PREVALENCE OF OXYTETRACYCLINE RESIDUE AND RESISTANT *ESCHERICHIA COLI* O157:H7 IN BEEF AND CHICKEN IN SELECTED CITIES OF SOUTH WESTERN NIGERIA

BY

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ABSTRACT

The use of antibiotics in livestock production is of food safety concern due to hazards of their residues and transfer of resistant bacteria along the food chain. There are few reported quantitative assessment of meat-borne antibiotic residues and resistant pathogens in southwestern Nigeria. Antibiotics usage in food animal production, antimicrobial residues screening, prevalence of *Escherichia coli* O157:H7 and levels of oxytetracycline residue in chicken and beef from Lagos, Ibadan and Akure were investigated.

A semi-structured questionnaire was administered to 30 poultry and 20 cattle producers purposively selected from the study areas to obtain data on types and sources of antibiotics used, knowledge of disease recognition and practice of withdrawal periods in food animals. Two hundred and fifty samples each of kidney, liver and muscles of slaughtered cattle from one abattoir and 200 samples each of breast muscle and liver from chicken markets and broiler farms in each study area were collected between January 2006 and December 2009. The samples were screened for antimicrobial residues using rapid microbial inhibition assay. Oxytetracycline residue levels were determined by high performance liquid chromatography. *Escherichia coli* O157:H7 was isolated and screened by culture and latex agglutination respectively. Antibiotics susceptibility of the isolates was performed using multi-disc diffusion method. Data were analysed using descriptive statistics and ANOVA at $p<0.05$. The residue levels were compared with Codex Alimentarius Maximum Residue Limits (MRLs) and residue prevalence in beef during the wet and dry seasons were also compared.

All the respondents administered antibiotics without veterinary prescription, with oxytetracycline being the most commonly used. Most (95.0%) of the producers never observed withdrawal periods and 75% of them did not know the importance of withdrawal

periods and hazards of antibiotic residues. Antibiotic residues prevalence in beef was 48.5%, 44.5% and 44.5% in Ibadan, Lagos and Akure respectively. In chicken, prevalence of 76.0% and 69.5% were obtained in Ibadan markets and farms compared to 70.0% and 61.0% in Lagos markets and farms respectively. Mean oxytetracycline residue concentrations of 1324.7±148.0, 856.6±118.0 and 651.7±101.3µg/kg were obtained in bovine kidney, liver and beef respectively with 37.8, 40.3 and 47.5% of these samples containing residues above MRLs. The levels in chicken liver and muscle were 1042.0±122.8 and 615.0±91.8µg/kg respectively of which 50.7% and 58.8% contained residues above MRLs. The prevalence of antimicrobial residue was significantly higher in chicken than in beef and during wet than dry season in beef. The prevalence of *E. coli* O157:H7 in beef from Ibadan and Lagos were 28.5% and 11.0%, while those of chicken from Ibadan and Lagos markets were 13.0% and 14.0%, and from Ibadan and Lagos farms were 18.0% and 13.0% respectively. All the isolates were resistant to one or multiple antibiotics, but the highest resistance of 91.1% was to tetracycline.

Indiscriminate antibiotics usage predisposes meat consumers to risks of antibiotic residues and resistant *Escherichia coli* O157:H7 in southwestern Nigeria. Regulatory control of antibiotics usage in livestock production, meat inspection and pharmaco-epidemiological surveillance of food animals is hereby recommended to ensure safe meat supply.

Key words: Antibiotic usage, Chicken, Beef, Oxytetracycline residue, *Escherichia coli* O157:H7

Word Count: 496.

DEDICATION

The work is dedicated to the Almighty God for the beautiful world he gives us and the power to dominate the creations including food animals to live well. To HIM be all the glory.

