PREVALENCE OF ANTIMICROBIAL RESISTANT KLEBSIELLA SPECIES IN POULTRY FEEDS, FEED INGREDIENTS AND FECAL SAMPLES IN IMO STATE, NIGERIA

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN ANIMAL MANAGEMENT OF THE DEPARTMENT OF ANIMAL SCIENCE AND TECHNOLOGY, SCHOOL OF AGRICULTURE AND AGRICULTURAL TECHNOLOGY

APRIL, 2016

CERTIFICATION

I certify that this work "The Prevalence of Antimicrobial resistant Klebsiella Species in Poultry Feeds, Feed Ingredients and Fecal samples in Imo State, Nigeria" was carried out by Ajayi, Nene Victoria (Reg Number 20054548608) in partial fulfilment for the award of degree of M.Sc in Animal Management of the Department of Animal Science and Technology of the Federal University of Technology Owerri.

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DEDICATION

This Work is dedicated to The Almighty God, The God of all wisdom who has helped me & brought the right people at the right time to assist in this Work.

ACKNOWLEDGEMENT

I sincerely thank the Almighty God for his love for me and my family and creating opportunity for me to carry out this study. All glory; be unto your name forever. I wish to acknowledge the assistance & support of my husband, Surv. Olusegun Ajayi, whose financial/moral support & encouragement made this study possible.

I am deeply indebted to my supervisor, Prof. I. C. Okoli for his immense support & assistance. His patience and encouragement has made this whole study possible. I am highly grateful to my co-supervisor, Dr. M.N. Opara for his advice.

I appreciate the heads of department who were helpful in the course of this programme especially the current HOD, Dr. E. B. Etuk. My sincere appreciation goes to Prof. A. B. I. Udedibie, Prof. Esonu, Prof. Anyanwu, Prof. Okeudo, Dr. Etuk, Prof. Mrs Ogundu, Prof. Uchegbu and all my lecturers.

I wish to thank Dr C. C. Ugwu for his assistance & encouragement, Mr Ifeanyi, Head Microbiology lab, ISEPA, Owerri for his assistance in the laboratory analysis and my collegue, Dr (Mrs) Irene Chima for her encouragement.

I also wish to thank the departmental staff and all those who in one way or the other helped to make this work successful. May God in His infinite mercy bless all of you according to his riches in glory.

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ABSTRACT

This study was carried out to determine the level of contamination of commercial poultry feed, feed ingredients and faecal samples with multi-drug resistant Klebsiella species in Imo State, Nigeria. Samples were collected from broiler starter, broiler finisher, growers and layers feed types of three feed brands labelled TFB, VFB and GFB. Faecal samples from broilers, layers and local chicken were collected from randomly selected farms, open market and local chicken in each zone using sterile swab stick to sample the chicken cloaca. These samples were collected for Orlu, Okigwe and Owerri zones of the state. In each zone, 6 collection centres were randomly selected. In each centre, 0.5kg of feed from thoroughly mixed randomly collected feed type was aseptically sampled, labelled appropriately and transported to the laboratory for analysis. This was repeated for each feed type of each brand. Samples were also collected from feed ingredients which included sorghum, maize, palm kernel cake, blood meal, groundnut cake, soya bean meal and fish meal. Samples were cultured on MacConkey and eosin methylene blue agar and identified through colonial morphology and biochemical tests. Antimicrobial susceptibility tests were carried out using disc diffusion method against a panel of ten antimicrobials with breakpoints as recommended by clinical laboratories standards institute. Data were analysed with means and percentages. Ninety one (75.8%) feed samples were positive for *Klebsiella species* after isolation procedures. Out of these, 25, 30 and 28 samples representing 62.5, 82.5 and 82.5% occurrence were recorded for TFB, VFB and GFB feed brands respectively. Twenty eight (46.67%), 46 (57.6%) and 43 (43%) of the energy and fibre source feed

ingredients, protein source feed ingredient and poultry droppings respectively were positive for Klebsiella species. The isolates from commercial feeds were most resistant to septrin (59.1%), nalidixic acid (53.4%) and augumentin (52.3%) and least resistant to gentamycin (37.5%), orfloxacin and ciprofloxacin (40.9%). All the isolates from feed ingredients were resistant to perfloxacin and nalidixic acid. The isolates were also highly resistant to orfloxacin and cephalexin 93.45% and 89.19% respectively and were least resistant to ciprofloxacin (33.78%) and streptomycin (66.22%).The isolates from poultry droppings showed highest resistance to septrin (87.1%), ampicillin(83.87%) and nalidixic acid(77.42%) but were least resistant to augumentin (29%), orfloxacin (19.35%) and cephalexin (16.13%). All the isolates from local chicken were resistant to perfloxacin, nalidixic acid and ampicillin but were equally sensitive to streptomycin, cephalexin and ciprofloxacin.The commercial feed brands in the study area and the feed ingredients were found to be highly contaminated with multi-drug resistant Klebsiella species and could be sources of difficult to treat Klebsiella infection for chicken, other animals and subsequently humans in the area.

Keywords: antimicrobial, resistance, klebsiella, poultry feeds, feed ingredients,.

CHAPTER 1

1.0: INTRODUCTION

1.1 Background Information:

Klebsiella species are Gram negative non-motile, facultative anaerobic rod-shaped bacteria. Klebsiella has been isolated from vegetation, soil and water (Neimela *et al.,* 1982). They are frequently isolated in infections of animals and humans where they cause various diseases (Wikipedia, 2014). These diseases include metritis in horses, mastitis in cattle and pneumonia in a wide variety of animals. Klebsiella can be found in 40% of normal humans and animals where they are commensals and could cause secondary infections or in immune compromised hosts. In chicken, it is responsible for health conditions like rhinitis, bronchopneumonia and gastroenteritis and could complicate other diseases especially, viral diseases which are very common in poultry. Cockroaches have been reported to play the role of vectors and could deposit the organism in feed and feed ingredients while crawling through them (Cotton *et al*., 2000). As poultry production becomes more intensified, the need to ensure that management and nutrition, particularly feed do not constitute hazard to animal health or productivity increases.

The extensive use of antimicrobial agents in animal feeds has resulted in the development and spread of resistant bacteria in the animals and the environment (Gorbach, 2001; WHO, 2002, Collignon *et al*., 2006;). These result in spread of resistant pathogens in animals through various pathways that may include

contaminated litter and animal manure which in turn may contaminate soil and farm produce or through direct animal contact with rodents, wild birds and animal feeds (Collignon *et al*., 2006; FAO/OIE/WHO, 2003; Okoli *et al*., 2005; Sapkota *et al.*, 2007). Many of these resistant bacteria are enteric pathogenic organisms and include pathogens of clinical importance in humans and / or animals such as Salmonella, *Staphylococcus aureus, E. coli* and *Klebsiella* (WHO, 2002; Okoli, 2004; Rao, 2006).

The emergence of antimicrobial resistance in humans has been linked to the spread of resistant bacteria in animals via animal direct contact (Collignon *et al*., 2006; Sapkota *et al*., 2007), through subsequent animal-based food products such as meat, eggs and milk (Sapkota *et al*., 2007; WHO, 2007) and the environment (Jenson *et al*., 2002; Chapin *et al*., 2005). Studies have shown that infections arising from resistant bacteria in humans emerged in the United States of America after specific antibiotics of human usage were approved for use in animal feeds and water (Gupta *et al*., 2004; Smith *et al*., 2007). It has however been shown on the contrary that in developing countries, antimicrobial resistance events in humans may be the drivers of the events in animal production (Okoli, 2006). Microbial contamination of animal feed remains a significant potential pathway for farm animals and subsequent entry into the Feed- chain (*Okoli et al.,* 2005a; Crump et al, 2006).

The global trade in animal feed and animal feed ingredient is substantial and far – reaching with more than 100 countries importing a total of 2 million tons (metric tons) of meat meal alone in 1999 (Crump *et al*., 2002). There is thus considerable potential for movement of contaminated feed and / or feed ingredients within and between countries as a result of the substantial global trade in animal feed and animal feed ingredients (Crump *et al*., 2002; Okoli, 2004; Okoli *et al*., 2005a). This could result in widespread and rapid dissemination of a pathogen to geographically dispersed animal herds and in turn a range of food products for human consumption (Crump *et al*., 2002).

Animal feeds serve as carriers of microbial contaminants such as moulds, bacteria and mycotoxins (Thar *et al*., 1957; Haggablom, 1993*; Okoli et al.,* 2006a; Maciorowski, 2007). Animal feed ingredients of both animal and plant origin are frequently contaminated by pathogenic bacteria (Crump *et al*., 2002, Okoli *et al.,* 2006a and b). Microbial contamination of animal feeds and feed ingredients is either through direct or indirect means. The primary mode of direct contamination includes the soil, rodents, and insects as well as standing crops and wild birds (Microbewiki, 2006). The indirect sources include contaminated water, sewage and animal manure used as fertilizers (Maciorowski *et al*., 2006; Okoli *et al*., 2004).

Animal feeds and feed ingredients have been shown to be contaminated by antibiotic-resistant bacteria, and thus act as vehicles for introduction of such

bacteria into the food chain (MAFF, 1993; Schwalbe *et al*., 1999, Hofacre *et al*., 2001, Okoli *et al*., 2005a). Several studies have shown that Microbial contamination of feeds and feed ingredients are regarded as likely sources of bacterial infections beyond the control of the poultry farmer (Wilson, 1990; Garland, 1996; Okoli, 2004). This is due to the likelihood of contamination at any point from the growing stage through the harvesting, processing and storage stages at the farm level (Maciorowski *et al*., 2006).

Many of the environmental bacterial contaminants of feeds and feed ingredients belong to the family Enterobacteriacea which include *E. coli* and Klebsiella (Maciorowski *et al*., 2006). A number of the species are endogenous members of the gut micro-flora which exist without producing harmful effects in animals but are capable of causing disease in humans (Mackenzie *et al*., 1976). Some of the bacteria have the ability to mutate and acquire resistance to antibiotics by internalizing genetic factors derived from other bacteria in the same environment (Okoli; 2004; Collignon *et al*, 2006). So when an antimicrobial agent is administered, the bacteria resistant to it would propagate faster than the non-resistant types (Barza, 2002, Collignon *et al*., 2006). A study carried by Okoli *et al*. (2005a) also revealed that in Owerri, Imo State, Nigeria, *E.coli* isolates from commercial poultry feeds and feed raw materials recorded very high resistance to cefuroxine, norfloxacin, cotrimaxazole, nalidixic acid and ampicilin with most organisms exhibiting multi-drug resistance.

Antimicrobial resistance.

The emerging problem of antimicrobial resistance due to non-human antimicrobial usage is of great concern to global agencies like WHO, FAO and OIE and major stake holders in the livestock industry (WHO, 2002; WHO, 2003). Antimicrobial resistance in *Klebsiella species* is of grave concern in internal medicine because of the involvement or implication of *Klebsiella pneumoniae* in hospital acquired infections (Obiemiwe, 2002; Paterson *et al*., 2004). Multidrug resistant bacteria with reference to Klebsiella is therefore a public health concern as well as an epidemiology issue which should reinforce the need for a more prudent use of antibiotics by farmers, veterinarians, animal scientists and physicians (WHO, 2002; Aiello and May,1998). Knowledge of the prevalence of *Klebsiella spp*. in poultry, poultry feeds and feed ingredients are grossly lacking in South Eastern Nigeria (Uwaezuoke *et al.*, 2008; Okoli, 2004; Chah *et al*., 2000 & 2003) which makes the study pertinent.

1.2 Problem Statement

The need to make sure that feed and its ingredients do not constitute a source of bacterial infection to poultry is very vital in production processes. This is against the back drop of the fact that insects example, cockroaches have been implicated as vector for Klebsiella species. Although these organisms are usually opportunistic pathogens, they could be a source of complication for other diseases of poultry especially viral diseases which are common in chicken.

It is therefore pertinent for us species in a given environment. to determine the level of contamination of poultry feed and feed ingredients with *Klebsiella*

The emerging problem of antimicrobial resistance due to the non-human antimicrobial usage and is particularly of grave concern in internal medicine because pathogenic organisms such as *Klebsiella pneumoniae* have been implicated in hospital acquired infections (Umeh, 2002; Paterson *et al*., 2004).

In a study carried out by Hofacre *et al*. (2001), some feed ingredients were shown to contain bacteria resistant to antibiotic such as ampicillin, amoxicillin and cephalothin. Several incidents have been reported in which human illness was traced back to contaminated animal feed. One of such is the outbreak of Salmonella infection in humans, traced back to consumption / ingestion of chickens fed with contaminated animal feed. The animal feed was derived from fish meal contaminated with *Salmonella enterica* Serotype. Contaminated feeds have also been shown to result in infection or colonization of food animals (Crump *et al*., 2002). Again poorly enforced import regulations, total absence of antimicrobial resistance monitoring and surveillance have been identified in Nigeria (Okoli *et al*., 2002)

These may be critical factors that drive antibiotic resistance among important public health and important economic pathogens such as Klebsiella in the Nigerian environment. Based on these problems, the following questions may be asked:

1. Do commercial poultry feeds, feed ingredients and fecal samples of poultry in Owerri, Imo state, Nigeria habour Klebsiella spp.?
2. If yes, what is the occurrence rate of Klebsiella spp. from feed, feed ingrdients and fecal samples of poultry in Owerri, Imo state, Nigeria?
3 What are the antimicrobial resistance profile of the Klebsiella species isolated from these sources?

