ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* IN POULTRY, KNOWLEDGE AND PRACTICE ABOUT ANTIMICROBIAL USE BY POULTRY VENDORS IN YOLA

BY

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ATTESTATION

I declare that the work in this thesis '**Antimicrobial Resistance of *Escherichia coli* in Poultry, Knowledge and Practice about Antimicrobial use by Poultry Vendors in Yola.'** was performed by me in the Department of Veterinary Public Health Medicine under the supervision of Prof J. K. P. Kwaga and Dr. J. Kabir. The information derived from literatures has being duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

This project thesis titled **'Antimicrobial Resistance of Escherichia *Coli* in Poultry, Knowledge and Practice about Antimicrobial use by Poultry Vendors in Yola'** meets the regulations governing the award of the degree of Master of Science Public Health and Field Epidemiology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to God.

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LIST OF ACRONYMS

E. coli- Escherichia coli

NVRI- National Veterinary Research Institute

NFELTP- Nigerian Field Epidemiology and Laboratory Training Programme

AMR- Antimicrobial Resistance

LBMs- Live Bird Markets

LGA- Local Government Authority

EMB- Eosine Methylene Blue

FAO- Food and Agricultural Organisation

H_2S- Hydrogen Sulphide

TSI- Triple Sugar Iron

EPEC- Enteropathogenic *E. coli*

EIEC- Enteroinvasive *E. coli*

EAEC- Enteroaggregative *E. coli*

EHEC- Enterohaemorrhagic *E. coli*

VTEC- Vero cytotoxigenic *E. coli*

HUS- Haemolytic Uraemic Syndrome

ETEC- Enterotoxigenic *E. coli*

DAEC- Diffuse Adherent *E. coli*

UTI- Urinary Tract Infection

MDR- Multi Drug Resistance

DNA- Deoxyribonucleic Acid

RNA- Ribonucleic Acid

VP- Voges Proskauer

TDA- Tryptophan Deaminase

ONPG- O-Nitrophenyl- β- d- galactopyranoside

µg- Microgram

PCR- Polymarase Chain Reaction

OIE- Office Internationale des Epizooties (World Organization for Animal Health)

SUMMARY

Escherichia coli is one of the most important food borne microorganisms that cause disease in animals and humans worldwide. Not all strains cause disease, but they are useful for testing of contamination of food and water and have been adopted as indicator organism for antimicrobial drug resistance among commensal gram-negative organisms. Antimicrobial drug resistance is associated with inappropriate use of veterinary antimicrobials in food animals especially in poultry production. LBM is a place located in markets in urban settlements where birds are housed and sold to the public. Poultry vendors try to keep their birds healthy through the use of antimicrobials. Hence, the need to study the resistance in *E. coli* isolated from these birds. Apparently healthy local chickens sold for food at live bird markets in Yola North and Yola South LGAs were examined for *E. coli* and their resistance to eight important antimicrobials. *E. coli* (n= 56) were isolated using Eosine Methylene Blue (EMB) and identified with conventional biochemical tests. Microbact 12 E was used to confirm 49 of the 56 isolates to be *E. coli*. In addition, knowledge and practices of the poultry vendors in LBMs about antimicrobials use was assessed. The results were as follows: 95.8% of the poultry vendors in LBM had not attended any formal training on poultry production and health, and 79.2% did not keep records of drugs they use. Hygiene score for cages showed 79% of cages were mildly contaminated/ dusty, 54% of drugs they used were purchased from the open markets without prescription and 68% of poultry vendors used their experience to determine the dosage for treatment of their birds. Only 10.4% of vendors consulted a veterinarian for the health needs of their poultry; 56% of poultry vendors did not practice hand washing and 98% did not use protective clothing such as coveralls. All the 49 *E. coli* isolates were resistant to Ceftiofur (100%), Erythromycin (100%) and Sulfamethoxazole (100%). The Percent resistance to other drugs were as follows:

Nalidixic acid (69.4%), Ciproflaxin (67.3%), Tetracycline (67.3%), Chloramphenicol (49%), and Gentamicin (34.7%). All the isolates were multi drug resistant showing 16 different patterns with each isolate being resistant to at least four drugs. The high prevalence of resistance among commensal *E. coli* in local chickens could be a significant source of resistance genes to other bacteria that share the same environment which could also be a source of direct contamination of poultry meat and LBM workers.

Key Words: Antimicrobial Resistance, *E. coli*, Poultry and Live bird Market

CHAPTER ONE

INTRODUCTION

1.1 Background

Antimicrobial Resistance (AMR) is a major global public health problem caused by inappropriate use of antimicrobials. In veterinary medicine, AMR is considered both from a human health perspective and from the perspective of protection of animal health and welfare and the environment. This leads to concerns about a number of important impacts in the area of public health, medical care, financial resources and food safety.[1] Since the introduction of antimicrobials as drugs against bacterial infections in humans and then veterinary medicine, AMR has been recognized as an existing phenomenon.[2,3] However, there is an increased occurrence of AMR in bacteria[4]. These concerns have been raised locally, nationally and internationally.[5] During the past decade, the emergence of drug resistant *E. coli* has dramatically increased. Consequently, the management of *E. coli* infections in both humans and animals that was previously straight forward has become more complicated. The risk of treatment failure is higher and cost of treatment increasing.[6,7]

Inappropriate antimicrobial use in veterinary and human medicine is considered the most important factor promoting the emergence, selection and dissemination of antimicrobial resistant bacteria. [8] Antimicrobials usage selects for resistance not only in pathogenic bacteria but also in non-pathogenic commensals. These commensals could serve as reservoirs for resistance determinants that could be transferred to other bacteria in the same ecosystem and as indicator organism for resistance among bacterial population.[9,10] Antimicrobials are used in agriculture as in humans to control bacterial infections.

However, in animals the use goes beyond therapeutics to use for prophylaxis and growth promotion. In intensively reared animals, antimicrobials may be administered to whole flocks rather than individuals in feed or water as growth promoters or for prophylactic treatment.[11] LBMs are special locations in markets where birds are housed and sold to the public, either for slaughter or for production purposes by poultry vendors. During the period of holding the poultry, poultry vendors use antimicrobials for prophylaxis to keep the birds healthy for sale. Whether this use is appropriate is not known but it is known that any form of use could lead to selection pressure for emergence of resistance.[12,13] Therefore, this research was conducted to investigate the prevalence and patterns of *E. coli* resistance to some selected antimicrobials among chickens sold at LBMs. This is because these organisms are usually present any time drugs are administered and could serve as an indicator organism for resistance in a bacterial population because of their continuous exposure to antimicrobial agents during treatment. In addition, there is a need to describe the knowledge and practices of antimicrobial use by poultry vendors.

1.2 Problem Statement

Antimicrobial resistance and the use of antimicrobial agents in veterinary medicine are complex issues that are currently a source of major international concern.[14] AMR has been increasing along with antimicrobial use. At the same time, the supply of new drugs to replace those rendered inefficient by the development has been dwindling, leading to concerns that we may soon lack efficient means to treat bacterial infections. Though the problem has received considerable interest, there are no indications that the situation is about to change.[3] Every application of antibacterial drugs in veterinary medicine may encourage selection for resistant bacteria which could be transferred via the food chain to humans.[15] An FAO report on poultry markets in Nigeria, highlighted a widespread misuse of antimicrobials by poultry vendors.[12] The use of antimicrobials for veterinary related purposes accounts for 50% of all use globally,[4,9,16] and most of this is used for growth promotion and prophylaxis mostly at sub- therapeutic levels. A study in poultry farms in Zaria, indicated that 100% of the farmers used antimicrobials in their farms for either prophylaxis and or therapy with all the farms never observing drug withdrawal periods [17]. There is an increasing incidence of antimicrobial resistance in the world today in animals and humans. If the resistance only affected the agriculture industry, it would be a huge problem, but it is also being felt in the human health arena. Hospitals are having problems with methicillin resistant *Staphylococcus aureus*. In recent years, the efficacy of human antimicrobial therapy has been challenged by the emergence of different resistant bacterial pathogens like vancomycin-resistant Enterococci, methicillin-resistant *Staphylococcus aureus*, multi resistant *Campylobacter* and multi resistant *Mycobacterium tuberculosis*. [18]

1.3 Relevance to Public Health

The use of antimicrobial agents in humans and food-producing animals has important consequences for human and animal health, as it can lead to the development of resistant bacteria and accumulation of drug residues. Antimicrobial drugs have been used in food animals for almost 50 years. Many of them are identical to or belong to classes which are used in human medicine, for example, penicillins, tetracyclines, sulphonamides, fluoroquinolones, aminoglycosides, macrolides and cephalosporins; and have been used either as therapeutic agents or as growth promoters to enhance feed conversion efficiency.[19] Moreover, resistant bacteria in animals can be transferred to humans usually through the consumption of food, but also through direct contact with food producing animals or through environmental spread. Ultimately, this can result in human infections with bacteria that are resistant to antimicrobial agents and that can therefore be difficult or impossible to cure.[20] Commensal bacteria are good indicator organisms for monitoring selection pressure of antimicrobial use and for resistance problems expected in pathogens. Monitoring the prevalence of resistance in indicator bacteria such as faecal *E. coli* in different animal populations, makes it feasible to compare the prevalence of resistance and to detect transfer of resistant bacteria or resistance genes from animals to humans.[9] *E. coli* antimicrobial resistance is internationally used to reflect resistance levels present in a bacterial population. The prevalence of resistance against the different antimicrobial agents gives a good impression of which resistances can be expected in pathogenic bacteria in the same ecosystem.

1.4 Research Questions

1. What are the antimicrobial resistance patterns and prevalence of *E. coli* isolated from poultry at LBM in Yola?

2. What are the practices of poultry vendors on antimicrobial use that promote inappropriate use?

3. What is the level of awareness of poultry vendors about antimicrobial use and resistance?

1.5 General Objective and Specific Objectives

1.5.1 General Objective

The main objective of this research was to isolate and identify *E. coli* from poultry sold in Yola and to determine the susceptibility of the isolates to selected antimicrobials agents. Additionally the knowledge and practices of the poultry vendors about antimicrobials use was assessed.