CERTIFICATION

This is to certify that this work was carried out by

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AT

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ADI	Acceptable Daily Intake
CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
CCP	Critical Control Point
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
EUCVM	European Union Center for Veterinary Medicine
FAO	Food and Agriculture Organization of the United Nations
FDA	US Food and Drug Administration
GAP	Good Agricultural Practice
GATT	General Agreement on Trade and Tariff
GDP	Gross Domestic Product
GVP	Good Veterinary Practice
HPLC	High Performance Liquid Chromatography
HACCP	Hazard Analysis Critical Control Point (System)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meetings on Pesticide Residues
LC-MS	Liquid chromatography – mass spectrometry

MRL	Maximum residual level
MRL	Maximum Residue Limit
MRLVD	Maximum residual level of veterinary drug
NAFDAC	National Agency for Food and Drug Admnistration and Control
OIE	Office International des epizooties/International Office of Epizooties
OTC	Oxytetracycline
SPS	Sanitary and phytosanitary measures
TBT	Agreement Agreement on the Technical Barriers to Trade
WHO	World Health Organization
WTO	World Trade Organization
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, Denmark
NARMS	National *Antimicrobial* Resistance Monitoring System, USA
JVARM	Japanese Veterinary Antimicrobial Resistance Monitoring System, Japan,
MARAN	Monitoring Antimicrobial Resistance And Antibiotic Usage Animals, The Netherlands
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance,
IIMAR	Indian Initiative for Management of Antibiotic Resistance,

SVARM	Swedish Veterinary Antimicrobial Resistance Monitoring
NORM-VET	Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance Monitoring in Norway

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Food security comprising of food availability and safety are major global challenges of the 21st century. The 1996 world food summit was concluded with declaration that "all countries of the world should rise up to the challenge of hunger eradication to feed the growing human population" (FAO, 1996). Nigerian livestock production is an integral part of agriculture contributing about 7% to the nation's Gross Domestic Product (Blench, 1999). The pressure of food demand in quantity and quality sought by consumers require a variety of intensive technologies and large scale agricultural production including livestock husbandry which provides animal protein in human diet.

Drugs, vaccines and some chemicals are employed in livestock to combat the challenges of infectious organisms and to enhance productivity. Commercial rearing of animals for food depends on the use of pharmacologically active compounds (drugs) which are beneficial to health, well-being and economics of the livestock industry. The five major classes of drugs used in food animals include: antibiotics; antiparasitic drugs; steroid anabolic growth promoters; ionophores; and topical antiseptics. Antibiotics are used in livestock prophylactically and therapeutically to combat infections by bacteria and also to improve productivity and as growth promoter antibiotics (GPA). Since the first discovery of penicillin by Alexander Fleming in 1928 (Fleming, 1929) and its subsequent clinical importance in the 1940s, antimicrobial agents have played revolutionary role in human and animal health (Abraham and Chain, 1940; Abraham *et al.*, 1941). The reported benefits of drug use in livestock result from the maintenance of good animal health thereby reducing the chance that pathogenic agents will spread to humans from animals.

Following the administration of these drugs to food animals, residues of the parent drugs or their metabolites may be deposited in edible portions (meat milk and eggs) of such animals (Schmidt and Rodrick, 2003). The potential health hazards of drug residues in food were categorised into three namely toxicological, microbiological and immunological effects (Van Dresser and Wilke, 1989). Oxytetracycline, streptomycin, penicillin and sulphamethazine have been reported as the most frequently used and detected antibiotics in tissues food animals (Van Dresser and Wilke, 1989; Rivere and Spoon, 1995; Phillips *et al.*, 2004; Posyniak *et al.*, 2005). Concerns on public health risks of residues in animal foods began with the linkage identified between outbreak of resistant bacterial infections in the people that consumed calves and milk with antibiotic residue in U. K. in 1960. Swann committee subsequently recommended stringent control of the use of antibiotics in food producing animals to protect public health (Swann *et al*., 1969).

A major public health concern about the presence of antibiotics residues in food is their contribution to development, spread and transfer of antibiotic resistant bacteria and genes along the food chain. There is also concern about continued dissemination and spread of antibiotic resistance from the co-mingling of antibiotic resistant bacteria with the natural micro-flora in animal and human gut leading to the disruption of gastrointestinal microbial ecology. This could results in food-borne zoonoses caused by multi-drug resistance (MDR) pathogens resulting from the selective pressure exerted by the exposure to antibiotics (Alanis, 2005; Kumai *et al*., 2005).