1.3 Study Objectives

The main objective of this study is to find out if antimicrobial resistant Kiebsiella species contaminate poultry feeds, feed ingredients and fecal samples of chicken in the study area.

The specific objectives were to:

1. *Determine the frequency of isolation of Klebsiella from poultry feeds, feed ingredients and poultry faeces from Imo State, Nigeria.*
2. *Determine the prevalence of antimicrobial resistance among Klebsiella Isolates from these sources.*
3. *Establish the antimicrobial resistance patterns of Klebsiella isolates*

1.4 **Justification of the Study**

The result of the study will help bring to the fore the information about the status of Klebsiella species as a contaminating organism in poultry feed and feed ingredients in the study area. The result will also help to close the gap in knowledge existing now with regards to the status of Klebsiella species as a

contaminating bacterial organism in poultry feed and feed ingredients in Imo state, Nigeria in comparism to other locations worldwide.

It will alert the poultry farmers, veterinarians and animal scientists on the danger feed and ingredients used in compounding feed could pose for poultry as a potential source of infection. The knowledge of the antimicrobial susceptibility testing will be a guide to veterinarians on the best line of treatment in the event of infection involving *Klebsiella species* of feed origin.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Klebsiella species*

Klebsiella is a non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. *Klebsiella pneumoniae* is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines (Ryan and Ray, 2004). The organism was named after the German microbiologist Edwin Klebs (1834–1913) who discovered it. It naturally occurs in the soil, and about 30% of strains can fix nitrogen in anaerobic conditions (Podschun and Ullmann, 1998). *Klebsiella pneumoniae* has been associated with 2–5% of nosocomial infections, particularly those involving the urinary and respiratory tracts (Podschum and Ullmann, 1998; Asensio *et al*., 2002). *Klebsiella* is among the five gram-negative pathogens most commonly encountered in hospital-acquired infections (Horan *et al*., 1988), and *Klebsiella pneumoniae* is the most frequently occurring species, accounting for 75 to 86% of *Klebsiella* species reported (De La Torre *et al*., 1985; Hansen *et al*., 1998 and Watanakunakurn, 1991). Much more rarely encountered are *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis*, which have been retained as separate species because of their association with specific diseases (Podschum and Ullmann, 1998).

Taxonomically, these two species are regarded as subspecies of *K. pneumoniae* based on DNA-DNA hybridization data (Orskov, 1984). *Klebsiella oxytoca* is the other well-established species, accounting for 13 to 25% of isolates (De La Torre *et al*., 1985; Hansen et al., 1998; Watanakunakurn, 1991). Members of the genus *Klebsiell* , especially *K. pneumonia* and *K. oxytoca*, are opportunistic pathogens associated with severe nosocomial infections such as septicaemia, pneumonia and urinary tract infections. *K. pneumonia* has been taxonomically subdivided into three subspecies namely; *K. pneumonia pneumonia*, *K. pneumonia ozaenae* and *K. pneumonia rhinoscleromatis* (Brisse and Verhoef, 2001). *Klebsiella rhinoscleromatis* and*K. ozaenae* cause two clinical forms of chronic rhinitis, namely rhinoscleroma and ozena, respectively (Malowany *et al*., 1972). Both diseases are endemic in areas with poor hygiene conditions and are commonly not detected in developed countries (Medina *et al*., 2003 and Evrard *et al*., 1998).

Members of the *Klebsiella* genus typically express 2 types of antigens on their cell surface. The first, O antigen is a component of the lipopolysaccharide (LPS), of which 9 varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties. Both contribute to pathogenicity and form the basis for serogrouping (Bagley, 1985). *Klebsiella* species are ubiquitous in nature (Bagley, 1985) and are scientifically classified into seven species as follows;

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Klebsiella*

Species: *K. pneumoniae*

K. oxytoca

K. terrigena

K. planticola

K. ozaenae

K. rhinoscleromatis

K. omithinolytica

(Podschun and Ullmann, 1998; Nordmann *et al*., 2009).

Klebsiella organisms can cause a wide range of diseases including pneumonia, urinary tract infections, septicemia, and soft tissues infections (Podschun and Ullmann, 1998). *K. pneumonia* is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae (Ryan and Ray, 2004). It is closely related to *K. oxytoca* from which it is distinguished by being indole-negative and by its ability to grow on both melezitose and 3-hydroxybutyrate. *K. pneumoniae* is the most medically important species of the group. *K. oxytoca* and *K. rhinoscleromatis* have also been demonstrated in human clinical specimens. In recent years, Klebsiella e have become important pathogens in nosocomial infections (Nordmann *et al*., 2009).

2.2 Pathogenicity of Klebsiella species

Klebsiella spp. has been identified as important common pathogens for nosocomial pneumonia (7 to 14% of all cases), septicaemia (4 to 15%), urinary tract infection (UTIs; 6 to 17%), wound infections (2 to 4%), intensive care unit (ICU) infections (4 to 17%), and neonatal septicaemias (3 to 20%) (Janda and Abbott, 2006). *Klebsiella* spp. can also cause bacteremias and hepatic infections and have been isolated from a number of unusual infection, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, muscle abscess, fascial space infections of the head and neck, and septic arthritis (Janda and Abbott, 2006). They are also important opportunistic pathogens, particularly among the immunocompromised.

Pathogenicity factors of *Klebsiella* spp. include adhesins, siderophores, capsular polysaccharides (CPLs), cell surface lipopolysaccharides (LPSs), and toxins, each of which plays a specific role in the pathogenesis of these species. Depending on the type of infection and the mode of infectivity, cells of *Klebsiella* spp. may adhere and attack upper respiratory tract epithelial cells, cells in gastrointestinal tract, endothelial cells, or uroepithelial cells, followed by colonization of mucosal membranes. Common underlying conditions include alcoholism, diabetes mellitus, chronic liver disease (cirrhosis), chronic renal failure, cancer, transplants, burns, and/or use of catheter (Janda and Abbott, 2006).

2.3 Bacterial Contamination of Feed and Feed Ingredient

2.3.1 Sources of contamination

Food poisoning and infection by bacterial and fungal genera pose obvious health threat to both animals and humans (Uwaezuoke and Ogbulie, 2008).

Ezekiel *et al*. (2011) reported the isolation of *Escherichia*, *Salmonella*, *Klebsiella* , *Enterobacter*, *Yersinia* and *Escherichia coli* from feed samples with *E. coli* accounting for 80% of the total isolates. This implicates feed as a source of enterobacterial infection for poultry. Talaro and Talaro (2002) reported that enterobacterial species could be acquired from comtaminated feed and water. Poultry feeds can be contaminated directly or indirectly through contact with soil, rodents, birds, dust, human carriers, sewage or water during processing and storage. Poultry feeds still remain a source of various bacteria that can be pathogenic to both human and animal health (Ezekiel *et al*., 2011). Nasrin *et al*. (2007) carried out an experiment to identify the bacteria isolated from poultry feces, litter, drinking water, feed and air of poultry farm and to determine their load. Klebsiella species was found in feed and feaces. Kilonzo-Nthenge *et al*. (2008) conducted a study to compare the presence and antimicrobial susceptibility of *Campylobacter*, *Salmonella* spp., and other enteric bacteria between chickens and guinea fowls. In that study, *Klebsiella oxytoca* and *Enterobacter sakazakii* were recovered only in chickens.

2.3.2: Survivability of *Klebsiella species* in the environment

Klebsiella spp. are often found in a variety of environmental sources such as soil, vegetation and water, contributing to biochemical and geochemical processes, and has been identified as a major component of the microflora in several types of stressed nonclinical environments (Huntley *et al*., 1976; Vasconcelos and Swartz, 1976; Seidler, 1981; Sjogren and Gibson, 1981; Niemelä *et al*., 1982). These bacteria have been recovered from aquatic environments receiving industrial wastewaters (Caplenas *et al*., 1981), plant products, fresh vegetables (Brown and Seidler, 1973), food with a high content of sugars and acids (Duncan and Razzell, 1972; Mundt *et al*., 1978), frozen orange juice concentrate (Fuentes *et al*., 1985), sugar cane wastes (Nunez and Colmer, 1968), living trees, plants and plant by-products (Brown and Seidler, 1973; Knittel *et al*., 1977; Caplenas *et al*., 1981). They are commonly associated with wood (Duncan and Razzell, 1972), saw dust and waters receiving industrial effluents from pulp and paper mills (Duncan and Razzell, 1972; Knittel, 1975; Dufour and Cabelli, 1976; Huntley et al., 1976) and textile finishing plants. As a consequence, the nasal cavities of many workers in paper and board mills proved to be contaminated by *Klebsiella* (Niemelä *et al*., 1985). *Klebsiella* spp. are also present in drinking water emanating from redwood tanks (Seidler *et al*., 1977). Isolates have been described several times in forest environments (Knittel *et al*., 1977; Bagley *et al*., 1978b), degrading kraft-lignin (Deschamps *et al*., 1980c), tannic acid (Deschamps *et al*., 1980b),

pine bark (Deschamps and Lebeault, 1980a), and condensed tannins (Deschamps and Lebeault, 1980a; Deschamps and Lebeault, 1981), or from living or decaying wood and bark or composted wood (Deschamps *et al*., 1979; Deschamps *et al*., 1983). *Klebsiella* strains from industrial effluents and a variety of human and bovine mastitis origins can multiply in pulp waste to levels exceeding 106 cells per ml. *Klebsiella* strains from vegetable surfaces or human infections could grow rapidly on the surfaces of potatoes and lettuce to numbers exceeding 103 organisms per gram of surface peel and leaf after 24 h incubation at room temperature (Knittel *et al*., 1977).

Klebsiella can frequently be isolated from the root surfaces of various plants (Pedersen, 1978; Haahtela *et al*., 1981). *Klebsiella pneumoniae*, *K. oxytoca* or *K. planticola* can fix nitrogen and are classified as associative nitrogen fixers, or diazotrophs (Mahl *et al*., 1965; Ladha *et al*., 1983). Isolation of nitrogen-fixing *Klebsiella* was reported from rice leaves (Thomas-Bauzon *et al*., 1982; Ladha *et al*., 1983), rhizosphere (Rennie, 1982), grassland soil (Line and Loutit, 1971), decaying wood (Seidler *et al*., 1972), paper mill liquors and effluents (Nielson and Sparell, 1976), and maize stem tissue (Palus *et al*., 1996). The endophytic lifestyle of two *K. pneumonia* strains was demonstrated using green-fluorescent protein labeling (Chelius and Triplett, 2000). These strains colonized the intercortical layers of the stem and the maturation region of the root. Association of *K. oxytoca* with barley rhizosphere during an entire vegetative period was demonstrated using a bioluminescence reporter plasmid

(Kozyrovska *et al*., 1994). Purified type 3 fimbriae, as well as type 1 fimbriae of nitrogen-fixing *Klebsiella* , mediate bacterial adhesion to plant roots (Korhonen *et al*., 1983; Haahtela *et al*., 1985b). However, type 3 fimbriae are more efficient than type 1 fimbriae in promoting adherence (Haahtela and Korhonen, 1985a). The bacteria adhere strongly to root hairs, and with a markedly lower efficiency, to the surface of the zone of elongation and to the root cap mucilage (Haahtela *et al*., 1986). No adhesion to the epidermal cell between root hairs was observed (Haahtela *et al*., 1986). A number of the *Klebsiella* strains reported from plant material might be *K. planticola* (Bagley *et al*., 1981). Strains of *K. pneumonia* sensu stricto, which are associated with plants (e.g., living or decaying wood and bark), differ from those associated with serious human infections.

These environmental *K. pneumonia* strains are most often able to utilize 5-ketogluconate as sole carbon source (P.A.D. Grimont, unpublished observation) and never have capsular types K1 to K6. Strains involved in serious infection do not utilize 5-ketogluconate and may have capsular types K1 to K6, as well as other capsular types. A comparison of three endophytic *K. pneumoniae* strains with two clinical strains (Dong *et al*., 2003) showed that the plant associated strains belong to KpIII (Brisse and Verhoef, 2001), whereas the two clinical strains belong to KpI and were not able to colonize the interior of wheat seedlings as efficiently as the endophytic strains. However, it should be noted

that KpIII strains may be found in clinical samples from hospitalized patients (Brisse and Verhoef, 2001).