1.5.2 Specific Objectives

1. To isolate and identify *E. coli* from apparently healthy chickens sold at LBMs.
2. To determine antimicrobial susceptibility of *E. coli* isolates from poultry at LBMs.
3. To assess the awareness and practices of poultry vendors regarding inappropriate antimicrobial use and resistance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Enterobacteriaceae

Enterobacteriaceae are a large group of gram-negative bacilli or coccobacilli non-spore forming, which are motile by peritrichous flagella or are non-motile. They have relatively simple nutritional requirements and are facultative anaerobes that ferment glucose to acid under anaerobic conditions. Many of them can be isolated from the intestinal tract. The medical importance of many of the members of this family has resulted in intense study of the group. Enterobacteria cause a variety of nosocomial and community-acquired infections including food borne illness in humans. As a result, their resistance to antimicrobials has profound implications. The major antimicrobial classes currently in use for enterobacterial infections are the β-lactams, quinolones, aminoglycosides, tetracyclines, and sulfonamides.[21,22]

2.2 *Escherichia coli*

The genus *Escherichia* is a large and diverse group of bacteria. Although most strains are non-pathogenic, others are pathogenic. *E. coli* are non-spore-forming, Gram-negative bacteria, usually motile by peritrichous flagella. They are facultative anaerobes with gas usually produced from fermentable carbohydrates. To date, *E. coli* is classed as a harmless member of the normal microbiota of humans located at the distal end of the intestinal tract. The organism is generally acquired at birth or by the faecal-oral route from the mother and also from the environment. Most strains of *E. coli* are not pathogenic. *E. coli* is the most common cause of acute urinary tract infections as well as urinary tract sepsis. It has also been known to cause neonatal meningitis and sepsis and also abscesses in a number of organ systems. *E. coli* may also cause acute enteritis in humans as well as animals.[23] *E. coli* is one of the most important food borne microorganism that could cause diseases in animals and humans worldwide. Those that do not cause disease are known as commensals are useful for testing of contamination of water and have been adopted as an indicator organism for antimicrobial drug resistance among gram-negative organism. Antimicrobial drug resistance is associated to inappropriate use of in food animals particularly in poultry production.

The biochemical characteristics of the genus *Escherichia* are shown in the characteristics of *E. coli*. Most strains of *E. coli* ferment lactose. They produce indole, and fail to hydrolyse urea. Hydrogen sulphide (H_2S) production is not detectable on triple sugar iron (TSI) agar, phenylalanine is not deaminated, and gelatine is not liquefied. Most strains decarboxylate lysine and utilize sodium acetate, but they do not grow on Simmons' citrate agar. They are also voges proskauer negative and methyl red positive.[24]

2.3. Gastrointestinal Infections in Humans

E. coli is the one of the most common causes of acute urinary tract infections and gastrointestinal disease in humans. It is known to cause neonatal meningitis and sepsis. *E. coli* may also cause acute enteritis in humans as well as animals.

2.3.1 Enteropathogenic E. coli (EPEC)

EPEC is a major cause of infant (less than 2 years of age) watery diarrhoea infections. It has, however, been documented as a major cause of watery diarrhoea in children less than 6 months of age specifically in developing countries. Mortality can be as high as 30%. The infectious dose of EPEC is very high, about 108–1010 organisms. The transmission of infection is directly from person to person with no evidence to date that it is transmitted in water.[23]

2.3.2 Enteroinvasive *E. coli* (EIEC)

Patients who become infected with EIEC present with watery diarrhoea, with a small proportion developing bloody diarrhoea. The infectious dose is high, between 106 and 1010 organisms. EIEC are closely linked to shigellae in that they both cause the bacillary dysentery using similar processes. The incubation period following ingestion is usually 1-3 days and the duration of infection is1–2 weeks.[23]

2.3.3 Enteroaggregative *E. coli* (EAEC)

EAEC causes a watery mucoid diarrhoea, which usually contains no blood and there is no fever. The infectious dose is generally high and it is primarily a disease of developing countries.[23]

2.3.3 Enterohaemorrhagic *E. coli* (EHEC) or Vero cytotoxigenic *E. coli* (VTEC)

Enterohaemorrhagic *E. coli* (EHEC) produces a shiga-like toxin that is cytotoxic to Vero cells. The commonest EHEC is O157. The incubation period following ingestion is 3–8 days and duration of infection is 1–12 days. Following ingestion of the required dose of less than 100 organisms symptoms develop which include watery and bloody diarrhoea associated with vomiting. EHEC can also cause two distinct conditions, haemorrhagic colitis and haemolytic uraemic syndrome (HUS). HUS is characterized by thrombocytopaenia, microangiopathic,haemolytic anaemia and renal failure.[23]

2.3.4 Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* (ETEC) cause gastroenteritis with profuse watery diarrhoea with abdominal cramps, vomiting and fever evident in a small percentage of patients. The severity of illness as a result of infection with ETEC varies from relatively mild and short-lived to a severe life-threatening illness. They are one of the major causes of death in children below the age of 5 years in developing countries. Adults and older children in tropical countries can also become infected by ETEC but, generally, these become asymptomatic carriers as a result of mucosal immunity. Individuals who do not acquire this sort of immunity develop a condition known as traveller's diarrhoea.[23]

2.3.5 Diffuse Adherent *E. coli (DAEC)*

Infection with DAEC causes watery diarrhoea, mostly in older children. DAEC like EAEC adhere to Hep-2 cells. The pattern by which DAEC attaches is, however, more diffuse than EAEC, a distinguishing feature. However, the pathogenic mechanisms for DAEC are to date still inconclusive.[23]

2.3.6 Urinary Tract Infections (UTI)

E. coli is the most common cause of UTI and kidney infection in humans which usually originate in the large intestines. They are able to adhere to epithelial cells in the urinary tract and thereby cause infections.[25]

2.3.7 Septicaemia and Meningitis

E. coli is one of the most common causes of septicemia and meningitis among neonates, acquired in the birth canal before or during delivery. *E. coli* also causes bacteraemia in adults, primarily from a genitourinary tract infection or a gastrointestinal source.

2.3.8 Infections in Chickens

E. coli is an important pathogen causing secondary bacterial infection in Poultry. It causes diseases such yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, colisepticaemia, coligranuloma and cellulitis in Poultry. Majority of *E. coli* colonise the avian gastrointestinal tract and other mucosal surface as commensals. [26]

2.3.9 Indicator Bacteria

Because of continuous exposure to antimicrobials during treatment the level of resistance among bacteria of the normal intestinal flora of humans and animals increases, constituting an enormous reservoir of resistance genes for pathogenic bacteria, zoonotic and other commensals. So the level of resistance in the endogenous flora is considered to be indicative for the selection pressure exerted by antimicrobial use in that population. Therefore, the prevalence of resistance of *E. coli* in the intestinal tract of different populations of animals could indicate resistance problems to expected in the bacterial populations in that animal population, environment and even humans.[9]

2.4 Epidemiology of *E. coli* Resistance

E. coli is commonly isolated from intestinal tract of animals and human beings[27]. Although most isolates are nonpathogenic they are considered indicators of feacal contamination of water. *E. coli* is also regarded as a useful indicator of Antimicrobial resistance and plays a prominent role in many existing surveillance systems for Antimicrobial resistance in animals and foodstuffs[54] *E. coli* are commonly found in the entire gastrointestinal tract of poultry and other mucosal surfaces they can cause other problems in humans such as Urinary tract infection.[26] Feacal contamination of meat at slaughter is one mechanism that can result in human exposure to these bacteria. While transmission rates remain unknown, the sheer volume of these bacteria makes many experts believe that the global threat from AMR may be more greatly impacted by this commensal reservoir than emerging resistance in pathogens. Expanding on the role as an indicator of meat contamination, *E. coli* from healthy animals have also been adopted as an indicator of AMR for gram-negative bacteria.[4] *E. coli* is used to study the selective pressure on the gram-negative bacteria population to develop and retain resistance. The intestinal normal flora is not the target of antimicrobial treatments, but these bacteria are exposed and become resistant.

2.5 Chickens as Reseviors of *E.coli* in Humans

Studies have shown that chickens are likely reservoirs for *E. coli* in humans.[7] Results from studies showed that chickens are a likely source of strains implicated in humans infections and that chickens could be major reservoirs. This raises a public health concerns about Poultry and poultry products been common reservoir of emerging antimicrobial resistances.[28] It is possible that the transmission of antimicrobial resistant bacteria to people who get in contact with these sources through ingestion or handling is common.[28] In theory, the birds serve as a vehicle for expanding the transmission of resistance bacteria to humans.[14]

2.6 Genetic Mechanisms of Resistance in *E. coli*

Gene transfer is the most common mechanism of transferring resistance. Resistance determinants in *E. coli* are typically on plasmids, but they may also be part of mobile genetic elements (transposons, integrons, and gene cassettes) which can move between plasmids or chromosomes in the same organism or to a new organism.[22]

2.6.1 Plasmids

Are self-replicating, extra chromosomal elements that contain genes for resistance, virulence, and other functions and are dispensable under certain conditions. Some larger plasmids are conjugative and can transfer between organisms, spreading along resistance genes.[22]

2.6.2 Transposons

Are mobile genetic elements that contain insertion sequences, with one or more resistance genes. They are not capable of autonomous self-replication, but can move from one site on the chromosome to another site on the same or different chromosome or plasmid and replicate along with it. Transposition is made possible by short inverted repeats of DNA.[29]

2.6.3 Integrons

Are mobile genetic elements of specific structure that consist of two conserved segments flanking a central region in which resistance gene cassettes are inserted.[29]

2.7 Major Antimirobial Classes used in Entrobacterial Infections

2.7.1 Beta Lactams

Beta Lactam constitute the most enduring and widely used class of antibacterials, encompassing a large number of mostly semisynthetic compounds. Their targets are peptidoglycan transpeptidases, cell wall–synthesizing enzymes located on the outer surface of the cytoplasmic membrane. These enzymes are ubiquitous in bacteria and are commonly detected by their ability to bind covalently and specifically to penicillin and other beta-lactam. The most important mechanism of resistance in enterobacteria is hydrolysis by beta lactamases, common bacterial enzymes related to the cell wall targets. cephalosporins are used in the treatment of septicemias, respiratory infections, and mastitis. Resistance to beta-lactam is mainly mediated by a large number of beta-lactamases which differ in their abilities to hydrolyse the various b-lactam antimicrobials.[29]

2.7.2 Quinolones

Resistance to quinolones is associated with changes in the target DNA gyrase or target protection by the proteins. Reduced accumulation in the cell, due to active efflux through the cytoplasmic membrane and decreased influx through the outer membrane, may facilitate the emergence of quinolone resistance. Quinolones are broad-spectrum, bactericidal . Resistance to fluoroquinolones is based either on mutations which render the target resistant to the drugs or on decreased intracellular drug accumulation. Enzymatic inactivation has not been observed so far, a wide variety of mutations have been detected in the various target genes of a wide range of gram-positive and gram-negative bacteria of human and veterinary importance.[29]

2.7.3 Aminoglycosides

The wide range of applications and the nature of the diseases treated makes aminoglycosides extremely important for veterinary medicine. Aminoglycosides are of importance in septicaemias, digestive, respiratory and urinary diseases. Gentamicin is indicated for *Pseudomonas aeruginosa* infections with few alternatives. Spectinomycin is used only in animals. Few economic alternatives are available. Aminoglycosides are bactericidal, broad-spectrum discovered in the 1940s. They are still widely used usually in combination with beta lactam agents, against problem pathogens despite their ototoxicity and nephrotoxicity. A number of subclasses have been identified, and semisynthetic derivatives less prone to enzymatic inactivation have been developed Resistance to aminoglycosides is mainly based on enzymatic inactivation by aminoglycoside-modifying enzymes. Moreover, decreased uptake of aminoglycosides and chromosomal mutations conferring high-level resistance to streptomycin has also been described.[29]