Other reported health hazards of antibiotics residues in food chain when consumed at violative levels include allergic or hypersensitivity reactions, carcinogenic, mutagenic and toxic effects in man (Dewdney *et al*., 1991; Berends *et al*., 2001; Nisha, 2008). Economic loses also arise from meat, milk and eggs condemnations at national and international trade levels when residues are detected at violative levels. Furthermore, milk contaminated with

even low concentrations of antimicrobial drug residues also create technological difficulties in the production of fermented milk products by inhibiting the growth of the starter cultures leading to economic loss in dairy industry (Heeschen and Blüthgen, 1991).

Developed nations are employing strict regulatory monitoring of antibiotics use and residues in order to safe guard against the associated health hazards (van den Bogaard, 1998). However, antibiotics are also widely and indiscriminate use in developing countries but the quantities being used are difficult to obtain due to lack of regulatory control of their uses (Mitema *et al*., 2001; Al Mustafa and Al Ghamdi, 2002). International, national and regional efforts in setting residues safety standards: maximum residue limits (MRLs) and acceptable daily intake (ADI) for the various drugs and chemicals used in livestock production are ongoing and are major requirements in food exports. Global food trade with little or no tariff and barrier (including drug residues) is a primary objective of the General Agreement on Tariffs and Trade (GATT) 1994 culminating in establishment of World Trade Organization (WTO) on January 1, 1995 with Codex Alimentarius Commission (CAC) as the international standard food safety regulatory body. The Codex Alimentarius Commission is a joint committee of Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) that sets and recommends food safety standards (codex) to promote international trade. Withdrawal periods are also established as the time required for the residue of toxicological concern to reach safe concentration before slaughtering of treated animals or harvesting milk and eggs for human consumption.

Veterinarians and livestock producers have ethical responsibility of prudent use of drugs in livestock and are expected to observe the withdrawal periods prior to the slaughtering of animals or harvesting of milk and egg for human consumption. When drug manufacturers and national legislative directions are followed by livestock producers and the veterinarians, drug residue levels in food of animal origin are expected to be within safe limits. But where levels

of residue exceed permitted maximum limits, they are usually caused by improper use and as such food should legally not be allowed into the food system. More so, carcasses of such animals can be carriers of food borne pathogens including *E. coli* that could be resistant to the antibiotics, thereby exposing the consumers to the associated public health risks of antibiotic residues and bacterial resistance. Several authors have isolated resistant foodborne pathogens from meat, in some part of Nigeria (Aibinu *et al*., 2007; Umolu, *et al*., 2006; Okeke *et. al.*, 2007).

This study therefore was aimed at investigating the practice of drug administration in poultry and cattle production and quantifying residues of oxytetracycline in the chicken and beef meant for human consumption in selected cities of south western Nigeria. Also the prevalence and antibiotic resistance patterns of *Escherichia coli* O157:H7 isolated from the meat was also investigated.

1.2 Research questions

(i) What are the patterns of veterinary drug usage in cattle and poultry production?

(ii) What is the prevalence of antibiotic residues in beef and chicken being consumed in selected cities of southwest Nigeria?

(iii) What are the levels of oxytetracycline residues in beef and chicken meant for human consumption in Akure, Ibadan and Lagos?

(iv) What is the prevalence of *Escherichia coli* O157:H7 contamination and its antibiotics resistance pattern in beef and chicken across the cities?

1.3 Objectives

The overall objective of this study is to determine the wholesomeness of beef and chicken with respect to the deposition of residue of oxytetracycline and contamination with *Escherichia coli* O157:H7 of meats from selected cities of southwest Nigeria

The specific objectives are:

(i) To assess the culture (i.e. knowledge, attitude and practice) of poultry and cattle producers in southwest Nigeria on the administration of antibiotics to their livsetock.

(ii) To determine the prevalence of antibiotic residues in beef and chicken in the cities of Ibadan, Akure and Lagos.

(iii) To quantify the levels of oxytetracycline residues in beef and chicken from Ibadan, Akure and Lagos.

(iv) To determine the prevalence and antibiotics resistance patterns of *E. coli* O157:H7 contamination of the beef and chicken samples from the cities.

1.4 Justifications

Beef and chicken have wide consumer acceptability and they constitute more than 60% of meat consumption in Nigerian cities. These meats are obtained mostly from commercial poultry and semi-intensive cattle production. Veterinary drugs including antibiotics are commonly administered to these animals indiscriminately.