2.4 *Klebsiella* in Animals

Klebsiella spp. can be found in a wide range of mammals (Gordon and FitzGibbon, 1999) and are also present in insects (Dillon *et al*., 2002) and possibly in many other animal groups. In locusts, *Klebsiella* may contribute, among other bacterial species, to the synthesis of the aggregation pheromone implicated in the change from a grasshopper-like solitary insect to the gregarious form (Dillon *et al*., 2002). Cockroaches were suggested to play the role of vectors in the hospital environment (Cotton *et al*., 2000).

Klebsiella pneumoniae is an important cause of metritis in mares. Capsular types K1, K2, and K5 were major causes of epidemic metritis in England, whereas type K7 was associated with sporadic, opportunistic genital infection (Platt *et al*., 1976). Outbreaks of metritis in mares were due to type K2 in the United States and France (Edwards, 1928; Tainturier and Richard, 1986). The stallion plays an important role in the transmission of *K. pneumoniae*. Type K7 was found on the preputial skin of stallions and may be part of the normal bacterial flora in this location (Platt *et al*., 1976). Thus it is important to determine the K-type of *Klebsiella* strains isolated from the genital tract of horses to detect stallion carriage of an epidemic strain versus carriage of less pathogenic *K. pneumoniae*. *Klebsiella* have been frequently associated with

bovine mastitis (Braman et al., 1973) and causes serious infections in other animals including dogs, Rhesus monkeys, guinea pigs, muskrats and birds (Wyand and Hayden, 1973; Fox and Rohovsky, 1975; Kinkler *et al*., 1976; Wilson, 1994; Roberts *et al*., 2000). Epidemics of fatal generalized infections among captive squirrel monkeys (*Saimiri sciureus*) in French Guyana and lemurs in a French zoo were due to *K. pneumonia* K5 and K2, respectively (Richard, 1989). Immunization of the monkeys (or lemurs) with the corresponding capsular polysaccharide was efficient in stopping the epidemic (Postal *et al*., 1988; Richard, 1989). The K5 and K2 capsular polysaccharide were probably developed for this specific incident.

2.5 Infections caused by *Klebsiella species*

2.5.1 Infections caused in animals

Klebsiella bacteria are an important cause of metritis and infertility in horses, mastitis in bovines, hematogenous osteomyelitis originating in pulmonary lesions in cattle, and accumulation of pus in the chest (pyothorax) in horses. *K. oxytoca* has been frequently isolated from insects. *Klebsiella species* have been isolated from the tissues of farmed crocodiles with hepatitis and/or sepsis as well as from oral cavities and cloacae of both healthy and diseased snakes. Bacterial bronchopneumonia or pneumonia caused by *Klebsiella* species is a common lung disease, particularly in dogs.

2.5.2 In chicken the incidence of enterobacteriosis has been reported to be increasing in Nigeria (Ezekiel *et al*., 2011).

2.5.3 Infections caused in humans

Infection occurs most often among middle-aged and older persons with debilitating diseases. This patient population is believed to have impaired respiratory host defenses, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases (COPD), glucocorticoid therapy, renal failure, and certain occupational exposures (such as paper mill workers). Many of these infections are obtained when a person is in the hospital for some other reason. The most common infection caused by *Klebsiella* bacteria outside the hospital is pneumonia, typically in the form of bronchopneumonia and also bronchitis. These patients have an increased tendency to develop lung abscess, cavitation, empyema, and pleural adhesions. It has a high death rate of about 50% even with antimicrobial therapy. The mortality rate can be nearly 100% for persons with alcoholism and bacteremia.

In addition to pneumonia, *Klebsiella* can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, bacteremia and septicemia. If a person has an invasive device in their body then contamination of the device becomes a risk; for example respiratory support equipment and urinary catheters put patients at

increased risk. Also, the use of antibiotics can be a factor that increases the risk of nosocomial infection with *Klebsiella* bacteria. Sepsis and septic shock can follow entry of the bacteria into the blood. Two unusual infections of note from *Klebsiella* are rhinoscleroma which is a chronic inflammatory process involving the nasopharynx and Ozena which is a chronic atrophic rhinitis that produces necrosis of nasal mucosa and mucopurulent nasal discharge. Research conducted at King's College, London has implicated molecular mimicry between HLA-B27 and two *Klebsiella* surface molecules as the cause of ankylosing spondylitis (Rashid and Ebringer, 2006). *Klebsiella* ranks second to *E. coli* for urinary tract infections in older persons. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma. Feces are the most significant source of patient infection, followed by contact with contaminated instruments (Jerome, 2008).

Respiratory disease

K. pnuemoniae is a leading cause of community-acquired and nosocomial pneumonia and lung abscesses. Infection of the upper lobe is more common. Symptoms include: fevers, chills, and leukocytosis with red currant jelly-like sputum (Janda and Abbott, 2006). Rare complications include lung infection involving necrosis and sloughing of the entire lobe.

K. ozaenae causes ozena, a primary atrophic rhinitis (AR) which involves chronic inflammation of the nose (Janda and Abbott, 2006). *K. rhinoscleromatis*

causes rhinoscleroma (RS), a chronic granulomatous infection which predominantly affects the cavity of the nose (Janda and Abbott, 2006).

Central nervous system (CNS) infections

K. pneumoniae and K. *oxytoca* cause community-acquired meningitis and brain abscesses. Clinical symptoms include: headaches, fever, and longer periods of unconsciousness, seizures, and septic shock. *K. ozaenae* is associated with rare cases of cerebral abscess and meningitis.

UTIs

Klebsiella spp. are a frequent cause of urinary tract infections (UTIs). Significant bacteriuria has been ascribed to *K. ozaenae*.

Hepatic disease

K. pnuemoniae is an important causative pathogen for pyogenic liver abscesses with symptoms including fever, right-upper-quadrant pain, nausea, vomiting, diarrhoea or abdominal pain, and leukocytosis. Abscesses occur predominantly in the right lobe and are solitary.

Other infections

K. granulomatis causes donovanosis or granuloma, a chronic ulcerative disease that primarily affects the genitalia. Symptoms include development of small papule or ulcer at the site of inoculation that later develop into large red ulcers (lesions) that extend along the moist folds of the genitalia (Janda and Abbott, 2006).

K. pneumoniae can cause various diseases including pneumonia. They cause destructive changes in the lungs leading to inflammation and hemorrhage which usually result to cell death (necrosis) that sometimes produces a thick, bloody, mucoid sputum (currant jelly sputum). Typically these bacteria gain access after a host aspirates colonizing nasopharyngeal microbes into the lower respiratory tract. As a general rule, *Klebsiella* infections are mostly seen in immune compromised hosts.

2.6 Antibacterial resistance in bacteria

2.6.1 Classification of antimicrobial agents

The antimicrobial agents in use in veterinary therapy are classified based on the following:

1. Their mode of action (Whether bacteriostatic or bactericidal).
2. Their spectrum of action, whether narrow-spectrum antimicrobial agents which are effective against a limited number of microbes or broad spectrum, effective against a wide range of gram-positive and gram-negative organisms. (Aiello & Mays, 1998)

Table 1: Classes of antimicrobial agent

SN	Class	Mode of action	Examples
1	Penicillins(β-lactam antibiotics)	Bactericidal	Penicillin, Amoxicillin, Ampicillin
2	Aminoglycosides	Bactericidal	Gentamycin, Streptomycin, Neomycin
3	Macrolids	Bacteriostatic(at high concentrations, Erythromycin is bactericidal)	Erythromycin, Tylosin
4	Tetracylines	Bacteriostatic	Oxytatracycline, Tetracycline, Chlortetracycline
5	Quinolones	Bacteriostatic	Oxyfloxacin, Pefloxacin, Nalidixic acid
6	Cephalosporins (β-lactam class)	Bactericidal	Cephalexin, Cefazolin
7	Sulfonamides	Bacteriostatic	Sulfadiazine, Sulmethazine (Sulmethazine)

(Aiello & Mays, 1998)

Approximately 10% (89 isolates) of *Klebsiella pneumoniae* isolated in 1985 from patients in intensive care units in Clermont-Ferrand exhibited a complex resistance phenotype towards antibiotics. They were resistant to amino-, carboxy- and ureidopenicillins, aminoglycosides (except gentamicin), chloramphenicol, sulphonamides, tetracyclines and, most importantly, to cephalosporins (except cefoxitin and latamoxef) and to aztreonam. The metabolic profile of fifty isolates was identical and seven were selected for further study. All the resistance characters in these isolates were transferable to

Escherichia coli by conjugation and were lost *en bloc* after treatment with ethidium bromide. Agarose gel electrophoresis of crude lysates of the wild types and their transconjugants indicated that the multiple resistances were mediated by a 95kb plasmid, pCF04. The seven isolates selected for study and their corresponding transconjugants, constitutively produced a plasmid-mediated β-lactamase with a pI of 6.3 that was much more active against third-generation cephalosporins than against cephalothin. The substrate profile and the isoelectric-focusing behaviour of this enzyme differed from those of other known plasmid-mediated β-lactamases, and the enzyme was designated CTX-1. A chromosomally-encoded SHV-1 (PIT-2) penicillinase (pI 7.7) was also present in the seven *K. pneumoniae* isolates but did not transfer. Resistance to aminoglycosides in the *K. pneumoniae* isolates was due to synthesis of a 6′-aminoglycoside acetyltransferase type IV. It was observed that *K. pneumoniae* produced a new β-lactamase. (Sirot *et al*., 1987)

Resistance rates were as follows: Ampicillin (62%), cefalotin (30%), tetracycline (14%), chloramphenicol 2%. aminoglycosides 0% and nalidixic acid 0%. Strains isolated from 'industrially reared animals' showed higher resistance rates than 'naturally reared' ones (Abdelmonem *et al*., 2009).

2.6.2 Resistance among Klebsiella spp. isolates

Multidrug-resistant enteric bacteria were isolated from turkey, cattle, and chicken farms and retail meat products in Oklahoma USA (Kim *et al*., 2005).

Among the isolated species, multidrug-resistant *Klebsiella pneumoniae* was prevalently isolated from most of the collected samples. Therefore, a total of 132 isolates of *K. pneumoniae* were characterized to understand their potential roles in the dissemination of antibiotic-resistance genes in the food chains. Multidrug-resistant *K. pneumoniae* was most frequently recovered from a turkey farm and ground turkey products among the tested samples. All isolates were resistant to ampicillin, tetracycline, streptomycin, gentamycin, and kanamycin. Class 1 Integrons located in plasmids were identified as common carrier of the *aadA1* gene, encoding resistance to streptomycin and spectinomycin. Production of B-lactamase in the *K. pneumoniae* isolates played a major role in the resistance to β-lactam agents. Most isolates (96%) possessed $bla_{\text{SHV-1}}$. Five strains were able to express both SHV-11 (pI 6.2) and TEM-1 (pI 5.2) β-lactamase. Transfer of these antibiotic-resistance genes to *Escherichia coli* was demonstrated by transconjugation. The bacterial genomic DNA restriction patterns by pulsed-field gel electrophoresis showed that the same clones of multidrug-resistant *K. pneumoniae* remained in feathers, feed, feces, and drinking water in turkey environments, indicating the possible dissemination of antibiotic-resistance genes in the ecosystem and cross-contamination of antibiotic-resistant bacteria during processing and distribution of products.

In a study by Burtram *et al*. (2012), 17 chicken samples collected from a vendor operating in an informal settlement in the Cape Town Metropolitan area, South

Africa were screened for antimicrobial-resistant Gram-negative bacilli using the Kirby Bauer disk diffusion assay. In total, six antibiotics were screened: ampicillin, ciprofloxacin, gentamicin, nalidixic acid, tetracycline and trimethoprim. Surprisingly, *Klebsiella ozaenae* was identified in 96 and *K. rhinoscleromatis* in 6 (n=102) of the samples tested. Interestingly, ~40% of the isolated *Klebsiella* spp. showed multiple resistances to at least three of the six antibiotics tested. Based on their result, Burtram, *et al*. (2012) concluded that *Klebsiella ozaenae* and *K. rhinoscleromatis* cause clinical chronic rhinitis and are almost exclusively associated with people living in areas of poor hygiene.

Miftode *et al*. (2008) reported that the increasing frequency of extended-spectrum beta-lactamases (ESBLs) producing enterobacteriaceae among nosocomial and community-acquired infections is an important problem for both microbiologists and clinicians, because of the difficulty in correctly detecting, reporting and treating such infections. Their study reports that in the Clinical Hospital of Infectious Diseases Iaşi, the most frequent etiological agents of urinary tract infections were: *E. coli* (64%), *Klebsiella spp*. (11%) and *Enterococcus spp*. (5%). The resistance rate of *E. coli* and *Klebsiella spp*. was 41% and 60%, respectively to amoxicillin-clavulanic acid, 29.6% and 72.5%, respectively to third generation cephalosporins, 26% and 24%, respectively to ciprofloxacin. The most active antimicrobial agents against cephalosporins resistant strains of *E. coli* and *Klebsiella spp* were

carbapenems (susceptibility rate 99 and 94%, respectively) and colimycin (susceptibility rate 89 and 83%, respectively). New antibiotic resistant strains of *K. pneumoniae* are appearing, and it is increasingly found as a nosocomial infection (Jerome, 2008).

