antimicrobials like aminoglycosides, quinolones, β -lactams, fosfomycin and sulfonamides, this resistance has spread to last resource antibiotics classes like carbapenems and polymyxins (Alarcon and Omenaca, 2004). Different mechanisms of resistance evolved against β -lactams and these include (a) alteration of target site, (b) efflux from the periplasmic space through specific pumping mechanism, (c) decreased penetration of the antibiotic to the target site (d) inactivation of these agents by production of beta-lactamases that is used mostly by *E. coli* and these enzymes are ancient compounds that emerge from environmental sources currently exceeding 2800 unique proteins (Bush, 2018). *E. coli* (Gram negative bacteria) from the medical point of view has evolved by production of different β -lactamases such as extended spectrum β -lactamases (ESBL), carbapenemases and AmpC β -lactamases (AmpC) and these present a different spectrum of hydrolytic activity that presents different types of β -lactams resistance (Alarcon and Omenaca, 2004).

3.3 Sample size determination

The sample size was calculated by using the formula by Thrusfield (2007). The parameters used were 95% confidence interval, estimated prevalence of *E. coli* in the study area as 88.4% and 5% desired level of precision as shown below;

$$n = \frac{Z^2 * p(1 - p)}{d^2}$$

Where:

n= Study sample size

Z = Critical value associated with 95% confidence interval =1.96.

p = Estimated prevalence of 88.4% of *E. coli* (Olila et al. 2021)

d = Margin of error = 0.05

$$n = \frac{1.96^2 * 0.884(1 - 0.95)}{0.05^2}$$

$$n = 157.4$$

$$n \approx 157 \text{ samples/ chicken}$$

Thus, the minimum sample size required was 157 local chickens. However, only one hundred samples (swabs) were considered due to financial constraint.

3.4 Sample collection, transportation and storage

The samples were collected from the homesteads that had five birds and above where by three swabs were picked irrespective of the total number of chicken in a home.

Local chicken were randomly selected for sampling. They were physically restrained by homestead care takers and under complete aseptic conditions, sterile cotton swabs were gently inserted and rotated inside the chicken's cloaca. The cotton swabs were then immersed into sample collection tubes containing Amis transport media, stored in a cool

box containing ice packs and immediately transported to the Mbale veterinary Diagnostic Laboratory for bacterial examination.

3.5 Laboratory procedure

3.5.1 Isolation of *E. coli*

The culture and isolation were performed according to methods described by (FAO, 1996). Swabs were transferred into falcon tubes containing 5ml of Buffered peptone water (to ensure enrichment) and incubated at 37°C for 24 hours. Using a sterile wire loop, each incubated sample was streaked on to MacConkey agar. The streaked culture plates were incubated at 37°C for 24 hours. The *E. coli* suspected colonies were identified as bright pink medium sized colonies with metallic sheen hence lactose fermenter in MacConkey agar.

3.5.1.1 Sub culturing

The bright pink medium sized colonies with metallic sheen were picked using sterile tooth picks from the original plates, inoculated onto the fresh MacConkey agar plates and streaked using sterile wire loop then incubated at 37°C for 24 hours in order to obtain a pure culture

3.5.1.2 Biochemical tests

Using a sterile wire loop, part of the selected pure colony was subjected to biochemical tests (Indole, methyl red, voscus proskar and citrate utilization tests) for *E. coli* confirmation. The *E. coli* isolates were indole (red ring on the surface of broth) and methyl red (red color) positive and Simmons citrate negative (green), from these results *E. coli* was confirmed positive.