2.7.4 Tetracyclines

Tetracyclines are broad-spectrum, bacteriostatic agents that also act by inhibiting protein synthesis. They bind reversibly to a single, high affinity site on the 30S ribosomal subunit and disrupt the codon-anticodon interaction between aminoacyl-transport RNA and messenger RNA, thereby inhibiting the binding of aminoacyl-transport RNA to the acceptor site on the ribosome. Their selective antibacterial toxicity may be due, at least in part, to selective, concentrative uptake by bacteria. The major mechanism for tetracycline resistance involves an inducible active efflux system whereby the intracellular concentration of these compounds is reduced.[29]

2.7.5 Sulphonamides

Sulfonamide resistance is due to an additional, plasmid-mediated, sulfonamide-resistant, dihydropteroate synthase target. Sulfonamides, the oldest, totally synthetic antibacterial agents, are competitive inhibitors of dihydropteroate synthetase by virtue of their active form being a structural analogue of the *para*-aminobenzoic acid substrate. Clinically, the most common and important mechanism of resistance to these bacteriostatic agents is altered, usually plasmid-mediated.[29]

2.7.6 Phenicols

Chloramphenicol is a bacteriostatic antimicrobial. It is considered a prototypical broad-spectrum Antimicrobial. It is both cheap and easy to manufacture which is frequently an antimicrobial of choice in the developing World. Chloramphenicol is effective against a wide variety of Gram-positive and Gram-negative bacteria. Due to resistance and safety concerns, it is no longer a first-line agent for any infection in developed nations, with the notable exception of topical treatment of bacterial conjunctivitis. Nevertheless, the global problem of advancing bacterial resistance to newer drugs has led to renewed interest in its use. In low-income countries, chloramphenicol is still widely used because it is inexpensive and readily available.[29]

2.7.7 Macrolides

Macrolides are a class of found in Streptomycetes. They are natural lactones with a large ring, consisting of 14 to 20 atoms. Macrolides bind to the 50S subunit of the bacterial ribosome and inhibit ribosomal translocation, leading to inhibition of bacterial protein synthesis. Their action is primarily bacteriostatic but may be bactericidal at high concentrations, or depending on the type of microorganism.[29]

2.7.8 Future Directions

Antimicrobial drugs have been widely used in human and veterinary medicine for more than 50 years, with tremendous benefits to both human and animal health. Because antimicrobial drug use contributes to the emergence of drug resistant organisms, these important drugs must be used judiciously in both animal and human medicine to slow the development of resistance. Antimicrobial resistance in humans and animals continue to be a growing public health concern worldwide. When a person or animal is infected with an antimicrobial-resistant bacterium, treatment becomes more difficult because standard antimicrobial therapies become less effective or may not work at all. Developing strategies for reducing AMR is critically important for protecting both public and animal health. Collaboration involving the public health, animal health, and animal agriculture communities on the development and implementation of such strategies is needed to assure that the public health is protected while also ensuring that such strategies are feasible and that the health needs of animals and humans are addressed.[30]

2.8 Antimicrobial in Animal Agriculture

Every year, hundreds of thousands of tons of antimicrobials enter the environment mostly through antimicrobials used in agriculture.[31,] Although precise quantities are still not available for many countries, it appears that about half of these are used to treat or prevent human infections and about half are used in agriculture. Moreover, the lifetime of an antimicrobial does not end with the person or animal or plant treated with the antimicrobial. An unknown amount of active antimicrobial is released into the environment by sewage treatment plants and runoff from manure.[32] Judicious use of antimicrobial drugs in agriculture is based on the principles of limiting medically important antimicrobial drugs to uses in food-producing animals including veterinary oversight or consultation.[31] The issue of antimicrobial misuse in agriculture is of global problem because of the spreading and developing resistance of most common bacteria to most antimicrobials. Antimicrobial resistance now has been universally identified as public health priority and necessary plan of action to combat resistance need to be put in place like better diagnostic tests promotion and evaluation antimicrobial use practice guidelines and restriction of antimicrobial use as growth promoters.[33] The use of antimicrobials for therapy, prophylaxis and growth promotion in animals can reduce their efficacy in animals and human medicine through the development of AMR strains of bacteria whether pathogenic or commensals. The risk may be represented by the loss of therapeutic efficacy of one or several drugs and include the emergence of multi-resistant microorganisms. Antimicrobial use in animals can contribute to the emergence of AMR which may be transferred to humans, thereby reducing the effectiveness of antimicrobial drugs for treating human disease[15]. It is critically important that antimicrobial drugs be used as judiciously as possible in an effort to minimize resistance development.[31]

2.8.1 Antimicrobials use in Animals

Questions regarding the use of antimicrobial drugs in food-producing animals have been raised and debated for many years. Unlike in human medicine, in food-producing animals are used for two different purposes, prevention and control of bacterial infections and growth promotion. The control and prevention of bacterial infections is achieved by either therapeutic, metaphylactic or prophylactic application of antimicrobials. For this, substances of mainly the same classes as used in human medicine are available for the treatment of food-producing animals. According to the number of animals present and the type of production, these treatments may be individual as in pet and companion animals, dairy cattle, horses and sows, and given by oral or parenteral ways. Nevertheless, in most cases, when large groups of animals have to be treated, as in poultry or swine production, they are applied via water or feed. With such mass production, when a limited number of animals have been identified as infected, rapid treatment of all animals of the respective group/herd/ flock is necessary to prevent further extension of the infection. This is referred to as metaphylaxis. In addition to these interventions, prophylaxis is a solely preventive measure, given individually or to groups of animals, which appears unavoidable under the use of , growth promotion, is specific to food producing animals. A specified number of substances licensed as growth promoters are given at low concentrations to improve growth during the entire growth period of animals. The available molecules and conditions of use are clearly defined in licences specifying the target animals, duration, and dosage.[31]

2.8.2 Use Antimicrobials of in Poultry Production

The purpose of poultry production is to increase production without any constraint. Therefore, the use of antimicrobials is considered a necessary tool for achieving maximum production. Antimicrobials are used for prevention, treatment and growth promotion in poultry production[14]. This use is not only for maximizing production but also to protect the consumers from zoonotic diseases. But this important use may result in the selection of resistance strains of bacteria. Antimicrobial resistance is a global problem that affects both humans and animals. The development of resistance is a consequence of the use of antimicrobials. Concerns about the use of antimicrobials in food producing animals involve food safety issues because meat products from animals have been identified as transfer vehicles for food borne illness in humans. It is imperative that everyone involved in food animal production, veterinarians and producers, as well as human health care providers work together in minimizing the development of antimicrobial resistance.[14] The development of resistance among bacterial populations exposed to antimicrobials is a growing public health issue. Applications of antimicrobials in poultry production bring about an increase in resistance to not only in pathogenic bacterial strains, but also in commensal bacteria. In this respect, gastrointestinal commensal bacteria constitute a reservoir of resistance genes for pathogenic bacteria. Their level of resistance is considered to be a good indicator for selection pressure for antimicrobial use and resistance.[14,34]

2.8.3 Acquired Resistance

Mutational resistance develops because of spontaneous mutation in a locus on the microbial chromosome that controls susceptibility to a given Antimicrobial. The presence of the drug serves as a selecting mechanism to suppress susceptible microorganisms and promote the growth of resistant mutants. Spontaneous mutations are transmissible vertically. Resistance can also develop because of transfer of genetic material between bacteria. Plasmids, which are small extrachromosal DNA molecules, transposons and integrons, which are short DNA sequences, can be transmitted both vertically and horizontally and can code for multi-resistance. It is estimated that the major part of acquired resistance is plasmid-mediated. The development of antimicrobial drugs, and particularly of , has played a considerable role in substantially reducing the morbidity and mortality rates of many infectious diseases. However, the fact that bacteria can develop resistance to has produced a situation where antimicrobial agents are losing their effectiveness because of the spread and persistence of drug-resistant organisms. To combat this, more and more with increased therapeutic and prophylactic action will need to be developed.[35]

2.8.4 Cross Resistance

Micro-organisms resistant to a certain antimicrobial may also be resistant to others that share a mechanism of action or attachment. Such relationships, known as cross-resistance exist mainly between agents that are closely related chemically (e.g. polymyxin B and colistin, neomycin and kanamycin), but may also exist between unrelated chemicals (e.g. erythromycin-lincomycin). Micro-organisms may be resistant to several unrelated . Use of one such Antimicrobial will therefore also select for resistance to the other . Resistance depends on different mechanisms and more than one mechanism may operate for the same Antimicrobial.[35]

2.8.5 Live Bird Markets

LBMs are essential for marketing poultry in many developing countries, and they are a preferred place for many people to purchase poultry for consumption throughout the world. LBMs are typically urban and have a permanent structure in which birds can be housed until they are sold. LBMs bring together a mixture of bird species that meet the preferences of their customers and that are commonly produced by multiple suppliers. The mixture of species, the lack of all-in–all-out management, and multiple suppliers are all features LBMs around the world. LBMs are not likely to be eliminated even though they present a disease risk to people[36]. Many birds produced for food in developing countries are marketed live to consumers. They flourish because they meet the needs of their customers. The problem of LBM and risk of disease exposure especially the occurrence of AMR cannot be used to argue for the closure of the LBM when you compare with the driving forces of consumer taste, tradition, and thousands of years of religious beliefs [36].

2.8.6 Poultry Production System

In Nigeria, the poultry population was estimated at around 150 million, with a large majority of local chickens and a minority of exotic breeds.[12] For analytical purposes, FAO has divided poultry production into four sectors: sector 1 Industrial integrated system, sector 2 commercial production system, sector 3 small-scale commercial production system, sector 4 village or backyard system. Nevertheless, the parameters that differentiate the four sectors vary from country to country.

Sector 1: Industrial integrated system with high level of biosecurity and birds/products marketed commercially examples are farms that are part of an integrated broiler production enterprise with clearly defined and implemented standard operating procedures for biosecurity.

Sector 2: Commercial poultry production system with moderate to high biosecurity and birds/products usually marketed commercially examples are farms with birds kept indoors continuously, strictly preventing contact with other poultry or wildlife.

Sector 3: Commercial poultry production system with low to minimal biosecurity and birds/products entering live bird markets examples are caged layer farms with birds in open sheds, a farm with poultry spending time outside the shed, a farm producing chickens and waterfowl.