Infection with carbapenem-resistant Enterobacteriaceae (CRE) or carbapenemase-producing Enterobacteriaceae is emerging as an important challenge in health-care settings. One of the carbapenem-resistant Enterobacteriaceae (CRE) is Carbapenem-Resistant *Klebsiella pneumoniae* CRKP. Over time, a progressive increase in CRKP has been seen worldwide; however, this new emerging nosocomial pathogen is probably best known for an outbreak in Israel that began around 2006 within their healthcare system (Berrie, 2007). In the USA, it was first described in North Carolina in 1996 (Yigit *et al*., 2001), since then CRKP has been identified in 24 states and is recovered routinely in certain hospitals in New York and New Jersey. It is now the most common CRE species encountered within the United States.

CRKP is resistant to almost all available antimicrobial agents, and infections with CRKP have caused high rates of morbidity and mortality, particularly among persons with prolonged hospitalization and those who are critically ill and exposed to invasive devices (e.g., ventilators or central venous catheters). The concern is that carbapenem is often used as a drug of last resort when battling resistant bacterial strains. The worry is that new slight mutations could

result in infections for which there is very little, if anything, healthcare professionals can do to treat patients with resistant organisms.

There are a number of mechanisms of Carbapenem Resistance in Enterobacteriaceae. These include (1) Hyperproduction of ampC beta-lactamase with an outer membrane porin mutation (2) CTX-M extended-spectrum beta-lactamase with a porin mutation or drug efflux, and (3) Carbapenemase production. When bacteria such as *Klebsiella pneumoniae* produce an enzyme known as a carbapenemase, they are referred to as carbapenem-resistant *Klebsiella pneumoniae* (CRKP) (LA County dept. of Pub. Health, 2013).

The most important mechanism of resistance by CRKP is the production of a carbapenemase enzyme, *blak*pc. The gene that encodes the *blak*pc enzyme is carried on a mobile piece of genetic material (a transposon; the specific transposon involved is called Tn4401), which increases the risk for dissemination. CRE can be difficult to detect because some strains that harbor *blak*pc have minimal inhibitory concentrations (MICs) that are elevated but still within the susceptible range for carbapenems. Because these strains are susceptible to carbapenems, they are not identified as potential clinical or infection control risks using standard susceptibility testing guidelines. Patients with unrecognized CRKP colonization have been reservoirs for transmission during nosocomial outbreaks.

The extent and prevalence of CRKP within the environment is currently unknown. The mortality rate is also unknown but is suspected to be within a

range of 12.5% to as high as 44% (Lledo *et al*., 2009). The likelihood of an epidemic or pandemic in the future remains uncertain. The Centers for Disease Control and Prevention (CDC) released guidance for aggressive infection control to combat CRKP. Place all patients colonized or infected with CRE or carbapenemase-producing Enterobacteriaceae on contact precautions. Acute care facilities are to establish a protocol, in conjunction with the guidelines of the Clinical and Laboratory Standards Institute (CLSI), (Terry, 2012), to detect nonsusceptibility and carbapenemase production in Enterobacteriaceae, particularly *Klebsiella* spp. and *Escherichia coli*, and immediately alert epidemiology and infection control staff members if identified. All acute care facilities are to review microbiology records for the preceding 6--12 months to ensure that there have not been previously unrecognized CRE cases. If they do identify previously unrecognized cases, a point prevalence survey (a single round of active surveillance cultures) in units with patients at high risk (e.g., intensive care units, units where previous cases have been identified, and units where many patients are exposed to broad-spectrum antimicrobials) is needed to identify any additional patients colonized with carbapenem-resistant or carbapenemase-producing *Klebsiella* spp. and *E. coli*. When a case of hospital-associated CRE is identified, facilities should conduct a round of active surveillance testing of patients with epidemiologic links to the CRE case (e.g., those patients in the same unit or patients who have been cared for by the same health-care personnel), (Lledo *et al*., 2009).

The reasons that the CDC is only recommending the detection of carbapenem resistance or carbapenemase production for *Klebsiella* spp. and *E. coli* are 1) this facilitates performing the test in the microbiology laboratory without the use of molecular methods and 2) these organisms represent the majority of CRE encountered in the United States. Effective sterilization and decontamination procedures are important to keep the infection rate of this antibiotic resistant strain, CRKP as low as possible.

2.7 Antibacterial drug usage in poultry in Nigeria and worldwide

2.7.1 Non therapeutic use of antimicrobial agents

In addition to being used in treatment of sick animals, antimicrobial agents are used for mass treatment to prevent infectious diseases or continuously in feed at very low doses for growth promotion (WHO, 2002). The use of antimicrobial agents for growth purposes has become an important part of intense animal husbandry, and has been practiced for about 59 years in the United States and other countries (WHO, 2002; Dibner and Richards, 2005). Early indicators of a beneficial effect on production efficiency in poultry and swine were reported by Moore *et al* (1946). The amount of antimicrobial agents used in food animals or agriculture is not known precisely. However, it is estimated that about half the total amount of antimicrobial agents produced globally is used in agriculture with emphasis in food animals (WHO, 2002). In many developing countries, the increase in meat production is mainly due to intensified farming which is often

coupled with increased antimicrobial usage for both disease therapy and growth promotion (WHO, 2002).

Driven primarily by increased use in poultry, the overall use of antimicrobial for non-therapeutic purposes seems to have risen by 50% since 1985 from 7.30 million kilograms to 11.16 million kilograms in 2001, in the United States (USC, 2002).

Antimicrobial agents used as growth promoters have been known in general to increase growth rate by 2 – 10 % and feed conversion efficiency by 3 – 9 %. Their effects are greater in young animals and have minimal effects on carcass composition other than that due to better growth rate (Aiello & Mays,1998). Some of the antimicrobial agents in use as growth promoters include avoparcin, nitrovin, tylosin, viginiamycin, bacitracin, monensin, sodium arsenilate, tetracycline, penicillin and erythromycin (Dibner and Richards, 2005).

Charles *et al*. (2001) reported that antibiotics are used in food animal production to treat diseases and also to improve performance. Antibiotics are not used on all farms, and antibiotic resistance is occasionally found on farms that do not use antibiotics. Rendered animal protein products are often included in poultry feeds and could potentially serve as a source of antibiotic-resistant bacteria. Sub-therapeutic doses of antimicrobial agents are administered routinely to poultry to aid growth and to prevent disease, with prolonged exposure often resulting in bacterial resistance (Burtram, *et al*., 2012). Crossover of antibiotic resistant bacteria from poultry to humans poses a risk to human health.

2.8 Methods of isolation and identification of Klebsiella spp

Abdelmonem *et al*. (2009) carried out a study on the identification and susceptibility to 8 selected antimicrobial agents of *Klebsiella* and *Enterobacter* bacterial species in 15 marketed meat samples (chicken, turkey-hen, beef, sheep, pig, dromedary, ostrich, and fish). The isolates were identified with the API 20E system, resulting in 7 clusters: *Enterobacter aerogenes* (2 isolates), *Enterobacter cloacae* (6), *Enterobacter sakazakii* (3), *Enterobacter spp* (14), *Klebsiella oxytoca* (5), *Klebsiella pneumoniae* (2) and *Klebsiella ornithinolytica* (12). The identities of isolates identified as *Enterobacter* and *Klebsiella spp.* were confirmed by Amplified Ribosomal DNA Analysis (ARDRA), using *Alu*I, *Msp*I, *Rsa*I restriction enzymes. Identification of isolates by ARDRA and API 20E system gave similar results with 90.2 % (44/51) of the collection.

2.8.1 Temperature and incubation conditions

Good growth results are usually obtained when *Klebsiella* cultures are incubated at 30–35°C. However, *Klebsiella* isolates from clinical specimens often have been observed to grow better at an optimal temperature for growth near 37°C. While conducting some tests (e.g., Voges-Proskauer test), growth at lower temperatures are more frequently best for positive results. *K. terringena*, *K. planticola*, *K. ornithinolytica* and *K. mobilis* were found to grow at 5^0C; all

the species except *K. planticola* and *K. terringena* grow at 410C while only *K. pneumonia* grow at 44.50C (Brisse *et al*., 2006).

2.8.2 The methods available for isolation of bacteria.

A number of methods are available for the isolation of bacteria. The following are a few important methods:

1. Surface plating

It is also called streak culture method. The specimen to be cultured is taken in a platinum loop. One loopful of specimen is transferred on to the surface of a well dried plate. Then it is spread over a small area at the periphery. The inoculum is then distributed thinly over the plate. This is done by streaking with the loop in a series of parallel lines in different segments of the plate. The loop should be flamed and cooled between different sets of streaks. After incubating the plate, it can be seen that growth is confluent at the original site of inoculation. But it becomes progressively thinner. In the final series of streaks, well separated individual colonies of bacteria can be obtained.

2. Enrichment and selective media

Bacteria can be isolated by growing in enrichment or selective media. In these media, substances which inhibit the growth of unwanted bacteria are added. So there is a growth of only the bacteria which is wanted.

3. Aerobic and anaerobic conditions

Aerobic and anaerobic bacteria can be separated by cultivation under aerobic or anaerobic conditions. The most widely used medium for anaerobic bacteria is Robertson's cooked meat medium. It contains fat-free minced cooked meat in broth. It is covered with a layer of sterile Vaseline.

4. Isolation by difference in temperature

Thermophile bacteria grow at 60° C. Some bacteria like N. magnitudes grow at 22° C. By incubation at different temperatures, bacteria can be selectively isolated.

5. Separation of vegetative and spore forming bacteria

Vegetative bacteria are killed at 80° C. But spore forming bacteria like tetanus bacilli survive at this temperature. So by heating at 80° C vegetative bacteria can be eliminated and spore forming bacteria can be isolated.

6. Separation of motile and non-motile bacteria

This can be achieved by using Craig's tube or a U tube. In the U tube, the organisms are introduced in one limb and the motile organism can be isolated at the other limb.

7. Animal inoculation

Pathogenic bacteria can be isolated by inoculation into appropriate animals, e.g. Anthrax bacilli can be isolated by inoculation into mice or guinea pigs.

8. Filtration

Bacteria of different sizes may be separated by using selective filters.

9. Micromanipulation

By means of micromanipulation, single bacterium can be separated and cultured.

2.9 Treatment of Klebsiella infections

As with many bacteria, the recommended treatment has changed as the organism has developed resistances. *Klebsiella* organisms are often resistant to multiple antibiotics. Current evidence implicates a plasmid as the source of the resistant genes. *Klebsiella* with the ability to produce extended-spectrum beta-lactamases ESBL are resistant to many classes of antibiotics. The most frequent resistances include resistance to aminoglycosides, fluoroquinolones, tetracyclines, chloram-phenicol, and sulfamethoxazole-trimethoprim (Deepti and Deepthi, 2010).

The choice of a specific antimicrobial agent or agents depends on local susceptibility patterns and on the part of the body that is infected. For patients with severe infections, a prudent approach is the use of an initial short course (48-72 h) of combination therapy, followed by a switch to a specific mono-therapy once the susceptibility pattern is known for the specific patient. If the specific *Klebsiella* in a particular patient does not have antibiotic resistance, then the antibiotics used to treat such susceptible isolates include

ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanate, ceftazidime, cefepime, levofloxacin, norfloxacin, gaitfloxacin, moxifloxacin, meropenem, and ertapenem. Some experts recommend the use of Meropenem for patients with ESBL producing *Klebsiella* . The claim is that meropenem produces the best bacterial clearing. The use of antibiotics is usually not enough. Surgical clearing (frequently done as interventional radiology drainage) is often needed after the patient is started on antimicrobial agents.

2.10 Prevention of Klebsiella infections

Infections involving Klebsiella species can be prevented through specific infection control measures. These involve strict hygienic practices in the selection and handling of feed stuff, preparation of feed and storage. The feed should be kept free from crawling insects and rodents which could be agents of contamination. Birds should be housed in a good ventilated pen with overcrowding highly avoided to reduce contact with infected chicken in the same pen.

Animal handlers should maintain strict hand washing practices and disinfection before and after handling birds in each pen. Adequate biosecurity measures should be carried out in all procedures in the farm.

To protect themselves and their families and also to avoid becoming infecting agent to the animals, animal handlers should encourage the use of hand gloves and ensure hand washing and disinfection:

- ✓ before preparing or eating food
- ✓ before touching their eyes, nose or mouth
- ✓ before and after all animal procedures
- ✓ after using restroom
- ✓ after blowing their nose, coughing and sneezing
- ✓ after touching farm surfaces and instruments like doorknobs, shovels, drinkers, feeders, live or dead chicken etc.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Location of study

This study was carried out over a period of four months in Imo State, which is located at the center of the South Eastern Zone of Nigeria. The coordinates are:

Latitude $4^0$45'N- $7^0$15'N

Longitude: $6^0$50'E- $7^0$25'E.