3.5.2 Antimicrobial susceptibility testing of *E. coli*

Antimicrobial susceptibility testing was done in accordance with methods described by Kirby-Bauer (Bauer et al., 1966). Briefly, fresh bacterial culture with 0.5 McFarland turbidity was swabbed onto the Mueller Hinton Agar (MHA) surface using sterile cotton buds. Using a pair of sterile forceps four Commercial antimicrobial discs were evenly embedded onto the inoculated agar incubated at 37°C for 24 hours (Gautam et al., 2019 and Kathleen et al., 2016). Antimicrobials were selected because they are commonly used by farmers; these were Ampicillin (10µg), Ciprofloxacin (5µg), erythromycin (15µg), and

Tetracycline (30µg) while ciprofloxacin is not used in poultry but local chicken can pick resistant genes from humans since they live in the same environment. The plates were placed in the incubator at 37°C for 24 hours. The observed zone of inhibition surrounding each drug disc was measured in millimeters using a ruler. For each drug the zone size was interpreted as susceptible (S) or resistant (R) based on the interpretation chart provided by Clinical and Laboratory Standards Institute (Walker, 1999). The absence of a zone of inhibition indicated resistance to the antimicrobial agent. The values were compared to the critical values of each antimicrobial disc to qualify the target bacteria as sensitive, resistant or intermediate in relation to colony growth and the results interpreted according to Clinical and Laboratory Standards Institute (Amer et al., 2018).

3.6 Data management and analysis

The collected data were entered into Microsoft Excel spreadsheet (Microsoft Excel 2010). The data were analyzed in STATA version 14.2 using frequencies and percentages. Associations were assessed using the chi-squared test at 5% level of significance. Results were presented in tables and figures.

CHAPTER FOUR

RESULTS

4.1 Prevalence of *Escherichia coli* in local chicken fecal samples

A total of 100 cloacal swabs were collected from local chickens across four parishes in Bumbobi Sub-County. *Escherichia coli* were isolated from 85 of the 100 samples, giving an overall prevalence of 85.0% (85/100).

The highest isolation rate was recorded in Busambe parish (92.0%, 23/25) and Bumbobi parish (88.0%, 22/25), while the lowest was observed in Bukhumwa parish (76.0%, 19/25). Bufuya parish recorded a prevalence of 84.0% (21/25). There was no statistically significant difference in the isolation of *E. coli* across the four parishes ($\chi^2 = 1.94$, $p = 0.585$).

Table 2: Prevalence of *Escherichia coli* in fecal samples of local chickens in Bumbobi Sub-County

Parish	Number sampled	Number positive	Prevalence (%)	p-value
Bumbobi	25	22	88.0	0.585
Busambe	25	23	92.0	
Bukhumwa	25	19	76.0	
Bufuya	25	21	84.0	
Overall	100	85	85.0	

4.2 Antibigrams of *Escherichia coli* isolates

The 85 *E. coli* isolates were tested against four antibiotics representing different classes: ampicillin (penicillins), erythromycin (macrolides), tetracycline (tetracyclines) and ciprofloxacin (fluoroquinolones).

The highest resistance was observed against ampicillin (89.4%, 76/85), followed by erythromycin (57.6%, 49/85) and tetracycline (13.0%, 11/85). Resistance to ciprofloxacin was low (2.4%, 2/85).

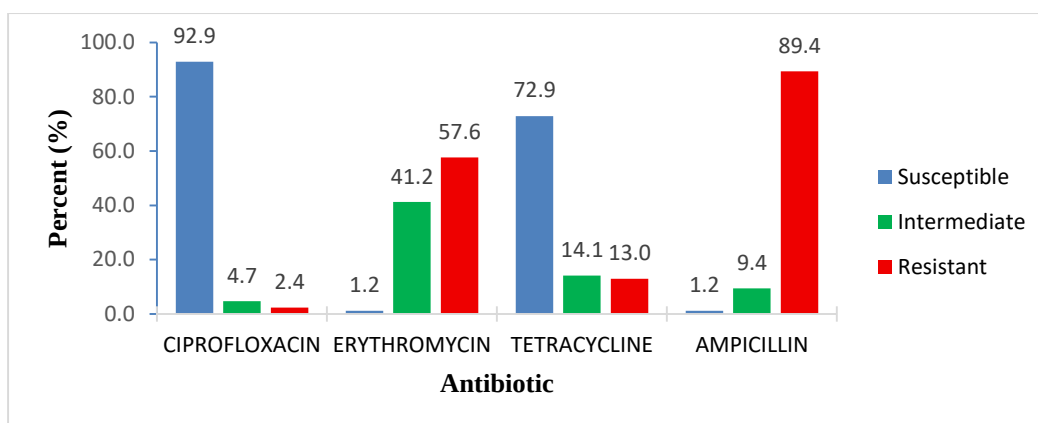


Figure 2: Antimicrobial resistance of the *E. coli* isolates to different antibiotics