Sector 4: Village or backyard production with minimal biosecurity and birds/products consumed locally.[37]

2.9 Knowledge and Practices

Knowledge is familiarity with something, which includes facts, information or skill acquired through experience or education. It can refer to the theoretical or practical understanding of a subject. However, no single agreed upon definition of knowledge exists, though there are numerous theories to explain it. While practice means to do or perform habitually or customarily; make a habit of a method, procedures, process or rule used in a particular field or profession. Misconceptions about Antimicrobial use among community members potentially lead to inappropriate use of in the community. Knowledge and practice are social cognitive factors at an individual level that influences health-related behavior, including the practice of using [38]. Knowledge by itself is not enough to change behaviour, but does play an important role in shaping beliefs and attitude regarding a particular behaviour. Consequently, in the context of Antimicrobial use, inappropriate knowledge of using correctly potentially leads to misconceptions leading to inappropriate use that leads to AMR. Information on knowledge and Practice among antimicrobial users in the developed, particularly among Poultry vendors is not common.[19]

CHAPTER THREE

METHODOLOGY

3.1 Study Area

The study area were Yola south and Yola north local government areas of Adamawa state. There are 3 registered daily LBMs where live birds from villages and farms are housed, slaughtered, dressed and sold daily to the public for consumption. The study sites are Yola town LBM, (E 012.48447 and N 09.20448) Jimeta modern market and Jimeta shopping complex market (E 012.43646 and N 09.27271). Adamawa state is located in Northeastern geopolitical zone of Nigeria.

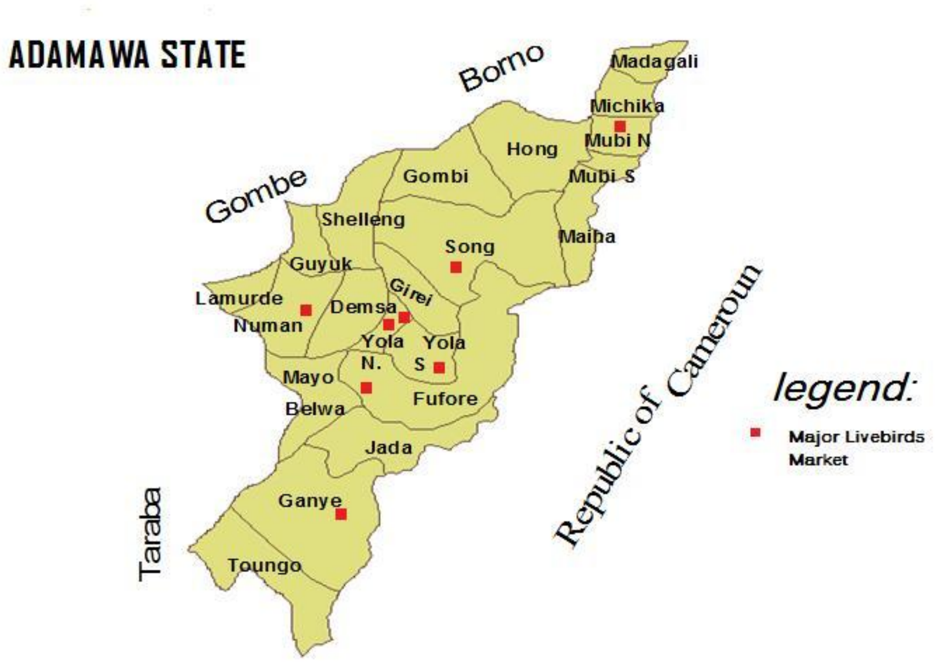

Figure 1: Map of Adamawa State showing the locations 8 registered daily Live Bird markets with the three Live Bird Markets located in Yola South and Yola North LGA.

3.2 Study Design

The study design is cross sectional study, designed to identify the factors contributing to inappropriate use of antimicrobials in poultry in Yola and to carry out a laboratory investigation of *E. coli* and their resistance to antibiotics.

3.3 Study Population

The study population are chickens housed in daily LBMs and the *E. coli* bacteria found in birds sold at daily LBMs. Information from poultry vendors in the market were collected. The study was conducted in these daily LBMs because they are located in densely populated areas and have the largest collection of birds and were purposively chosen based on the location and size. LBMs serve as points where poultry made up of different species, ages and from different production systems are sold to the public. In these daily markets birds are usually stressed and transmission of various diseases among different species can readily occur. Drugs are administered to birds for various reasons. Fig. 2 below shows how birds from different production systems are collected at daily LBMs for sale. Birds from traditional, commercial and industrial sectors could end up in LBMs where consumers could purchase them.[12]

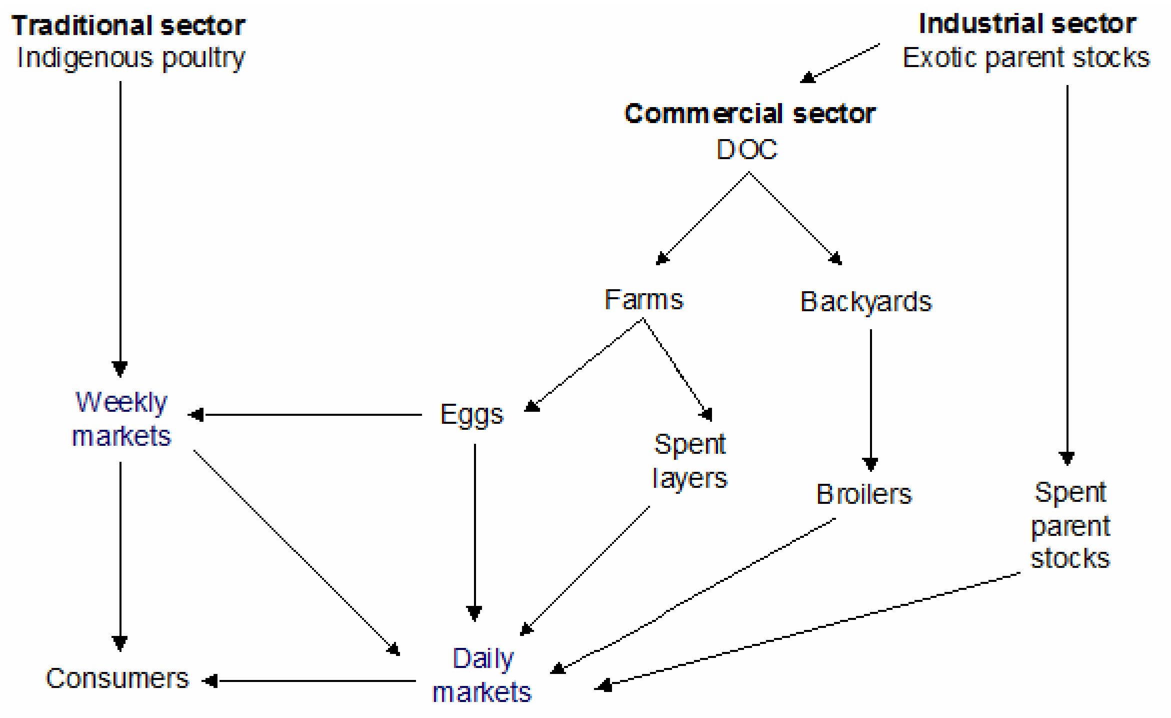

Figure 2: Nigerian poultry market chain[12]

3.4 Sample Size Determination

Sample size was determined based on the OIE terrestrial animal health code for harmonisation of National AMR Surveillance and Monitoring Programmes recommendation for sample size calculation in chapter 6.7 article 6.7.3. A 10% prevalence was chosen in this research so that sample size would be large enough to allow detection of existing resistance and not excessively large to avoid waste of resources. [39]

$$N = \frac{Z\alpha^2 pq}{d^2}$$

- N=sample size, p= prevalence q=1-p

a. Sample size for *E. coli* culture

A precision of 5% and confidence level of 95% and 10% prevalence of *E. coli* resistance in local chickens were selected.[39]

$$N = \frac{1.96^2(0.1)(0.9)}{0.05^2}$$

$= 138.4$ minimum sample size to be collected from poultry.

- All poultry vendors who had cages with birds were interviewed.

3.5 Sampling Technique

Individual birds were conveniently selected from each cage as they were available and cloacal swabs were collected using sterile cotton swabs from chickens between January and February 2013. A maximum of 5 samples from each cage were collected. A total of 236 samples were collected from 48 poultry vendors. The samples were collected into sterile samples bottles and were stored and transported in ice packs from the Yola to National Veterinary Research Institute, Bacteriology Research Laboratory Vom for processing and stored at 4^oC. All poultry vendors who have cages with birds were interviewed.

3.6 Study Instruments

A semi-structured questionnaire was used, to collect information on socio-demographic knowledge and practices of poultry vendors about antimicrobials usage and development of AMR.

3.7 Data Collection and Management

A semi-structured questionnaire was administered to all poultry vendors having cages with birds. Data generated was analysed descriptively using IBM SPSS statistic 20 software.

3.8 Laboratory Methods

3.8.1 Isolation and Identification of *E. coli* Strains

Individual samples were collected in sterile sample bottle and were transported on ice pack to the laboratory for analysis. At the laboratory 5ml of peptone water was added to each individual sample bottle containing sample swab and incubated for at 37^{o}c for 18-24 hours. The samples were then inoculated on Eosin Methylene Blue (EMB) (Oxiod) and incubated at 37^{o}c for 24 hours for cultural isolation and identification. Colonies that appeared as greenish metallic sheen on EMB were presumed to be *E. coli.*

3.8.2 Biochemical Tests

The presumptive *E. coli* isolates were subjected to further biochemical tests which included indole, methyl red, voges proskauer, citrate, urease, triple sugar iron agar , oxidase and nitrate tests.

3.8.3 Microbact 12E

The Microbact 12E is a standardised micro-substrate system designed to simulate conventional biochemical substrates used for the identification of *Enterobacteriaceae*. Organism identification is based on PH change and substrate utilization of twelve different biochemical substrates. An 18- 24 hour pure culture of the organism on appropriate Agar (EMB) is needed. An oxidase test was performed on the organism to be identified (oxidase negative organism can be identified with microbact 12E). Two to three colonies were suspended in 2.5ml of normal saline and four drops were added into each well. Oil overlay was applied to wells 1, 2 and 3 with mineral oil (Lysine, Ornithine and H_2S) resealed and incubated at 37^{o}c for 24 hours. After 24 hours of incubation, appropriate reagents were added to wells 8, 10, and 12, two (2) drops each of VP1 and VP2 (voges proskauer) to well 10 and observed for 15- 30 minutes and 1 drop of TDA in well 12 which was interpreted immediately. Results were recorded in report forms. Twelve substances were tested; lysine, ornithine, hydrogen sulphide, glucose, mannitol, xylose, ONPG (o- Nitrophenryl B- D- galactopyranoside), indole, urease, voges proskauer, citrate and TDA. Three (3) substances formed one group with each substrate assigned a number; when a substrate gave a positive result, the corresponding number for that group was summed up and recorded. The results of the 3 consecutive 3 wells were added and a four digit code was then obtained, which was imputed into the computer identification software, which immediately gave the probable identity of the organism tested as a percentage. The microbact software permits 75% cut off point for a probable identification. All test organisms that had a probable identification less than 75% were not accepted as *E. coli.*

3.8.4 Evaluation of in Vitro Susceptibility of Identified Isolates to Antimicrobial Agents

Disk diffusion method was used to determine the susceptibility of the isolates to 8 important antibacterial agents which included the following: Nalidixic acid (30µg), Ciprofloxalin (5µg), Ceftiofur (30µg), Gentamicin (10µg), Tetracycline (30µg), Chloramphenicol (30µg), Erythromycin (15µg), and Sulphamethoxazole (25µg). Mueller-Hinton (Oxoid) agar plates were prepared and used according to the manufactures instruction. A fresh culture was prepared in tryptone soy broth to a turbidity of 0.5% Mcfarland standard. A sterile swab was dipped into the broth of tryptone soy (Oxoid) culture, excess broth drained by pressing on the inner side to the tube and used to streak the Mueller Hinton agar plates in three directions at 180^o until the entire surface was streaked. The plates were allowed to dry at room temperature for 15 minutes and the antimicrobial disc dispensed into the plates using multiple disc dispensers (Oxoid). The disks were further pressed with a sterile forceps to ensure complete contact with media. The plates were incubated at $37^{o}c$ for 18 hours. The zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk was measured by holding the Petri plate a few inches above a black, nonreflecting background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. The zone of inhibition were measured to the nearest millimeter and interpreted based on interpretation of zone diameter of test culture provided by CLSI (2006). The intermediate susceptibility was interpreted as susceptible.