Also known as the Eastern Heartland, Imo State shares boundaries with Anambra to the North, Rivers State to the South, River Niger & Delta State to the West and Abia to the East (until 1991 was part of Imo State). The vegetation is a typical rainforest type with two seasons; rainy (April – October) and dry (November – March). The annual mean rainfall is 2,500mm with a temperature range of 25 -35^{0}C and humidity of 70 -80% (Okoli 2004, Wikipedia 2016).

3.2 Sample collection

Feeds: – Feed samples of broiler starter, broiler finisher, growers and layer diets were randomly and aseptically collected from each three commercial feed brands labelled TFB, VFB and GFB. A set of feed sample comprising of BS, BF, LM, GM samples were collected from the three zones of the State (Orlu, Owerri and Okigwe) from randomly selected feed sellers. In each zone, six collection centres were randomly selected. In each collection centre, samples of each feed type of the different feed brands were collected by collecting 0.5 kg

each, from four different bags, thoroughly mixed together, and then 0.5 kg was weighed out of the mix. The collected samples were properly labeled and transported aseptically to the laboratory for analysis.

Table 3.1 Distribution of samples collected

VFB

No of feed samples	Zone A (Okigwe)	Zone B (Orlu)	Zone C (Owerri)		
6	BS	BS	BS	2	
6	BF	BF	BF	2	
6	GM	GM	GM	2	
6	LM	LM	LM	2	
		8		8	8

TFB

No of Feed Samples	Okigwe (Zone A)		Zone B (Orlu)		Zone C (Owerri)	
6	BS	2	BS	2	BS	2
6	BF	2	BF	2	BF	2
6	GM	2	GM	2	GM	2
6	LM	2	LM	2	LM	2
24		8		8		8

GFB

No of Feed Samples	Okigwe (Zone A)		Zone B (Orlu)		Zone C (Owerri)	
6	BS	2	BS	2	BS	2
6	BF	2	BF	2	BF	2
6	GM	2	GM	2	GM	2
6	LM	2	LM	2	LM	2
24		8		8		8

BS = Broiler Starter diet; BF = Broiler Finisher ; GM = Growers' mash
VFB = Vital Feed Brand; TFB = Top Feed Commercial Brand ; GFB = Guinea Feed Brand
Grand total

Feed ingredients: – Six samples of each feed ingredient were randomly and aseptically collected in each of the three zones from selected marketers. In each collection centre, 0.5 kg was weighed out from four different bags as was done of feed sample collection. After a thorough mix, 0.5 kg was weighed out. Feed ingredient samples collected were palm kernel cake (PKC), sorghum and maize (energy and fiber materials) and maize offal, blood meal, groundnut cake (GNC), soya bean meal (SBM) and fish meal (protein materials). Collected samples of feed ingredients were properly labeled and aseptically transported to the laboratory for analysis.

Faecal sample: – faecal samples were aseptically collected in the three zones of the State from randomly selected farms, open markets (Relief market, Orie Okporo and Eke Okigwe) and local birds. Six replicate faecal dropping samples of each of broilers, growers, layers and local birds were collected with sterile swab stick from the cloaca, labeled accordingly and taken to the laboratory for analyses.

3.3 Culture and isolation of *Klebsiella spp*

The laboratory determination of the samples was carried out at the microbiology laboratory of the Imo State Environmental Protection Agency (ISEPA) Owerri. Ten grams of each sample were dissolved in 100 ml of sterile distilled water and allowed to stand for about 30 minutes. A loop full of the diluent from each sample was plased on MacConkey agar plates and streaked out to get pure culture and incubated at 37^0C for 24 hours. After incubation, the samples were observed for colour. The pink coloured colonies showing lactose fermentation were subcultured on eosin methylene blue agar to differentiate between *Klebsiella species* and *E. coli* species. The presumptive *Klebsiella species* were stocked from where antimicrobial susceptibility testing was carried out.

3.4 Antimicrobial Susceptibility Tests

Antimicrobial sensitivity tests were carried out on the Klebsiella isolates using the Disc Diffusion method (Bauer *et al*., 1966). The isolates were put into Nutrient Broth Medium and incubated at 37^{o}C for four hours and transferred to the Mueller-Hinton Agar plates (NCCLS, 1999 now known as Clinical and Laboratory Standards Institution (CLSI)). The plates were then incubated at 37^{o}C for 24 hours with the antibiotics susceptibility disc carefully placed on the surface of the plate. After 24 hours, the antibiotic resistance patterns were noted. The antibiotic susceptibility Disc used is a product of Optu Laboratories Nig. Ltd.

3.5 Antimicrobial agents tested

The Klebsiella isolates were tested for antimicrobial resistance against the following antimicrobial agents;

Oxafloxacin...10mcg; Perfloxacin 10mcg; Ciprofloxacin 10mcgm; Nalidixic acid 30mcg; Gentamycin 10mcg; Streptomycin 30mcg; Augumentin 30mcg; Ampicilin 30mcg; Cephalexin 10mcg Trimethoprim (Septrin) 30mcg

3.6 Experimental design and statistical analysis

The experiment was carried out in a Completely Randomized Design (CRD). The data collected were subjected to analysis of variance (ANOVA) to determine significant differences among treatment means according to Steel & Torrie (1980). Where there were significant differences between means, the

means were separated using the Duncan's Multiple Range Test. The standard error of means was calculated as follows:

$$SEM = SD/\sqrt{N}$$

Where,

SEM = Standard error of mean

SD = Standard deviation

$\sqrt{N}$ = Square root of the population size.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Frequency of isolation of Klebsiella species from different poultry feed sources

4.1.1 Frequency of isolation of Klebsiella species from commercial feeds

The result of the occurrence of *Klebsiella species* in commercial feeds are presented in table 4.1. From the result, 91 (75.8%) samples were positive for *Klebsiella species* after isolation procedures. Out of these, 25, 30 and 28 samples respesenting 62.5, 82.5 and 82.5% occurrence were recorded for TFB, VFB and GFB feed brands respectively.

Out of the 25 isolates from TFB, 8, 6, 6 and 5 isolates respresenting 80, 60, 60 and 50% were isolated from broiler stater (BS), broiler finisher (BF), growers' mash (GM) and layers mash (LM) respectively (Table 4.2).

Of the 30 isolates from VFB brand, 10, 8 and 12 isolates respresenting 100, 80 and 80% were isolated from BS, BF and GM respectively. There was no LM sampled from this brand, so there was no isolates from the feed type (Table 4.3).

Out of the 28 isolates from the GFB brand, 14, 6 and 8 isolates representing 93.3, 60 and 80% were isolated from BS, BF and LM respectively. There were no samples of the GM, therefore, there was no isolates from the feed type (Table 4.4).

The overall 75.8% occurrence of *Klebsiella species* in commercial feed brands in Nigeria is quite high indicating the need for appropriate biosecurity measures in the commercial feeds production process. The range of 80-100% occurrence in broiler starter rations against 50-80% in LM, GM and BF rations show that BS may contain more of the ingredient vectors of the *Klebsiella species.*

According to Jones (2005), the overall mission of feed formulation and manufacturing should be to provide customers with efficiently manufactured feeds that are correctly delivered to their facilities and should consistently contain the available materials required by animals for body maintenance, growth or reproduction, having considered the quality characteristics of the feed raw materials.

In Nigeria however, there is hardly any serious effort directed at evaluating and regulating the quality and hygienic status of animal feeds being released to the public (Uchegbu *et al*., 2008). This explains the high occurrence of Klebsiella in the tested feed samples which is supported by the earlier reports of Okoh *et al*., (2005), Nasrin *et al*., (2007) and Ezekiel *et al*., (2011).

Table 4.1: Overall frequency of occurrence of Klebsiella species in commercial feeds

Feed Brand	No. Sampled	No. (%) Isolated
TFB	40	25 (62.5)
VFB	35	30 (82.5)
GFB	35	28 (82.5)
Total	110	91 (75.8)

TFB = Top Feed Brand, VFB =Vital Feed Brand, GFB = Guinea Feed Brand

Table 4.2 Frequency of occurrence of Klebsiella species in different feed types of TFB

Feed Type	No. Sampled	No. Isolated	Percentage occurrence
BS	10	8	80
BF	10	6	60
GM	10	6	60
LM	10	5	50
Total	40	25	62.5

BS = Broiler starter; BF = Broiler finisher; GM = Grower mash; LM = Layer mash.

Table 4.3 Frequency of occurrence of Klebsiella species in different feed types of VFB

Feed Type	No. Sampled	No. Isolated	Percentage occurrence
BS	10	10	100
BF	10	8	80
GM	15	12	80
LM	-	-	-
Total	35	30	85.7

BS = Broiler starter; BF = Broiler finisher; GM = Grower mash; LM = Layer mash.

Table 4.4 Frequency of occurence of Klebsiella species in different feed types of GFB

Feed Type	No. Sampled	No. Isolated	Percentage occurrence
BS	15	14	93.3
BF	10	6	60
GM	-	-	-
LM	10	8	80
Total	35	28	80

BS = Broiler starter; BF = Broiler finisher; GM = Grower mash; LM = Layer mash

4.1.2 Frequency of isolation of Klebsiella species from Feed ingredients

The result of the frequency of isolation of *Klebsiella species* from feed ingredients is presented in table 4.5 and 4.6. From the result, 46.67% of the energy and fibre sources feed ingredients harboured *Klebsiella species* (table 4.5), while 57.6% of the protein source feed ingredients were positive for *Klebsiella species* (Table 4.6). Among the energy and fibre sources feed ingredients, 8 (40%), 10 (50%) and 10 (50%) of PKC, sorghum and maize respectively were positive for *Klebsiella species* while 10 (50%), 10(50%), 14 (70%) and 12 (60%) of the fish meal, soyabean meal, groundnut cake and blood meal feed ingredients were positive for *Klebsiella species* respectively.

These results when compared with those of finished feeds seemed to indicate that poor processing and handling during manufacturing may be responsible for the higher *Klebsiella* prevalence in the finished feeds. It is also interesting that the highest frequency of *Klebsiella* occurrence was recorded for GNC (70%) and blood meal (60%). These were probably the ingredients contributing to the higher occurrence rates observed in BS rations. The present result also support the earlier report high microbial contamination of feed raw materials produced in Nigeria (Okoli et al., 2005; 2006; 2007).

Table 4.5 Frequency of occurrence of *Klebsiella* species in energy and fibre source feed ingredients

Feed Ingredient	No. Sampled	No. Isolated	Percentage occurrence
PKC	20	8	40
GC(S)	20	10	50
Mz	20	10	50
Total	60	28	46.67

PKC = Palm kernel cake; GC(S) = Guinea corn(Sorghum); Mz = Maize

Table 4.6 Frequency of occurrence of *Klebsiella* species in protein source feed ingredient

Feed Ingredient	No. Sampled	No. Isolated	Percentage occurrence
F M	20	10	50
SBM	20	10	50
GNC	20	14	70
BM	20	12	60
Total	80	46	57.6

FM = Fish meal; SBM = Soya bean meal; GNC = Groundnut cake; BM = Blood meal;

4.1.3 Frequency of isolation of Klebsiella species from Poultry Droppings

The results of the frequency of isolation of *Klebsiella species* from poultry droppings are presented in table 4.7. From the result, 43 (43%) of the 100 samples were positive for *Klebsiella species*. From these, 7 (35%), 6 (30%), 10 (50%), 8 (40%) and 12 (60%) of the droppings from starter broilers, finisher broilers, growers, layers and local chicken respectively, had *Klebsiella species* isolated from them.

On overall comparison, *Klebsiella species* were highest isolated from VFB and GFB feed brands (82.5%) each and least isolated from poultry droppings (43%) (Figure I)

The higher occurrence of the organism in local chicken is expected since these are usually free range fowls that hardly receive any veterinary attention (Okoli et al., 2004). The relatively low occurrence recorded in broilers (30-35%) may be attributed to the effects of frequent antibiotic dosing used to control diseases in such poultry in Nigeria (Okoli et al., 2002).

The overall Klebsiella occurrence results show that commercial feeds act as vectors of the organism in farms more than feed ingredients. It is also probable that the high prevalence is driven by human contamination during feed preparation and handling. However, considering the high occurrence rates recorded in the feeds and feed ingredients, it is seems that the organism colonizes poorly in chicken or that the drug use habits of farmers help to keep

their prevalence low in any commercial chicken type population (Okoli, 2004). These results again highlight the critical importance of controlling micro-organism population in commercial feeds. While different treatments such as heating, acidification and irradiation have been used to control microbial population in commercial poultry rations in developed economies, such simple procedures are not practiced in Nigerian poultry feed industry.