Overall, 94.1% (80/85) of the isolates were resistant to at least one of the tested antibiotics. The most frequent resistance pattern was resistance to two antibiotics (47.1%, 40/85), followed by resistance to one antibiotic (36.5%, 31/85) and resistance to three antibiotics (10.6%, 9/85). Only 5.9% (5/85) of the isolates were susceptible to all four antibiotics. The distribution of resistance profiles did not differ significantly across the four parishes ($p = 0.730$) as summarized in table below.

Table 3: Antimicrobial resistance profiles of *Escherichia coli* isolates from local chicken fecal samples in Bumbobi Sub-County

Resistance profile	Bumbobi (n=22)	Busambe (n=23)	Bukhumwa (n=19)	Bufuya (n=21)	Overall (n=85)
Susceptible to all (0 drugs)	3 (13.6%)	1 (4.3%)	0 (0.0%)	1 (4.8%)	5 (5.9%)
Resistant to 1 drug	6 (27.3%)	10 (43.5%)	6 (31.6%)	9 (42.9%)	31 (36.5%)
Resistant to 2 drugs	9 (40.9%)	9 (39.1%)	12 (63.2%)	10 (47.6%)	40 (47.1%)
Resistant to 3 drugs	4 (18.2%)	3 (13.0%)	1 (5.3%)	1 (4.8%)	9 (10.6%)

4.3 Multidrug resistance among *Escherichia coli* isolates

Multidrug resistance (MDR) was defined as resistance to three or more classes of antibiotics. Of the 85 *E. coli* isolates, 9 (10.6%) were multidrug-resistant.

The highest proportion of MDR isolates was recorded in Bumbobi parish (18.2%) followed by Busambe parish (13.0%). Bukhumwa and Bufuya parishes recorded MDR rates of (5.3%) and (4.8%), respectively (Table 4.3). There was statistically significant difference in the distribution of MDR isolates across the parishes ($p < 0.05$).

Table 4: Multidrug resistance among *Escherichia coli* isolates from local chicken fecal samples in Bumbobi Sub-County

Parish	MDR, n (%)	Non-MDR, n (%)	Total
Bumbobi	4 (18.2)	18 (81.8)	22
Busambe	3 (13.0)	20 (86.9)	23
Bukhumwa	1 (5.3)	18 (94.7)	19
Bufuya	1 (4.8)	20(75.2)	21
Overall	9 (10.6)	76 (89.4)	85

MDR = resistance to three or more classes of antibiotics.

CHAPTER FIVE

DISCUSSION

The development of anti-microbial resistance in many bacterial organisms constitutes a serious problem in the control of infectious diseases. Anti-microbial use and especially misuse has been found to be the most important selecting force in bacterial antibiotic resistance. Although there is no linear relationship between the amounts of anti-microbial used and the development of resistance, increased use of an anti-microbial often results in decreased susceptibility among the exposed bacteria(Okoli, 2006).

The high resistance of *E. coli* to Ampicillin, Erythromycin and Tetracycline could be attributed to the use of these antimicrobials for long term in veterinary practice. Tetracycline, Ampicillin and Erythromycin are common antibiotics prescribed for poultry treatment in Uganda probably most of the time, poultry farmers use these medicines without advice of the veterinarian. This finding is similar to a previous study carried out in Tanzania where high prevalence of *E. coli* resistance to Tetracycline and Ampicillin resistance was observed in *E. coli* isolated from poultry (Rugumisa et al., 2016).

This study showed resistance of *E. coli* isolates to Ciprofloxacin to be very low .This could probably be due to the limited use of this drug in livestock production in the study area and therefore less exposure of the birds to the drug (Majalija et al., 2010). Similar findings were reported by (Sarba et al., 2019) who analyzed *E. coli* in poultry from central Ethiopia.