3.9 Ethical Consideration

Approval of the Ministry for Animal Production was obtained before the commencement of sampling. A visit was paid to the LBMs in the company of Ministry for Animal Production officials where the purpose of the research was explained to the poultry vendors. Verbal consent of each poultry vendor was also obtained. Confidentiality was assured and maintained.

CHAPTER FOUR

RESULTS

A total of 48 poultry vendors were interviewed. The poultry vendors had a mean age of 44 years with a standard deviation of 12.9 years. The results in Table 4.1 show the sex distribution of poultry vendors and their level of education; with 93.8% being males, while 25% and 27.1% had no formal education and possessed primary education only respectively.

Table 4.1: Demographic characteristics of poultry Vendors, Yola, 2013.

Variable	**Frequency**	**Percent (%) N= 48**
Sex		
Male	45	93.8
Female	3	6.3
Education Level		
None	12	25
Primary	13	27.1
Secondary	8	16.7
Tertiary	8	16.7
Arabic Education	7	14.6

Table 4. 2: Formal Training and duration of work as Poultry vendors in Yola 2013.

Variable	Frequency	Percent (%)
Duration of working with poultry		
1-5 years	7	14.6
6-10 years	14	29.2
11-20 years	12	25
20 And above	15	31.3
Formal Training in poultry production and health		
Yes	2	4.2
No	46	95.8

The results in Table 4.2 show that 95.8% of poultry vendors had no formal training in poultry production and health.

Table 4. 3: Knowledge of AMR among Poultry Vendors in Yola 2013.

Knowledge	Frequency	Percent (%)
Have you heard about AMR?		
Yes	39	81.3
No	9	18.8
Have you heard of drug withdrawal periods?		
Yes	18	37.5
No	30	62.5
Will you consult a veterinarian if he/she is available?		
Yes	22	45.8
No	25	52.1
Do you Know where to find a Veterinarian?		
Yes	22	45.8
No	26	54.2

Table 4.3, shows 81.3% of poultry vendors have heard of antimicrobial drug resistance and 62.5 % have not heard of drug withdrawal periods.

Table 4.4: Practice of Poultry Vendors in Yola 2013.

Practice	Frequency	Percent (%)
Do you keep records of your poultry business?		
Yes	7	18.8
No	38	79.2
Do you practice drug withdrawal periods?		
Yes	5	10.4
No	40	83.3
Are birds of different age groups kept in the same cage?		
Yes	28	58.3
No	20	41.7
Are birds of different species kept in the same cage?		
Yes	22	45.8
No	26	54.2
Do you administer drugs to your birds whether they are sick or not?		
Yes	14	29.2
No	34	70.8

Table 4.4 shows 79.2% of poultry vendors do not keep records of the drugs they use and 83.3% do not practice drug withdrawal periods.

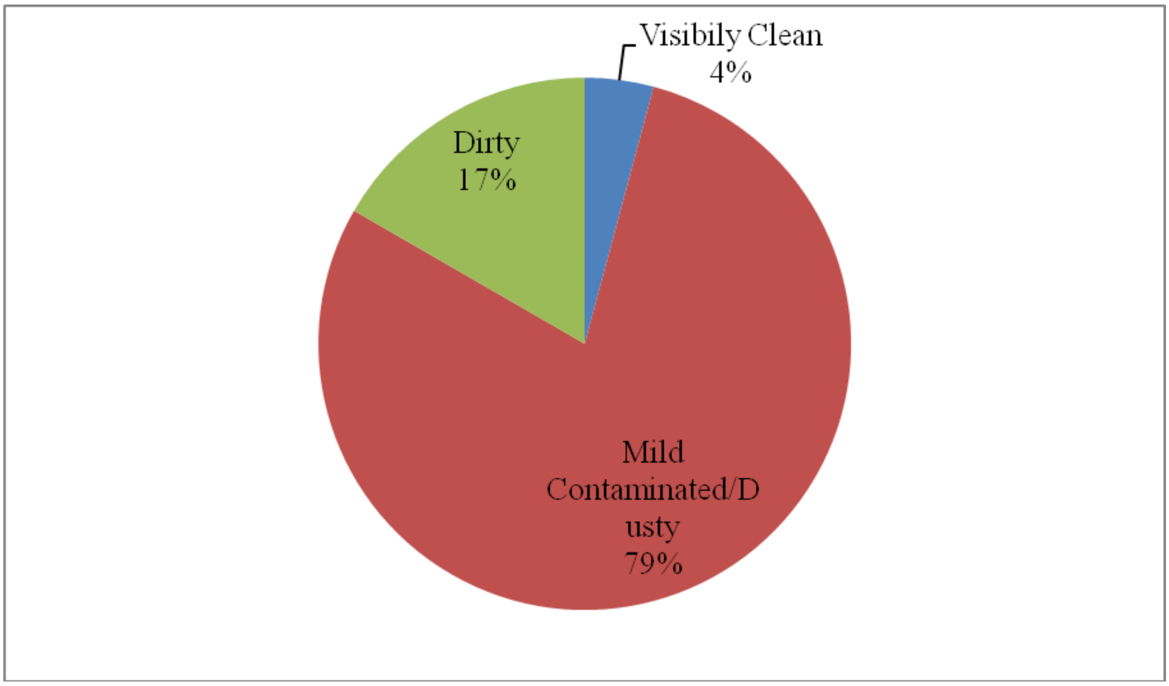

Figure 4.3: Hygiene Score for Cages at LBMs Yola, 2013.

Figure 4.3 on cage hygiene: shows that 17% of cages were dirty and 79% were slightly contaminated and dusty.

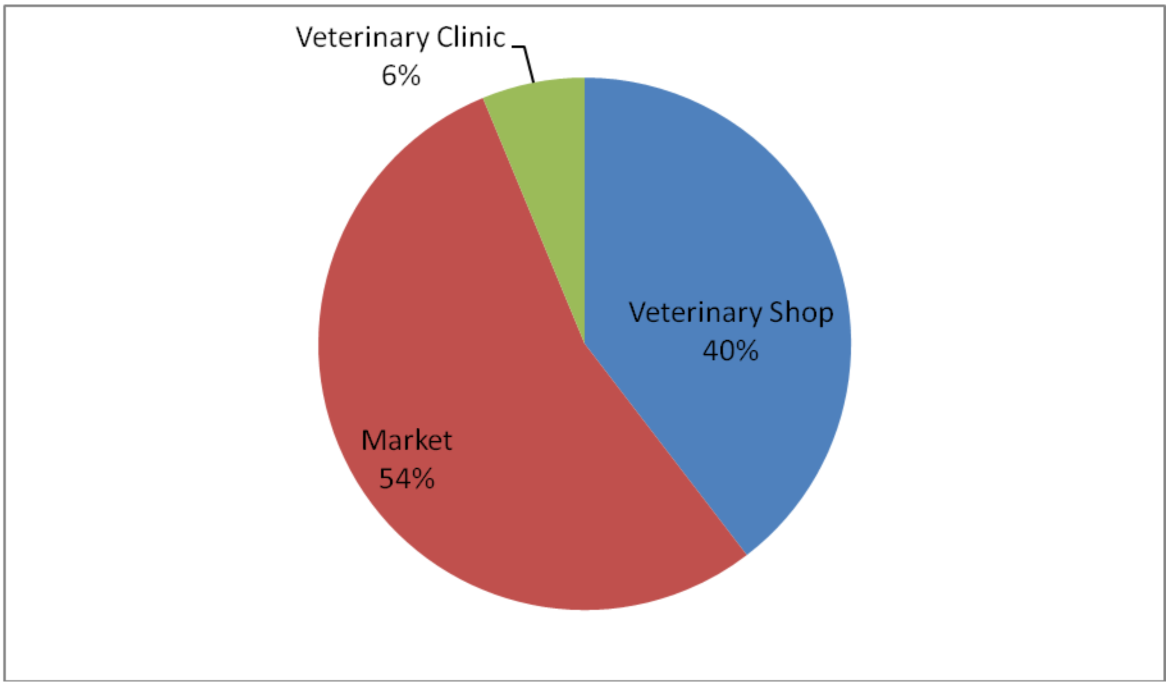

Figure 4.4: Source of Veterinary Drugs Used at LBMs in Yola, 2013.

Figure 4.4 shows that the source of veterinary drugs indicated that only 6% of drugs used are sourced from veterinary clinic, while the remaining sources are market driven and therefore prone to abuse.

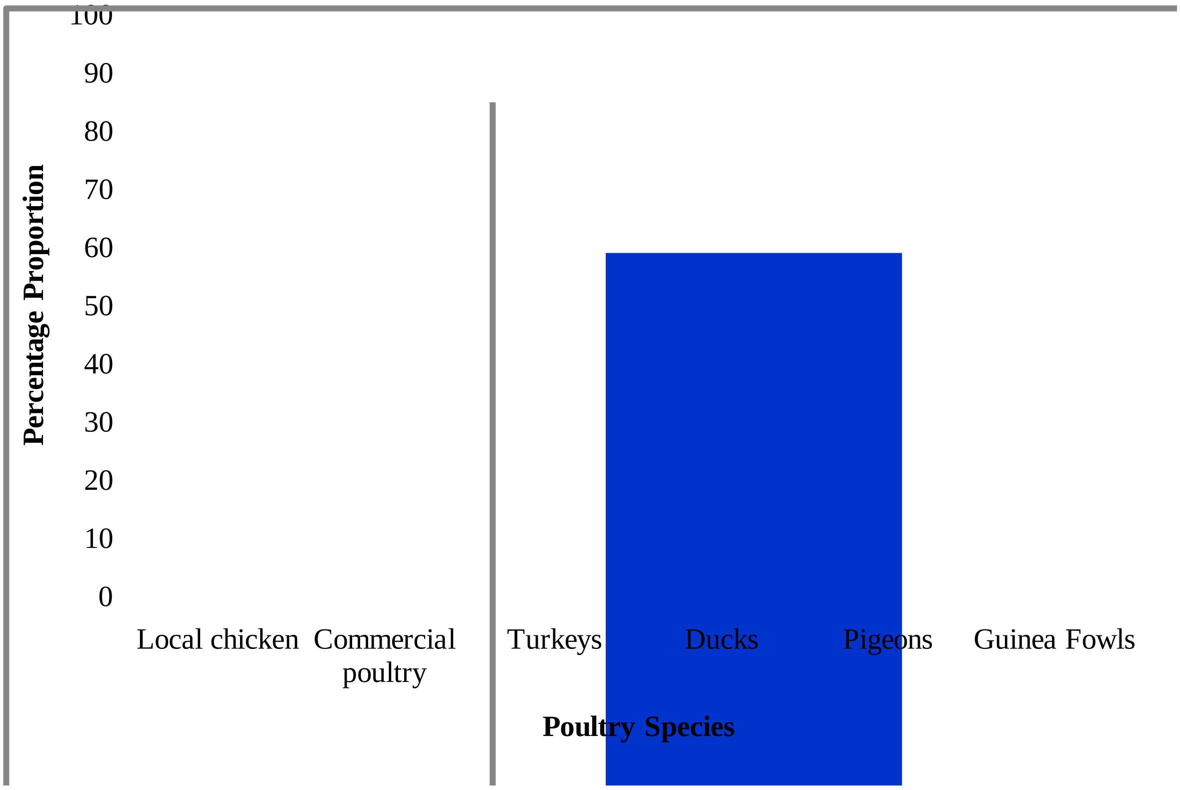

Figure 4. 5: Common Poultry Species sold at LBMs in Yola 2013.