Table 4.7 Frequency of isolation of *Klebsiella* species from poultry droppings

Poultry Type	No. Sampled	No. Isolated	Percentage occurrence
SB	20	7	35
F B	20	6	30
G	20	10	50
L	20	8	40
L C	20	12	60
Total	100	43	43

SB = Starter Broiler; FB =Finisher Broiler; G =Grower; L =Layer; LC =Local Chicken

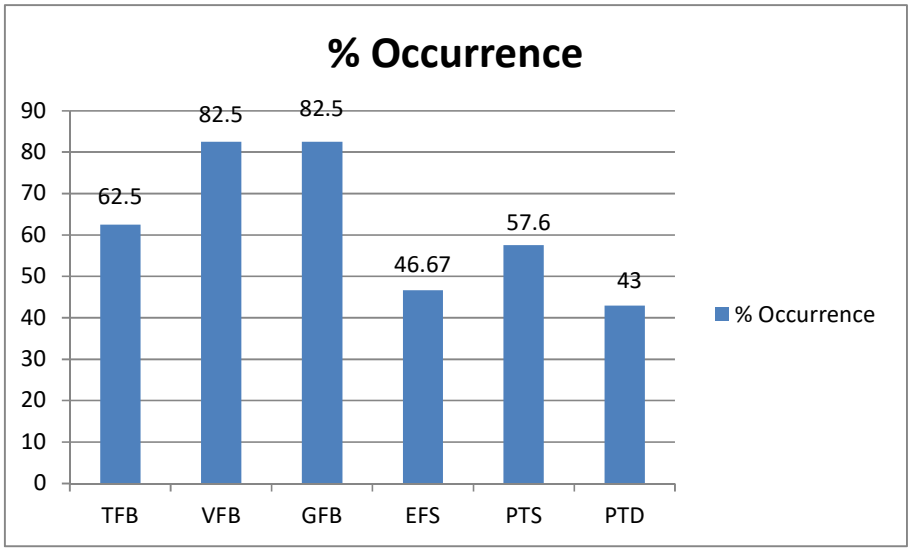

Fig. I: Comparison of frequencies of occurrence of *Klebsiella* species in the different sources

4.2: Resistance profile of Klebsiella species from commercial feeds, feed ingredients and poultry droppings

4.2.1 Commercial feeds

The Anti-microbial profile of the Klebisella isolates in feed brand, TFB exhibited varying degree of resistance of antimicrobial drugs tested (see table 4.8)

The Klebisella isolates in the TFB Feed brand showed a low to moderate degree of resistance from zero resistance (of Ciprofloxacin CPX) to 40% resistance (to CEP) cephalexin and Augumentin ((AU)

Isolates in the TFB feed brand from all the feed types exhibited zero resistance to ciprofloxacin (CPX). Isolates from the layer's mash exhibited zero resistance to all the antimicrobial agents tested while Klebisella species isolated from the other 3 (three) feed types showed varying degree of resistance to all other the antimicrobial agents with the exception of ciprofloxacin with the lowest rate of 12.5% (broiler starter diet) to the highest of 100% (from Grower's mash).

Klebisella isolates exhibited a resistance rate of 16% to the quinolone class of drugs (Ofloxacin, perfloxacin, and Nalidixic acid) with zero resistance to ciprofloxacin.

The isolates were very resistant to Augumentin (AU) Cephalexin (CEP) and Ampicilin (PN) at the rate of 28% (to Ampicilin) and 40% (Augumentin (AU) and Cephalexin (CEP) while the degree of resistance to (Gentamycin and streptomycin) and septrin was from 28% (Septrin) 24% (Streptmycin) 16%

(Gentamycin) ie Septrin (28% > steptomycin (24%) > Septrin . The resistance profile indicates that isolates in the Grower's Mash were highly resistant to the anti microbial agents (ranging from 50% to 100%) while the layers mash isolates were least resistant followed by Broiler Starter (12.5% to 50%) LM<BS<BF< GM.

VFB

The resistance profile of Klebisella profile of Klebisella is presented in **Table 4.9**

Samples of layer's mash of the VFB brand were not available both in the feed Distribution Centres or the Open Market.

The Klebisella isolates showed a moderate to high resistance of 66.67% to Ofloxacin (OfX) followed by 73.33% (Ciprofloxacin and Nalidixic acid) and 80% Perfloxacin.

Resistance by Augumentin (AU), Cephalexin (CEP) and Ampicilin (PN) ranged from 33.33% (to CEP) to 63.33% (Augumentin and Ampicilin) respectively.

On the other hand, Klebisella isolates exhibited moderate resistance of 46.67% and 56.67% respectively to streptomycin and Gentamycin and a high resistance of 80% to septrin.

GFB

The profile of the antimicrobial resistant isolates from GFB is presented in Table 4.10

The resistance profile of the Klebisella isolates in general showed a range in 42.80% to 75% samples of Growers mash (GM) were not available for the testing. On the whole the isolates exhibited a moderate resistance of 42.80% to 50% with a high resistance rate of 75% to Nalidixic acid (NA) against the Ofloxacin Perfloxacin and Ciprofloxacin while showing a high resistance of 60.71%, 71.43 and 67.86 (Augumentin, Cephalexin and Ampicilin respectively.

The Klebisella specices were high resistant to septrin and streptomycin at the rate of 75% while moderate resistance of 4286% was exhibited against Gentamycin.

Details of the profile reveal that isolates in the Broiler Finisher (BF) and layer's mash (LM) were highly resistant to the antimicrobial agents.

Comparison of antimicrobial resistance profile of Klebisella species in selected commercial feeds shows that the Klebisella in the TFB brand exhibited low resistance rates (below 50%) to Ciprofloxacin and 40% (to Augumentin and Cephalexin

While The VFB and GFB brands exhibited similar high resistance reaction to the following antimicrobial agents namely:

- Ampicillin (63.33%; 67.86%)
- Augumentin (63.33%; 60.71%)

- Nalidixic Acid (73.33% ; 75%)
- Septrin, SXT (80% ;75%) respectively.

Cephalexin (CEP) had moderately resistance rate to both TFB and VFB brands at 40% and 33 % respectively.

The resistance profile of the different brands as follows TFB < GFB < VFB This pattern shows that the TFB brand has the lowest resistance rate while the highest rate was exhibited by the VFB brand (37.5% & 59.1%)

Reasons for the high profile in the feed types and/or brands could be procurement of raw feed ingredients/materials from contaminated sources, contaminated processing equipment, lack of inadequate use of treatment methods (use of heat, acidification etc) (Nielsen, 1992, Haggblom, 1993; Dorey, 2001). In an earlier study of antimicrobial resistance of E. coli isolates from commercial feeds in the study area, Okoli et al (2005) reported the organism exhibited the highest resistance to Ampicillin (68.2%), Nalidixic Acid (54.6.%). The findings are moderate and similar to those reported by Uwaezuoke et al (2008) in their studies on other bacteria species and fungi as well as the study carried out by Ezekiel et al, 2011.

These highest the critical importance of commercial feed as vectors or resistance factors in poultry farms in Nigeria

4.2.2 Selected Feed Ingredients

Studies on the anti-microbial resistance profile of Klebsiella isolates from selected feed ingredients (Table 3) reveals that Klebsiella isolates from palm kernel cake (PKC), fish meal (FM), soya bean meal (SBM), groundnut cake (GNC), blood meal (BM), guinea corn (GC) and maize (M) recorded 100 % resistance to pefloxacin (PEF) and nalidixic acid (NA). Klebsiella isolates from FM, SBM, GNC, GC and M recorded higher resistance profile to oxfloxacin(OFX) than *Klebsiella* isolates from BM.

Zero resistance to ciprofloxacin resistance profile to oxfloxacin (OFX) than *Klebsiella* isolates from PKC which was higher than resistance profile of cin were recorded among *Klebsiella* isolates from PKC, GNC, BM and M, while high resistance to CPX were recorded for *Klebsiella* isolates from FM (100 %), SBM (80.00 %) and GC (70.00 %).

Klebsiella isolates from FM, SBM and BM recorded 100 % resistances to augumentin (AU), which were higher than those recorded for *Klebsiella* isolates from PKC (62.50 %), GNC (64.29 %), GC (70.00 %) and M (70.00 %), which were similar.

Resistance profile of Klebsiella isolates to cephalexin (CEP) were 100 % and higher in FM, SBM, BM, GC and M, than in PKC (62.50 %), and GNC (64.29 %) which were similar. *Klebsiella* isolates from FM, SBM, GNC and BM recorded 100 % resistance to ampicilin (PN), which were similar ($P>0.05$) to that recorded in Klebsiella isolates from PKC (87.50 %), but higher than

resistance profile of Klebsiella isolates from maize (50.00 %) which is higher than that recorded for guinea corn (0.00 %).

Klebsiella isolates from FM, SBM and BM recorded 100 % resistance to gentamycin (CN), which were higher than their resistance profiles recorded for PKC (62.50 %), GNC (64.29 %), GC (70.00 %) and maize (70.00 %), which were similar.

Streptomycin was 100 % resisted by *Klebsiella* isolates from FM, which was higher than resistance profiles of *Klebsiella* isolates from SBM (80.00 %), GNC (64.29 %), BM (66.67 %), GC (70.00 %) and maize (70.00 %). Klebsiella isolates from PKC recorded zero resistance to streptomycin.

Klebsiella isolates from FM and SBM recorded 100 % resistance to Septrin (SXT), which were higher than resistance profile of *Klebsiella* isolates from GNC (64.29 %), BM (66.67 %), GC (70.00 %) and maize (70.00 %), which were similar, but higher than resistance profile of *Klebsiella* isolates from PKC (37.59 %).

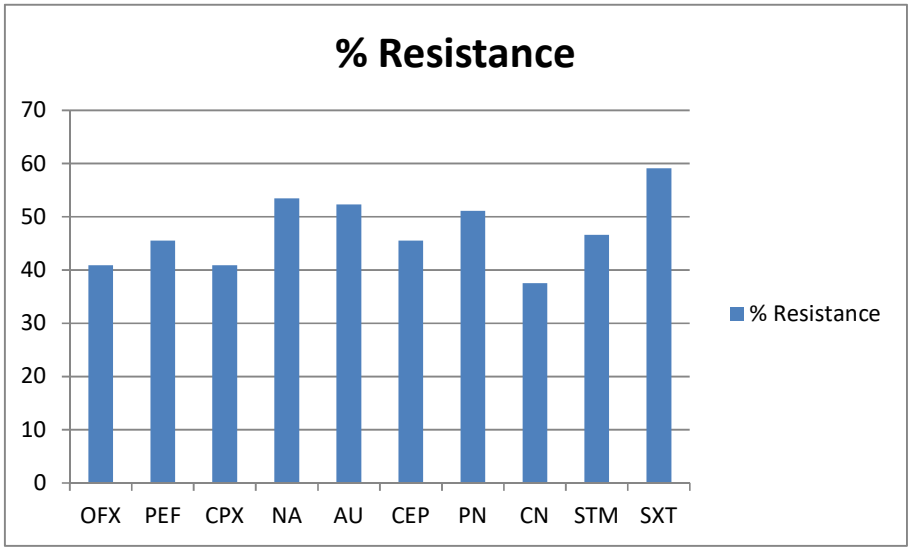

Fig. II: Overall mean antimicrobial resistance of Klebsiella isolates from commercial feeds

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin.

Table 4.8: Antimicrobial resistance profile of Klebsiella species from TFB

Antibiotics	No(%) of isolates resistant				
	BS	BF	GM	LM	TOTAL
OFX	4(50)	0(0)	0(0)	0(0)	4(16)
PEF	4(50)	0(0)	0(0)	0(0)	4(16)
CPX	0(0)	0(0)	0(0)	0(0)	0(0)
NA	4(50)	0(0)	0(0)	0(0)	4(16)
AU	1(12.5)	3(50)	6(100)	0(0)	10(40)
CEP	4(50)	0(0)	6(100)	0(0)	10(40)
PN	4(50)	0(0)	3(50)	0(0)	7(28)
CN	1(12.5)	0(0)	3(50)	0(0)	4(16)
STM	0(0)	3(50)	3(50)	0(0)	6(24)
SXT	1(12.5)	3(50)	3(50)	0(0)	7(28)

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin. BS = Broiler starter; BF = Broiler finisher; GM = Grower mash; LM = Layer mash.