In this study, some of the *E. coli* isolates were resistant to more than three classes of antibiotics. This could be due to use of many antibiotics at the same time by farmers. The resistance of *E. coli* isolates to different antibiotics (Erythromycin, Tetracycline and Ampicillin) in this study indicates a threat to public health. Similar findings were reported in Iran by (Rahman et al., 2020) who did the same study in commercial chicken meat.

CHAPTER SIX

CONCLUSION AND RECOMMEDATIONS

6.1 Conclusions

This study revealed that *E. coli* isolates from local chicken were highly resistant to ampicillin and erythromycin antibiotic drugs. More than half of the *E. coli* isolates were multidrug resistant as they exhibited resistance to two or more antibiotic classes. These findings highlight the threat of AMR to public health and the need for target interventions to curb the continued escalation of AMR in poultry farming.

6.2 Recommendations

The occurrence of AMR in *E. coli* from local chicken underscores the need to sensitize local chicken farmers on proper use of antibiotics to mitigate the spread of AMR.

Future studies should investigate the drivers and molecular epidemiology of AMR in *E. coli* from local chicken to avail basis for target interventions.

REFERENCES

- Altenbuchner, J., & Mattes, R. (2004). *Escherichia coli*. *Production of recombinant proteins: novel microbial and eukaryotic expression systems*, 7-43.
- Gedeno, K., Hailegebreal, G., Tanga, B. M., Sulayeman, M., & Sori, T. (2022). Epidemiological investigations of Salmonella and *Escherichia coli* associated morbidity and mortality in layer chickens in Hawassa city, Southern Ethiopia. *Heliyon*, 8(12).
- Guabiraba, R., & Schouler, C. (2015). Avian colibacillosis: still many black holes. *FEMS microbiology letters*, 362(15), fnv118.
- Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology*, 15(1), 1-23.
- Jakaria, A., Islam, M. A., & Khatun, M. M. (2012). Prevalence, characteristics and antibiogram profiles of *Escherichia coli* isolated from apparently healthy chickens in Mymensingh, Bangladesh. *Microbes and Health*, 1(1), 27-29.
- Kabiswa, W., Nanteza, A., Tumwine, G., & Majalija, S. (2018). Phylogenetic groups and antimicrobial susceptibility patterns of *Escherichia coli* from healthy chicken in Eastern and Central Uganda. *Journal of veterinary medicine*, 2018(1), 9126467.
- Khoo, L., Hasnah, Y., Rosnah, Y., Saiful, N., Maswati, M., & Ramlan, M. (2010). The prevalence of avian pathogenic *Escherichia coli* (APEC) in peninsular Malaysia. *Malays. J. Vet. Res*, 1(1), 27-31.
- Kika, T. S., Boci, J., Cocoli, S., Puvaca, N., Cabeli, P., Shoshi, N., & Camarda, A. (2023). Avian Pathogenic *Escherichia coli* Resistance Patterns According to Poultry Species in Albania, a Two Years Study. *Albanian j. agric. sci*, 22(1), 1-6.
- Kilari, V. B., & Oroszi, T. (2024). The misuse of antibiotics and the rise of bacterial resistance: a global concern. *Pharmacol Pharm*, 15(12), 50823.
- Mandal, J., Acharya, N. S., Buddhapriya, D., & Parija, S. C. (2012). Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant *Escherichia coli*. *Indian Journal of Medical Research*, 136(5), 842-849.