Figure 4.5 shows that local chickens are the most common poultry species sold in LBMs.

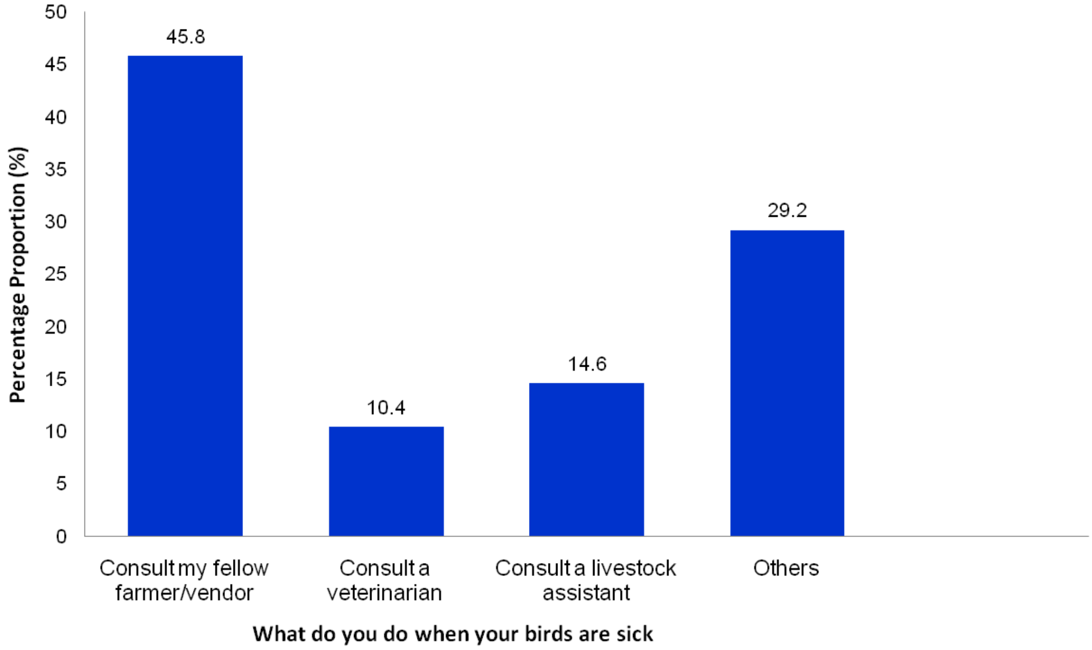

Figure 4.6: Veterinary Care seeking Practices of Poultry Vendors in LBMs Yola 2013.

Figure 4.6 shows that 45.8% of poultry vendors consulted among themselves when their birds are sick.

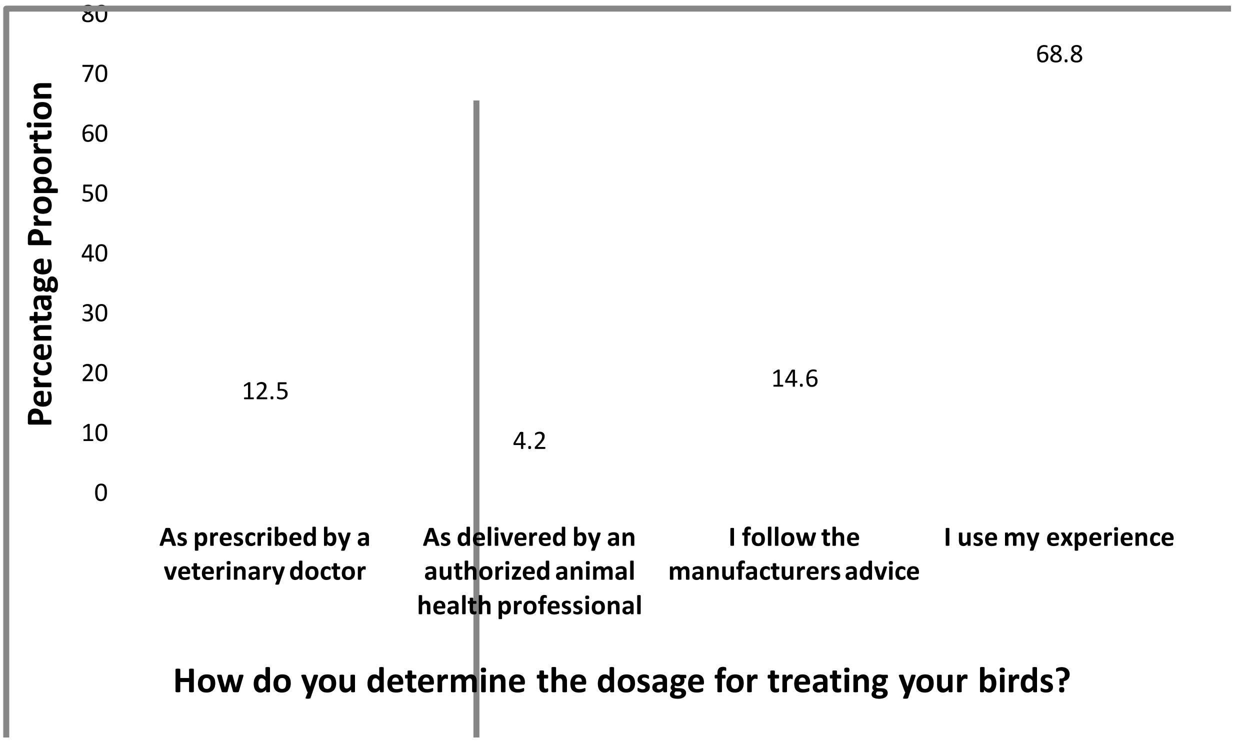

Figure 4.7: Dosage Determination Practices of Poultry Vendor at LBMs Yola 2013.

Figure 4.7 shows that 68.8% of poultry vendors use their experience to determine the dosage to use in treating their birds.

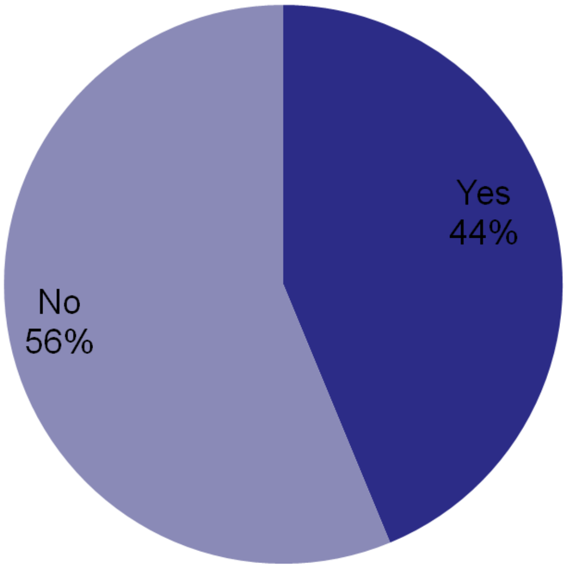

Figure 4.8: Hand washing Practices of Poultry Vendors at LBMs in Yola 2013.

Figure 4.8 shows that 56% of poultry vendors do not practice hand washing.

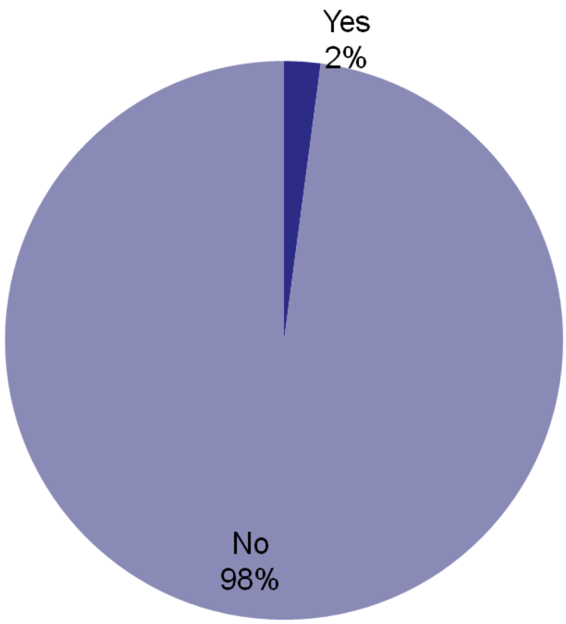

Figure 4. 9: Use of Protective clothing by Poultry Vendors at LBMs in Yola 2013.

Figure 4.4 shows that 98% of poultry vendors do not use any protective clothing.

Table 4. 5: *E. coli* identification using Microbact 12E (N=56)

Percent probability of being *E. coli*	Frequency	Percent (%)
Less than 75 %	7	12.5
Greater than 75%	49	87.5
Total	56	100

The isolates which had probability of being *E. coli* less than 75% were not accepted as *E. coli*, 87.5% of the 56 isolates were confirmed with microbact 12E.

Table 4.6: Antimicrobial susceptibility of identified *E. coli* (N=49)

	Disk Potency (µg)	Percent Resistance	Percent Susceptible
Erythromycin	15	100	0
Ceftiofur	30	100	0
Sulfamethoxazole	25	100	0
Nalidixic acid	30	69.4	30.6
Tetracycline	30	67.3	32.7
Ciprofloxacin	5	65.3	34.7
Chloramphenicol	30	49	51
Gentamicin	30	34.7	65.3

E. coli isolates were 100% resistant to Erythromycin, Ceftiofur and Sulfamethoxazole.

Table 4.7: Antibiograms of *E. coli* isolated from Poultry at LBMs in Yola 2013.