Table 4.9: Antimicrobial resistance profile of Klebsiella species from VFB

Antibiotics	No(%) of isolates resistant				
	BS	BF	GM	LM	TOTAL
OFX	0(0)	8(100)	12(100)	-	20(66.67)
PEF	4(40)	8(100)	12(100)	-	24(80)
CPX	4(40)	8(100)	10(83.33)	-	22(73.33)
NA	4(40)	6(75)	12(100)	-	22(73.33)
AU	0(0)	7(87.5)	12(100)	-	19(63.33)
CEP	0(0)	0(0)	10(83.33)	-	10(33.33)
PN	0(0)	7(87.5)	12(100)	-	19(63.33)
CN	0(0)	7(87.5)	10(83.33)	-	17(56.67)
STM	7(70)	7(87.5)	0(0)	-	14(46.67)
SXT	7(70)	7(87.5)	10(83.33)	-	24(80)

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin. BS = Broiler starter; BF = Broiler finisher; GM = Grower mash; LM = Layer mash.

Table 4.10: Antimicrobial resistance profile of Klebsiella species from GFB

Antibiotics	No(%) of isolates resistant				
	BS	BF	GM	LM	TOTAL
OFX	0(0)	5(83.33)	-	7(87.5)	12(42.86)
PEF	0(0)	5(83.33)	-	7(87.5)	12(42.86)
CPX	0(0)	6(100)	-	8(100)	14(50)
NA	7(50)	6(100)	-	8(100)	21(75)
AU	5(35.71)	5(83.33)	-	7(87.5)	17(60.71)
CEP	7(50)	6(100)	-	7(87.5)	20(71.43)
PN	7(50)	5(83.33)	-	7(87.5)	19(67.86)
CN	0(0)	5(83.33)	-	7(87.5)	12(42.86)
STM	7(50)	6(100)	-	8(100)	21(75)
SXT	7(50)	6(100)	-	8(100)	21(75)

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin. BS = Broiler starter; BF = Broiler finisher; GM = Grower mash; LM = Layer mash

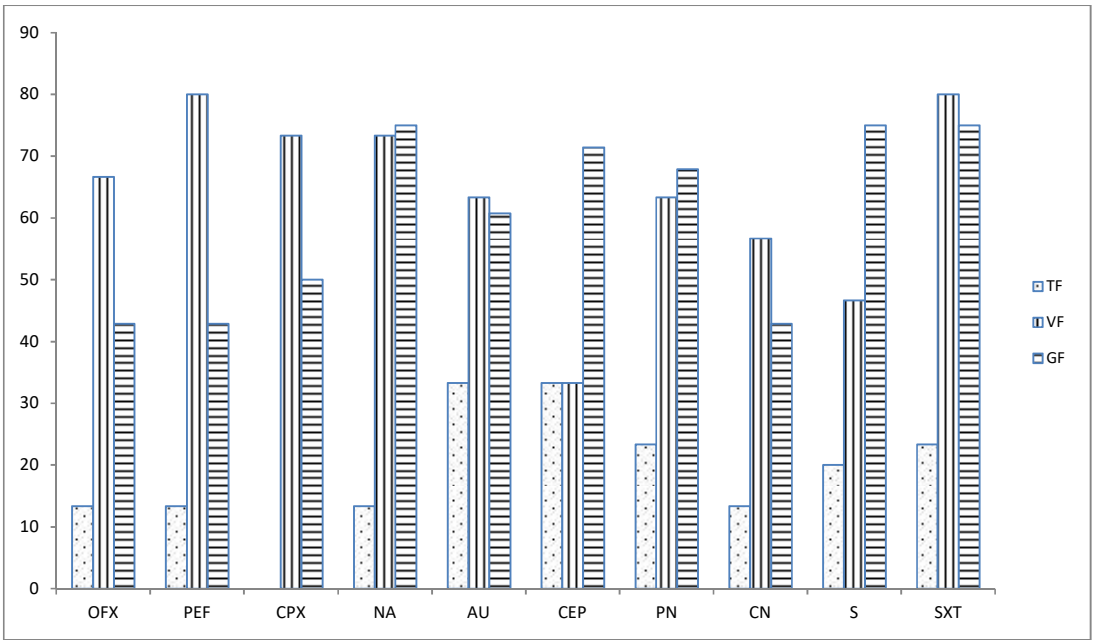

Fig III: Comparison of antimicrobial resistance profile of Klebsiella isolates from selected commercial feed.

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin. TF = Top feed; VF = Vital feed; GF = Guinea feed.

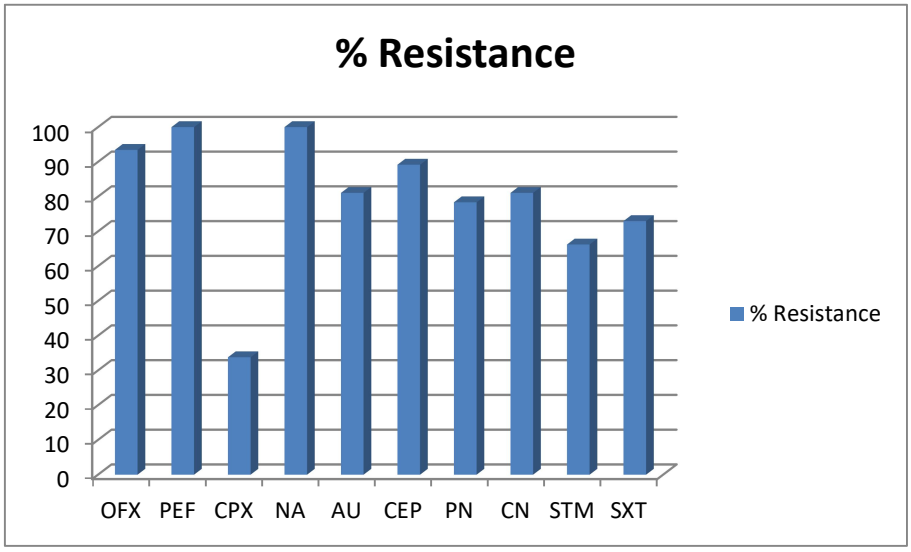

Fig. IV: Overall mean antimicrobial resistance profile of Klebsiella isolates from feed ingredient

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin. BS = Broiler starter; BF = Broiler finisher; GM = Grower mash; LM = Layer mash.

Table 4.11: Antimicrobial resistance profile of Klebsiella species from energy and fiber source feed ingredients

Antibiotics	No(%) of isolates resistant			
	PKC	GC	M	Total
OFX	7(87.5)	10(100)	10(100)	27(96.43)
PEF	8(100)	10(100)	10(100)	28(100)
CPX	0(0)	7(70)	0(0)	7(25)
NA	8(100)	10(100)	10(100)	28(100)
AU	5(62.5)	7(70)	7(70)	19(67.86)
CEP	5(62.5)	10(100)	10(100)	25(89.29)
PN	7(87.5)	0(0)	5(50)	12(42.86)
CN	5(62.5)	7(70)	7(70)	19(67.86)
S	0(0)	7(70)	7(70)	14(50)
SXT	3(37.5)	7(70)	7(70)	17(60.71)

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin. PKC = Palm kernel cake; FM = Fish meal; SBM = Soya bean meal; GNC = Groundnut cake; BM = Blood meal; GC = Guinea corn; M = maize. SEM = Standard error of mean.

Table 4.12: Antimicrobial resistance profile of Klebsiella species from protein source feed ingredient

Antibiotics	No(%) of isolates resistant				
	FM	SBM	GNC	BM	Total
OFX	10(100)	10(100)	14(100)	8(66.67)	42(91.3)
PEF	10(100)	10(100)	14(100)	12(100)	46(100)
CPX	10(100)	8(80)	0(0)	0(0)	18(39.13)
NA	10(100)	10(100)	14(100)	12(100)	46(100)
AU	10(100)	10(100)	9(64.29)	12(100)	41(89.13)
CEP	10(100)	10(100)	9(64.29)	12(100)	41(89.13)
PN	10(100)	10(100)	14(100)	12(100)	46(100)
CN	10(100)	10(100)	9(64.29)	12(100)	41(89.13)
S	10(100)	8(80)	9(64.29)	8(66.67)	35(76.1)
SXT	10(100)	10(100)	9(64.29)	8(66.67)	37(80.43)

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin. PKC = Palm kernel cake; FM = Fish meal; SBM = Soya bean meal; GNC = Groundnut cake; BM = Blood meal; GC = Guinea corn; M = maize. SEM = Standard error of mean.

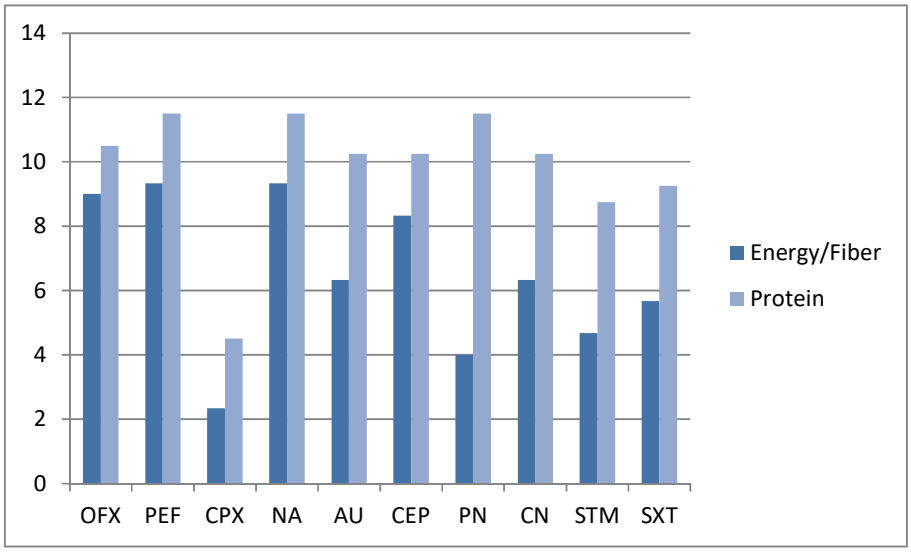

Fig. V: Comparison of mean antimicrobial resistance profile of energy/fiber and protein feed sources

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin.

The comparison of average resistance profile of Klebsiella isolates from selected feed ingredients in Nigeria are presented in figure V. The resistance profile of Klebsiella isolates from the feed ingredients followed the trend of FM > SBM > BM > GC > M > GNC > PKC.

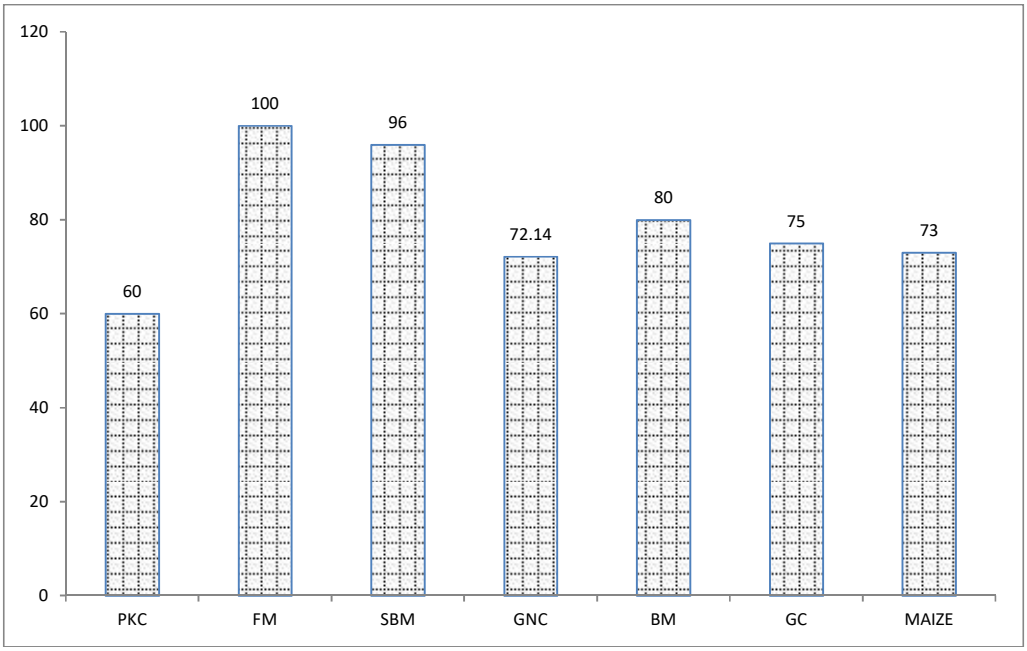

Fig. VI: Overall mean antimicrobial resistance profile of feed ingedients PKC = Palm kernel cake; FM = Fish meal; SBM = Soya bean meal; GNC = Groundnut cake; BM = Blood meal; GC = Guinea corn; Mz = Maize

4.2.3 POULTRY DROPPINGS

The antimicrobial resistance profile fecal droppings are presented on Tables 4:13-4-14. (Table 4:13 and Table 4:14

The different sources of Poultry Dropping were from broiler starter birds, broiler finisher Grower and layer birds as well as from local birds.