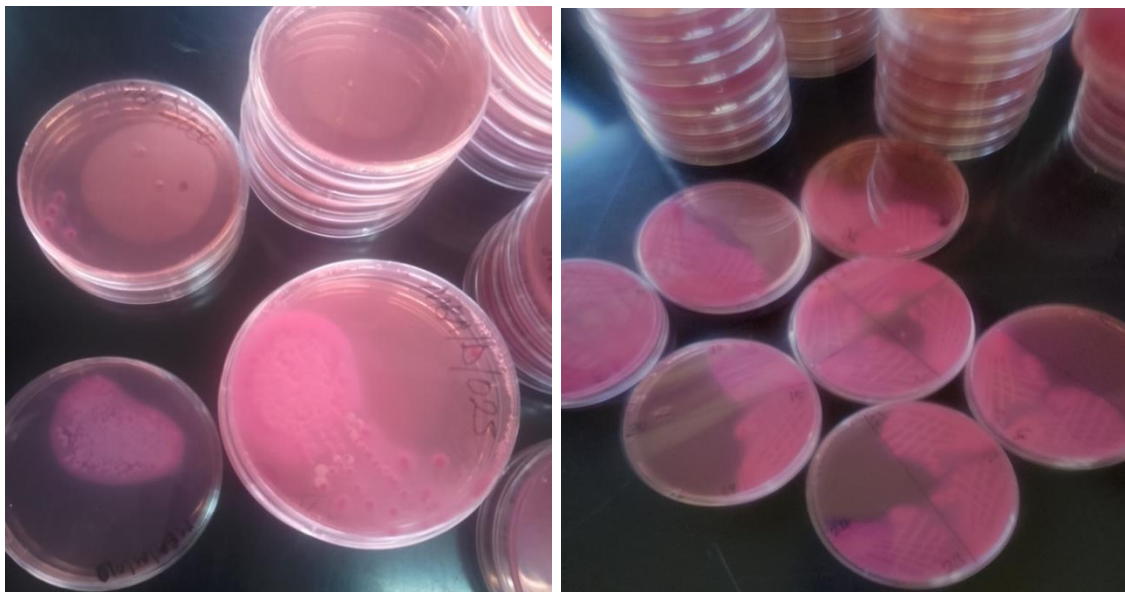
- Mohamudha, P. R., Harish, B., & Parija, S. (2012). Molecular description of plasmid-mediated AmpC β -lactamases among nosocomial isolates of *Escherichia coli* & *Klebsiella pneumoniae* from six different hospitals in India. *Indian Journal of Medical Research*, 135(1), 114-119.
- Okoli, I. (2006). Anti-microbial resistance profiles of *E. coli* isolated from free range chickens in urban and rural environments of Imo State, Nigeria. *Online J Health Allied Scs*, 1(3).
- Olonitola, O. S., Fahrenfeld, N., & Pruden, A. (2015). Antibiotic resistance profiles among mesophilic aerobic bacteria in Nigerian chicken litter and associated antibiotic resistance genes. *Poultry science*, 94(5), 867-874.
- Parija, S. C., Banerjee, T., & Biswas, R. (2026). Enterohemorrhagic *Escherichia coli* (EHEC) Infections. In *Textbook of Bacterial Zoonoses* (pp. 301-312). Springer.
- Rafiq, K., Sani, A. A., Hossain, M. T., Hossain, M. T., Hadiuzzaman, M., & Bhuiyan, M. A. S. (2024). Assessment of the presence of multidrug-resistant *Escherichia coli*, *Salmonella* and *Staphylococcus* in chicken meat, eggs and faeces in Mymensingh division of Bangladesh. *Heliyon*, 10(17).
- Rugumisa, B. T., Call, D. R., Mwanyika, G. O., Mrutu, R. I., Luanda, C. M., Lyimo, B. M., Subbiah, M., & Buza, J. J. (2016). Prevalence of antibiotic-resistant fecal *Escherichia coli* isolates from penned broiler and scavenging local chickens in Arusha, Tanzania. *Journal of food protection*, 79(8), 1424-1429.
- Sarba, E. J., Kelbesa, K. A., Bayu, M. D., Gebremedhin, E. Z., Borena, B. M., & Teshale, A. (2019). Identification and antimicrobial susceptibility profile of *Escherichia coli* isolated from backyard chicken in and around ambo, Central Ethiopia. *BMC veterinary research*, 15(1), 85.
- Shehata, A. A., & Hafez, H. M. (2024). Colibacillosis. In *Turkey Diseases and Disorders Volume 1: Bacterial and Fungal Infectious Diseases* (pp. 29-45). Springer.
- Yassin, A. K., Gong, J., Kelly, P., Lu, G., Guardabassi, L., Wei, L., Han, X., Qiu, H., Price, S., & Cheng, D. (2017). Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. *PloS one*, 12(9), e0185326.

APPENDECES

Appendix I: Research Evidence pictorials



Sample collection





McFarland solution on Mueller Hinton agar



Embedding of drugs



Measuring the area of clearance