Antibiograms	Frequency	Percent (%)
E,EFT,NA,RL,TE,C,CIP,CN	9	18.4
E,EFT,NA,RL,TE,CIP,	6	12.2
E,EFT,NA,RL,TE,C,CN	4	8.1
E,EFT,NA,RL,TE,C,CIP	4	8.1
E,EFT,NA,RL,TE,CIP,CN	3	6.1
E,EFT,RL,TE,CIP,	3	6.1
E,EFT,NA,RL,CIP,CN	2	4.1
E,EFT,NA,RL,TE,CN	2	4.1
E,EFT,NA,RL,CIP,CN	2	4.1
E,EFT,NA,RL,C,CIP,CN	2	4.1
E,EFT,RL,TE,C,CN	2	4.1
E,EFT,NA,RL,TE,	2	4.1
E,EFT,NA,RL,CIP,	2	4.1
E,EFT,NA,RL,CN	2	4.1
E,EFT,NA,RL,	2	4.1
E,EFT,RL,TE,	2	4.1
TOTAL	**49**	**100**

E, Erythromycin, EFT Ceftiofur, NA Nalidixic Acid, RL Sulfamethoxazole, TE Tetracycline, C Chloramphenicol, CIP Ciprofloxacin and CN, Gentamicin

Sixteen different resistance patterns were observed.

Other Findings

It was found that human antimicrobial preparations were being used by the poultry vendors to treat their birds. This finding was observed during sample collection at the LBMs.

CHAPTER FIVE

DISCUSSION

Appropriate uses of antimicrobials for therapy, prophylaxis or growth promotion depends on the knowledge and practices of the antimicrobial user. Users of antimicrobials should have information on disease prevention, management strategies, the ability of antimicrobials to select for resistance and the need to observe responsible use in agreement with the provisions of the marketing authorisations.[40] This is why training is required for users of antimicrobials. From the study it was observed that poultry vendors were using drugs without these information. All antimicrobials must be used only on prescription by a qualified person. This is because prescription for antimicrobial agents should indicate precisely the treatment regime, dose, treatment intervals, duration of the treatment, withdrawal period and the amount of drug to be delivered. Also medicines should only be used within the expiry date and for the condition for which they were prescribed. At the same time they should be keeping records of bacteriological and susceptibility tests including the name of the prescribed drug, batch number, name of prescriber, supplier, date of administration, identification of the animal or group of animals to which the drug was administered, reasons for treatment, dosage, withdrawal periods, and effectiveness of therapy. From these it is clear that the responsibilities of antimicrobial users is enormous and requires training and supervision.[40]

In this study, it was observed that 93.8% poultry vendors in LBMs in Yola were men which is probably a factor of the culture of the locality, where more men are engaged in outdoor activities than women as is common in northern Nigeria. This is in contrast to the southern Nigeria, where women are more commonly engaged in the LBMs.[12] It was observed that 25% of poultry vendors had no formal education and 27.1% had only primary education. This low level of education among poultry vendors with over 50% having less than primary education could affect their practices thereby influencing appropriate use of antimicrobials. Also 95.8% of the poultry vendors in LBMs, had not attended any formal training in poultry production and health such that they will have learnt how to use antimicrobials appropriately. Thus there is a likely chance of abuse of drugs because they have not been trained. This situation is made worse by the fact that 79.2% kept no record of drugs they use. It is important to note that record keeping is a vital part of any business operations. Records of drugs used by those who keep food producing animals is very necessary for monitoring the quantities of drugs used in animal husbandry. This is useful when a more accurate and locally specific information is required as recommended by OIE.[39] Hygiene score of the cages in LBMs were found to be 17% dirty and 79% slightly contaminated/ dusty and therefore could affect the health of the birds and increase the use of antimicrobials. This finding is similar to the findings in Belgium broiler farms by Persoons *et al.* where dirty farms were found to use more antimicrobials and thereby increased the incidence of AMR.[41]

The practices of mixing birds of the different species and age groups were observed in 45.8% and 58.3% of poultry vendor practices respectively. It is advisable to separate birds to limit transmission of disease among different species and age groups and so limit the need to use antimicrobials. This finding is similar to the finding highlighted by FAO assessment of Nigerian LBMs.[12] The major driver for antimicrobials resistance in bacteria is inappropriate use of antibiotics which is influenced by overcrowding and poor sanitation. These factors are common in LBMs where poultry of the different species and age groups are housed together increasing stress and the occurrence of disease, leading to increased use of antibiotics and subsequently resistance selection as was highlighted by Van Den Bogaard *et al*.[11] This study also found that 54% antibiotics were purchased from the open market which is similar to findings among farmers in Zaria where 100% of drugs were sourced from the open market.[17,42] All antimicrobials should be controlled and not to be sold without a prescription from a veterinarian. The finding in this study means antimicrobials can be accessed without any form of control. This also means that profit making could be the drive for sales of antimicrobial agents.

Veterinary health seeking practices of poultry vendors showed that only 10.4% consult veterinary doctors for the health care needs of their poultry and this could be because 54.2% do not know where to find a veterinarian. There is therefore a need to station veterinarians and livestock extension officers who could provide the services required at LBMs. Dosage determination practices is very important, because it will determine appropriate use of antimicrobials; 68.% were observed to be using their experience to determine the dosage for treatment of their poultry a situation in which antimicrobials use is likely to be abused.

Keeping hands clean through hand washing is one of the most important steps that can be taken to avoid contracting diseases and spreading pathogens to others. Many diseases and conditions are spread by not washing hands with soap and clean water. In this study it was found that 56% of poultry vendors did not practice hand washing. The use of protective clothing is an essential hygiene practice for people who are in contact with animals, that may get exposed to infectious agents shared by animals and man. However, 98% of poultry vendors did not use protective clothing like coveralls. This is probably due to insufficient information about the risk of contracting zoonotic disease from animals to man. From the study, it was observed that local chickens were the commonest poultry species in the LBMs among which the study of the AMR was carried out. Findings from this study indicated poultry vendors in general did not have a thorough knowledge and did not observe acceptable practices with regard to the use of antimicrobials. Hence, education programs need to be designed for poultry vendors and farmers to develop an understanding of AMR.

The identification of an organism to be used in any AMR studies is very important, as such the *E. coli* isolates used in this study were confirmed using microbact 12E. Fourty nine out of the 56 isolates of *E. coli* identified with conventional biochemical tests were confirmed to be *E. coli* using Microbact 12E. The use of Microbact 12E for the identification of the *E. coli* was less tedious, rapid and easier than the conventional biochemical test. The convenience of using Microbact 12E suggests that it could possibly replace the conventional biochemical method, since the principles by which they work are the same, which is the utilization of substrate and colour change.

All the 49 *E. coli* were (100%) resistant to Ceftiofur, Erythromycin, and Sulfamethoxazole, while other antimicrobials resistance were Nalidixic acid(69.4%), Ciprofloxacin (65.3%), Teracycline (67.3%), Chloramphenicol (49%), and Gentamicin (34.7%). All the isolates were multi drug resistant showing 16 different resistance patterns, with each isolate being resistant to at least four antimicrobials. These findings were similar to the findings of several studies which found high *E. coli* resistance to different antimicrobials.[43,44,45,46] While this finding may not mean an implication for treatment failure among commensal *E. coli,* it may indicate widespread resistance among other pathogens like *Salmonella.* It may also indicate widespread misuse of antimicrobials. A study in Nigeria found multidrug resistant *E. coli* with 19 patterns which is similar to our finding of 16 resistance patterns among strains from local chickens.[47] This high resistance is also an indication of the resistance to be expected in other microorganisms found in these animals, environment and possibly the workers. Ceftiofur, represent the third generation cephalosporin and beta lactam antibiotics, it is used in veterinary medicine.[48] The findings of very high resistance to this drug is similar to the findings of Donalson *et al.* in *E coli* isolated from calves that have not received any treatment with ceftiofur.[45] It is known that *E. coli* strains that produce extended spectrum Amp-C-like b-lactamases, have been found to be cross resistant to a range of b-lactam antimicrobials, including amoxicillin-clavulanic acid (penicillin), ceftriaxone (cephalosporin), and cefoxitin (cephamycin).[7] Thus, resistance to antimicrobials not used routinely could co-select for resistance to those that are in use. Ceftiofur is not a common antibiotic in use in Nigeria and was not observed in drug stores in a survey of drug in stores by Geidam *et al*.[42] However, other beta lactams for which cross resistance could occur like penicillin and ampicillins were observed by Geidam *et al*.[42] The high frequency resistance observed could therefore be most probably due to cross resistance with other drug from the same class that share the same mechanism of action. Erythromycin is a macrolide that is commonly found in drug stores for use in poultry in Nigeria as it was found in 9.4% of drug stores by Geidam *et al*.[42] The very high resistance observed were similar to the that of Khan *et al.* [49] where all isolates were resistant to erythromycin. Therefore, the high resistance observed in this study could be mostly due to inappropriate use of this drugs. Another macrolides that is in use in poultry is tylosin to which cross resistance could occur with Erythromycin.

Sulphamethoxazole is a sulphonamide which are widely used in the treatment of animals, specifically for the treatment of coccidiosis which is a common disease of poultry. It was observed by Geidam *et al.* in drug shops which means it is in use in Nigeria.[42] The very high resistance to sulfamethoxazole observed in this study could be due to misuse of sulphonamides. However, sulpha resistance can co-select for resistance to other antimicrobials. Similarly, because resistance determinants often aggregate in linked clusters, antimicrobials of a different class, or substances such as heavy metals and disinfectants, can select for multiple-antimicrobial resistant bacteria.[7] In this study, it was observed that human preparations particularly the sulpha drugs were being used by the poultry vendors to treat their birds when they notice any illness. (See plate III). This is possibly because the vendors can buy these drugs without prescription and can be easily accessed without restriction. Therefore, the resistance observed in this study could both be due to the abuse of animal and human preparations abuse.

Tetracycline had a high resistance prevalence which is similar to the findings by Persoons *et al.* who found high resistance to tetracycline among *E. coli* isolated from cloaca of broiler chickens.[50] The resistance observed could be due to wide spread use of this drug. It was observed to be present in 27.2 % of drug stores by Geidam *et al.* which constituted the most commonly found drug for use in animals.[42] It was observed also that human preparations of this drug are being used by poultry vendors because of ease of purchase (See plate III). Therefore the abuse of human preparations could be contributing to the high resistances observed.

Ciprofloxacin and Nalidixic acid are third generation flouroquinolones and first generation quinolones respectively. These drugs are not commonly used in veterinary medicine but other drugs in the same family observed to be available for sale to veterinary users, included norfloxacin and enrofloxacin[42] inappropriate use of which could lead to resistance to other members of the same family. Gentamicin is an aminoglycoside which has wide range of applications making it a very important drug. It is not commonly used in poultry. This could be the reason for the low resistance of 34% observed against this drug. It was observed in 1.3% of drug stores by Geidam *et al*.[42] Chloramphenicol has been banned for use in poultry, but it is still found in the market and still being used. Its resistance rate was 49%, and was observed in 2.2% of drug stores by Geidam *et al*.[42] It is also known that fluoroquinolone resistant *E. coli* strains could show resistance to other drugs such as ampicilin, tetracycline, chloramphenicol, trimethoprin, sulphamethoxazole and gentamicin,[51,47] meaning there could be wide spread cross resistance among the eight selected antimicrobials used in this study and this could be the reason for the high resistance of *E. coli* commensals observed.