The Klebsiella Isolates from fecal droppings of broilers (both broiler and starter and broiler finisher) exhibited a high resistance profile ranging 66.67% (to Augmentin) to 100% resistance. The Isolates were highly resistant to the following drugs: Ofloxacin Pefloxacin Nalidixic acid, Amplicillin and Gentamycin

The profile of droppings from the growers and layers showed a moderate to high resistance of ranging from 50% to 100% for Growers and for the Layers, the lowest value of 37.5% and the highest of 100%. Both birds types exhibited very high resistance to Nalidixic acid and Septrin.

The Antimicrobial resistance profile of fecal droppings from local birds as represented in Fig vlll shows that the klebsiella isolates were highly resistant to Ciprofloxacin- Ampicillin-Oxfloxacin as well as Ampicillin at rate of 100% and least resistant to Septrin at the rate of 50%. The Isolates displayed zero resistance to Ciprofloxacin CPX (Cephalexin, CEP) and Streptin.

All the Isolates from all the sources of poultry dropping were highly resistant to Nalidixinc acid with a rate of 100% resistance. They were also resistant to Septrin at the rate of 50% (local chicken), 71.43% (Broiler starter), 66.67 (Broiler finisher and 100% (Growers and Layers) and Ampilcillin, 37.5% (Layers), 100% (local chicken, Broiler starter and finisher and Grower).

The antibiotic resistance in all the Isolates could be explained in term of usage in the study location.

The low resistance may be due to the fact that those antibiotics may not be in common use. The high prevalence of resistance could be attributed to heavy dependence of these antibiotics for therapeutic and sub- therapeutic uses in poultry. The prevalence of multi antibiotic resistance Isolates appear to be higher in poultry than in local chicken as a result of heavier dosing of the broilers to enhance weight gain and for therapeutic purposes compared to local chicken that are rarely given antibiotics.

The findings are in consonance with other studies that confirm the high incidence of antibiotic-resistant bacteria recovered from poultry linked to heavy dependence of antibiotics for therapeutic and sub- therapeutic uses in poultry. (Okoli, 2004, Okoli, 2006, Ajayi et al

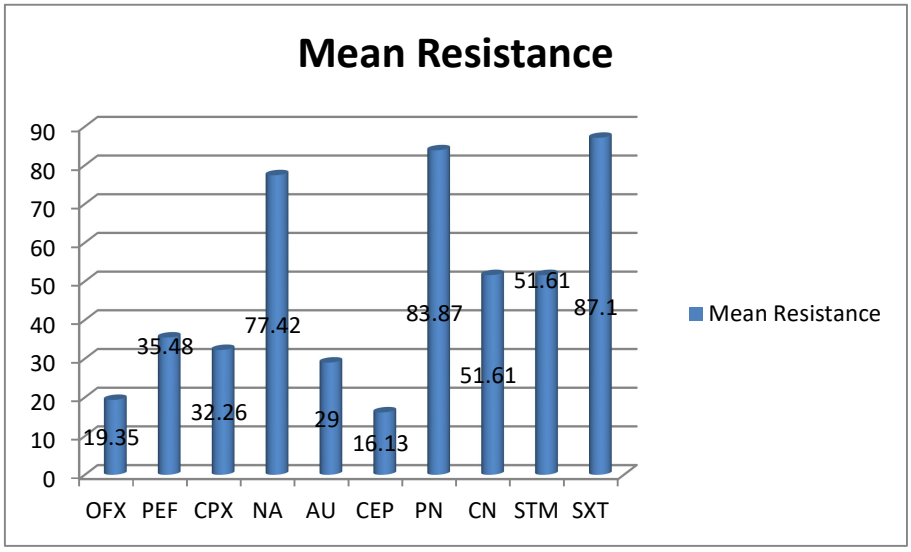

Fig. VII: Overall mean antimicrobial resistance profile of Klebsiella species from poultry droppings

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin.

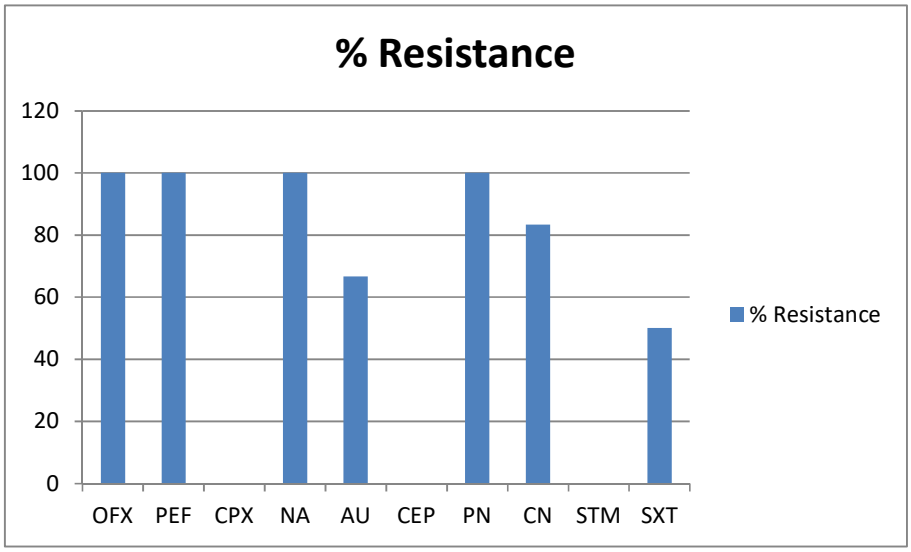

Fig. VIII: Overall mean antimicrobial resistance profile of Klebsiella species from local chicken

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin

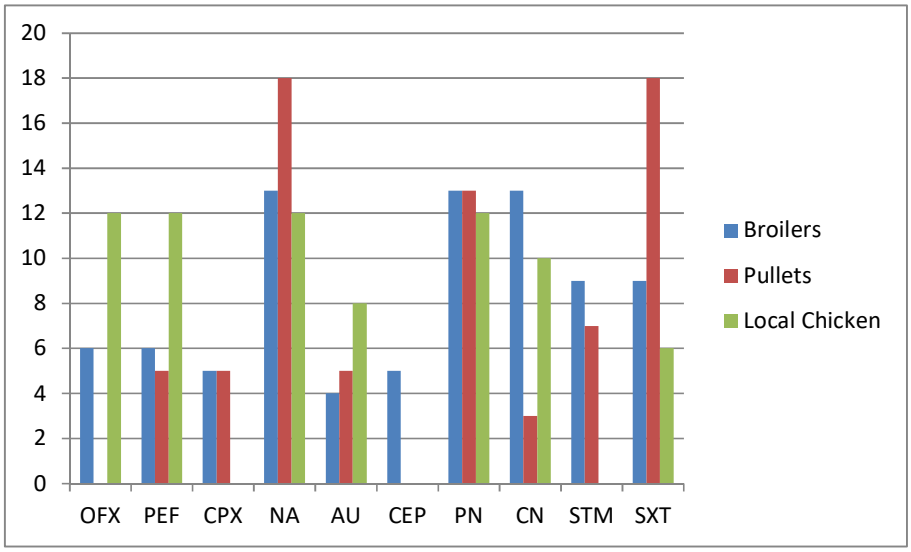

Fig. IX: Comparison of resistance profile of Klebsiella species from broilers, pullets and local chicken

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin.

Table 4.13 Antimicrobial resistance profile of Klebsiella species from poultry droppings of broilers

Antibiotics	No(%) of isolates resistant		
	Starter	Broiler	Total
OFX	0(0)	6(100)	6
PEF	0(0)	6(100)	6
CPX	5(71.43)	0(0)	5
NA	7(100)	6(100)	13
AU	0(0)	4(66.67)	4
CEP	5(71.43)	0(0)	5
PN	7(100)	6(100)	13
CN	7(100)	6(100)	13
STM	5(71.43)	4(66.67)	9
SXT	5(71.43)	4(66.67)	9

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin

Table 4.14 Antimicrobial resistance profile of Klebsiella species from poultry droppings of growers and layers

Antibiotics	No(%) of isolates resistant		
	Growers	Layers	Total
OFX	0(0)	0(0)	0
PEF	0(0)	5(62.5)	5
CPX	0(0)	5(62.5)	5
NA	10(100)	8(100)	18
AU	5(50)	0(0)	5
CEP	0(0)	0(0)	0
PN	10(100)	3(37.5)	13
CN	0(0)	3(37.5)	3
STM	7(70)	0(0)	7
SXT	10(100)	8(100)	18

OFX, Ofloxacin; PEF, Perfloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin. SEM = Standard error of mean.

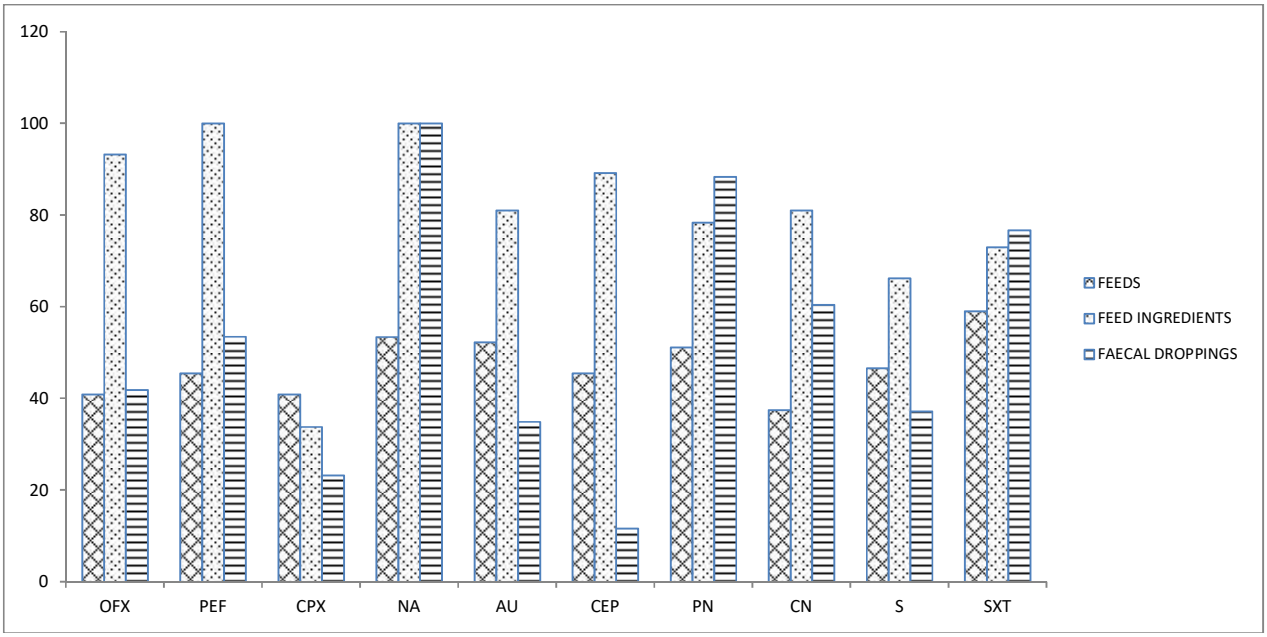

Fig. X: Comparism of total resistance profiles of Klebsiella isolates from selected feeds, feed ingredients, and poultry faecal droppings.

OFX, Ofloxacin; PEF, Pefloxacin; CPX, Ciprofloxacin; NA, Nalidixic acid; AU, Augumentin; CEP, Cephalexin; PN, Ampicilin; CN, Gentamycin; S, Streptomycin; SXT, Septrin.

CHAPTER 5

5.0 CONCLUSSION AND RECOMMENDATION

5.1 CONCLUSSION

This study has demonstrated that commercial feeds, feed ingredients and faecal droppings are vehicles through which multi-drug resistant Klebsiella species can be disseminated in the poultry farm. It also reveals that the different feed types and brands do not pose same level of danger in relation to dissemination of multi-drug resistant Klebsiella .

5.2 RECOMMENDATION

It will be of great help in controlling the spread of pathogenic and multi-drug resistant Klebbsiella species if maximum hygiene is practiced in livestock farms, especially in the handling of feeds and disposal of wastes. Commercial feed millers in Nigeria should ensure proper screening of their feed raw materials, and also implore appropriate treatments such as heating and acidification (Nape and Murphy, 1971; Cox *et al*., 1986) in order to reduce bacterial load such as Klebsiella species in feeds.

CONTRIBUTION TO KNOWLEDGE

The status of Klebsiella organisms as a contaminant of poultry feed, feed ingredients and poultry environment in Imo State has not been documented. This study has therefore closed the gap in knowledge about the status of Klebsiella species as a possible contaminant of poultry feed, feed ingredients and poultry environment. It has also exposed the else-while unknown prevalence of antimicrobial resistant Klebsiella species in poultry feed, feed ingredient and poultry environment which poses danger to the poultry industry, other animals and public health.

The study also highlighted the susceptibility of these organisms to commonly used antibiotics thereby guiding animal health of these organisms to guiding animal health officers and veterinarians on the best line of treatment in the eventful occurrence of Klebsiella infection of poultry in Imo State.

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