The high prevalence of resistance among commensal *E. coli* in local chickens could be a significant source of resistance genes to other bacteria, that share the same environment and a source of direct contamination of poultry meat and LBM workers. Contrary to our findings low resistance of *E. coli* isolates from broilers were observed in Belgium,[52] a variation which may be due to differences in the way antimicrobials are used. Whereas in Belgium there is control of use of antibiotics as opposed to Nigeria where there is no control of use of antibiotics. Studies of AMR of intestinal *E. coli* from different animal species show an increase in the prevalence of resistance.[45,53,54,55] This means that there could be an increase due to inappropriate use of antimicrobials.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion the occurrence of antimicrobial resistance among *E. coli* in LBMs may be wide spread. This may be due to inappropriate use of antimicrobials. To stop the emergence and ultimately reduce resistance levels among bacteria in poultry, it is first necessary to identify the practices that influence the development of resistance. Poultry vendors need to be educated on the use of drugs, the need to keep records and to practice personal hygiene. It is also clear from this study that there is an urgent need to work to protect and preserve antimicrobials by using them appropriately whether in veterinary medicine, human medicine or plant agriculture. The findings from this study is a wakeup call to the scientific community on the need for a critical review of the usage of antimicrobial agents in livestock in Nigeria and the importance of taking definite steps by relevant authorities to curtail the indiscriminate use of antimicrobial agents.

6.2 Recommendations

The following recommendations are made based on the findings of this study,

1. Adamawa State Ministry for Livestock Production;
    - Should conduct trainings for poultry vendors at LBMs on poultry production including the appropriate use of antimicrobials.
    - Public veterinary services should be involved in the transfer of information and carry out extension activities in all aspects of AMR, leaflets could be prepared and distributed to public veterinary services, drug marketers, poultry farmers and vendors.

- Develop and work out principles for appropriate use of antimicrobials suited for the locality.
- Regulate the sales of antimicrobials in the open market to prevent inappropriate use and slow AMR.

2. Veterinarians;

- Organise awareness campaigns for consumers on food safety and AMR.
- Prescribe antimicrobials only based on laboratory results and when necessary.

3. Poultry Vendors;

- Use antimicrobials only on prescription by a Veterinarian
- Practice personal hygiene and use protective clothing like coveralls and hand gloves when handling poultry

4. Federal Ministry Agriculture and Rural Development;

- Prepare a legislation on veterinary antimicrobials pertaining to sales, prescription, utilization, and import, for presentation to parliament. This is a very important issue for the everyday life of all Nigerians.

- There is need to carry out further studies to determine genes encoding for the various antimicrobials resistance found in the *E.coli* isolates.
- Establish a surveillance system for AMR particularly in poultry.

5. General Recommendations

- The study should be extended to cover the state and other states of the country.
- There is need to carry out further studies to determine the genes encoding for various antimicrobials resistance found in the E coli isolate.

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LIST OF PLATES

Plate 1: Microbact Identification of *E. coli*

S/No	ID SYSTEM	ISOLATE No	OCTAL CODE	IDENTIFICATION ORGANISM	PERCENT PROBABILITY	RESULTS
1	12E	14	6650	*Y.enterocolitica*	32.95	Not Selected
2	12E	18	6670	*E.coli*	59.67	Not Selected
3	12E	8	6670	*E.coli*	59.67	Not Selected
4	12E	49	6670	*E.coli*	59.67	Not Selected
5	12E	53	6670	*E.coli*	59.67	Not Selected
6	12E	219	6670	*E.coli*	59.67	Not Selected
7	12E	217	7670	*E.coli*	74.63	Not Selected
8	12E	140	7660	*E.coli*	76.56	Selected
9	12E	232	7660	*E.coli*	76.56	Selected
10	12E	7	6660	*E.coli*	76.77	Selected
11	12E	60	6660	*E.coli*	76.77	Selected
12	12E	27	6660	*E.coli*	76.77	Selected
13	12E	33	6260	*E.coli*	76.77	Selected
14	12E	5	6660	*E.coli*	76.77	Selected
15	12E	21	6660	*E.coli*	76.77	Selected
16	12E	42	6660	*E.coli*	76.77	Selected
17	12E	197	6660	*E.coli*	76.77	Selected
18	12E	235	6660	*E.coli*	76.77	Selected
19	12E	2	6660	*E.coli*	76.77	Selected
20	12E	34	6660	*E.coli*	76.77	Selected
21	12E	174	6260	*E.coli*	76.77	Selected
22	12E	50	6660	*E.coli*	76.77	Selected
23	12E	115	6260	*E.coli*	76.77	Selected
24	12E	20	6660	*E.coli*	76.77	Selected

25	12E	52	6660	*E.coli*	76.77	Selected
26	12E	25	6660	*E.coli*	76.77	Selected
27	12E	17	6660	*E.coli*	76.77	Selected
28	12E	27	6660	*E.coli*	76.77	Selected
29	12E	45	6660	*E.coli*	76.77	Selected
30	12E	56	6660	*E.coli*	76.77	Selected
31	12E	135	6660	*E.coli*	76.77	Selected
32	12E	191	6660	*E.coli*	76.77	Selected
33	12E	119	6660	*E.coli*	76.77	Selected
34	12E	178	6660	*E.coli*	76.77	Selected
35	12E	44	6660	*E.coli*	76.77	Selected
36	12E	213	6660	*E.coli*	76.77	Selected
37	12E	207	6660	*E.coli*	76.77	Selected
38	12E	204	6660	*E.coli*	76.77	Selected
39	12E	195	6660	*E.coli*	76.77	Selected
40	12E	32	6660	*E.coli*	76.77	Selected
41	12E	38	6660	*E.coli*	76.77	Selected
42	12E	40	6660	*E.coli*	76.77	Selected
43	12E	190	6660	*E.coli*	76.77	Selected
44	12E	214	6660	*E.coli*	76.77	Selected
45	12E	59	6660	*E.coli*	76.77	Selected
46	12E	117	6660	*E.coli*	76.77	Selected
47	12E	39	6660	*E.coli*	76.77	Selected
48	12E	13	6660	*E.coli*	76.77	Selected
49	12E	34	6660	*E.coli*	76.77	Selected
50	12E	36	6660	*E.coli*	76.77	Selected
51	12E	35	6660	*E.coli*	76.77	Selected
52	12E	41	6660	*E.coli*	76.77	Selected

53	12E	224	6660	*E.coli*	76.77	Selected
54	12E	3	6660	*E.coli*	76.77	Selected
55	12E	4	6760	*E.coli*	96.39	Selected
56	12E	9	6760	*E.coli*	96.39	Selected

Plate II: Questionnaire

KNOWLEDGE AND PRACTICES AMONG POULTRY VENDORS IN LIVE BIRD MARKETS

INFORMED CONSENT

Greetings. My name is ____ and I am a resident of the Nigeria Field Epidemiology and Laboratory Training Program, Ministry of Animal production , Yola.

My team is conducting a study on antibiotic resistance in chickens. We would also be collecting information and cloacal swab from your chickens which will be tested for the presence resistance in bacteria. Taking the swab from the chickens is very safe for your chickens and will not harm them. You will be told the result of this study later, if you so wish to receive such information.

Whatever information you provide will be kept strictly confidential. Participation in this study is voluntary. However, we hope that you will participate in this study since your views are important.

Date of Interview: ---

Name of Market:-------------------------------------- **L.G.A**:--------------------------------

Demographic Data (Please Tick appropriate Option)

1. Age: ----------- 2. Sex: ☐ M /☐ F

3. Educational Level: ☐None, ☐ Primary, ☐ Secondary, ☐ Tertiary, ☐ Arabic education

4. How long have you been working at the market or with poultry?

< 1 year ☐, 1-5 years ☐, 6-10 years ☐, 11- 20 years ☐, > 20 years ☐

5. Have you attended any formal training on poultry production and health? Yes ☐ No ☐

KNOWLEDGE AND PRACTICES

1. What types of poultry do you keep or Sell?
    a. Local chicken(Native) ☐
    b. Commercial Poultry (Broilers) ☐
    c. Commercial poultry(Layers) ☐
    d. Turkeys ☐
    e. Ducks ☐
    f. Pigeons ☐
    g. Guinea Fowls ☐
    h. Others(specify) : ______________________
2. What is the source of your birds?
    a. Farms ☐
    b. Village markets ☐
    c. I raise my birds myself ☐
    d. Others (specify): ______________________
3. Do you keep records of your poultry business activity? Yes ☐ No ☐
4. What do you do when your birds are sick?

Consult my fellow farmer/ vendor ☐ Consult a veterinarian ☐ Consult a livestock Assistant? ☐ Treat them myself. Others (specify) : ______________________

5. Do you know where to find Veterinary Doctor that can treat your birds?

Yes ☐ No ☐

6. How often do you administer drugs to your birds?

Daily, ☐ Weekly, ☐ Monthly, ☐ Others (please specify): ______________________

7. Have you heard about Antimicrobial drug resistance? Yes ☐ No ☐
8. Have you heard about drug withdrawal periods? Yes ☐ No ☐
9. Do you practice drug withdrawal periods? Yes ☐ No ☐
10. How do you determine the dosage for treating your birds?
    a. As prescribed by a veterinary Doctor ☐
    b. As delivered by an authorized animal health professional ☐
    c. As instructed by the Pharmacist/vendor☐

d. I follow the manufacturer advice ☐

e. I use my experience ☐

f. Other (Specify) : ______________________

11. How do you administer your drugs?

    a. By injection ☐ b. In feed☐ c. In drinking water ☐ d. Other (specify)______________________

12. Do you administer drug to your poultry whether sick or not?

    Yes ☐ No ☐

13. Where do you get your drugs from?

    a. From and veterinary shop ☐ b. From the market ☐ c. From a veterinary clinic ☐ d. Others (specify) : ______________________

14. Will you consult a veterinary doctor if you know where to find them?

    Yes ☐ No ☐

15. Hygiene scores for cages by observation.

    Visibly clean ☐ Mild contaminated/ dusty ☐ Dirty ☐

16. Are birds of different species kept in the same cages?

    Yes ☐ No ☐

17. Are birds of different age groups kept together? Yes ☐ No ☐

18. Do you wash your hand after handling your birds? Yes ☐ No ☐

19. Do you use protective clothing? Yes ☐ No ☐

Plate III: Human Preparations of Tetracycline, Sulphonomide and Paracetamol about to be used by Poultry Vendors to treat his chickens.

Plate IV: Poultry meat preparation in a typical LBM