Antibiotic use in food animals is of global and public health concerns and also a critical regulatory function of the codex alimentarius commission for SPS regulations in World Trade Organisation (WTO) to which Nigeria subscribes. Compliance with such international standards will ensure participation in international food and meat trade. There is no

regulatory monitoring of the use of veterinary drugs, no national antibiotics residues and resistance monitoring or surveillance and there are no residue limits (MRL or ADI) set for veterinary drugs in foods in Nigeria (Aliu, 2004). Previous studies in Nigeria have shown qualitative presence of antibiotics residues in meat. There is need for quantitative analysis of residues in theses food using more sensitive and specific detection methods such as HPLC.

In addition, meat-borne bacteria due to contamination of the meat with fecal material or ingesta during the slaughtering process have been repeatedly isolated with *E. coli* as an indicator organism. These bacteria are also resistant to most commonly used antibiotics and can subsequently be transfer to human beings via the food chain. *E. coli* O157:H7 is one of the emerging food-borne pathogens that have been isolated from beef and chicken worldwide. Consequent to the practices of floor slaughtering and meat processing in most abattoirs in Nigeria there is the need to investigate the prevalence of meat contamination with such zoonotic pathogens and its antibiotics resistance patterns.

The results from this study would supply the hitherto unavailable baseline information on the quantitative evaluation of oxytetracycline residue and associated resistant *E. coli* O157:H7 in the meat commonly consumed in Nigerian cities on which risk assessment of antibiotics use in food animals in Nigeria could be based. The knowledge of the residue levels in such meat and its control would aid Nigeria's participation in international meat trade under the WTO.

1.5 Study hypotheses

(i) H_O1: There is no prudent and responsible use of antibiotics in poultry and cattle production in southwest Nigeria.

(ii) H_O2: The use of antibiotics in poultry and cattle cannot lead to the presence of high levels of residue of antibiotics in meat.

(iii) H_O3: The prevalence of antibiotic residues in beef and chicken in southwest Nigeria are not significantly different.

(iv) H_O4: There is no significant difference in the prevalence of antibiotic residues in beef during dry and wet season.

(v) H_O5: The levels of oxytetracycline residue in beef during the wet and dry seasons are not significant different.

(vi) H_O6: There is no significant difference in the levels of oxytetracycline residue in beef and chicken in the different cities.

(vii) H_O7: The mean concentrations of oxytetracycline in beef and chicken in south western Nigeria are not above the codex maximum residue limits (MRLs).

(viii) H_O8: The prevalence of beef and chicken contaminations with *E. coli* O157:H7 across the cities abattoirs are not significantly different.

1.6 Description of study Area

The southwest Nigeria is one of the six geopolitical zones of Nigeria. The zone comprises of Lagos, Ogun, Oyo, Ondo and Ekiti States and is located within latitude $6^o\ 25^1$ to $8^o\ 03^1$N and longitude $3^o\ 02^1$ and $5^0\ 30^1$E. It comprised of tropical rainforest and derived savannah favouring commercial livestock production. Poultry production is the major commercial livestock farming constituting a significant portion of commercial poultry in Nigeria and more than 80% of the breeders stocks in Nigeria are reared in this region. Also agro-pastoral Fulanis who engaged in cattle rearing are scattered across the derived savannah zone of southwestern Nigeria where their animals enjoy the availability of green grass and water all year round. Lagos Ibadan and Akure are among the state capital cities in the south western Nigeria (figure 1.1) were selected to have a wide cosmopolitan coverage for this work based

on different population density and demand for meat. Poultry farming exist mostly at the peri-urban area and outskirts of these cities with some backyard holdings within the cities. Feed, equipments and veterinary inputs are located at wholesale and retail shops and feed mills in the cities. There is also a high concentration of government and private veterinarians in these cities. There are several wholesalers and retailers of livestock inputs and veterinary drugs in the city. Also central abattoirs (such as Araromi abattoir in Akure, Bodija abattoir in Ibadan and Oko-Oba abattoir in Lagos) exist along with slaughter slabs scattered within the cities and open meat retail markets where cattle and goats are regularly slaughtered.

1.7 Limitations of study

The kidney of chicken was not available for this study because it was not easily accessible for both screening and quantitative assays, Also chicken samples were not obtained from Akure because there were few broiler grow-out farmers and no chicken slaughter market in the city.

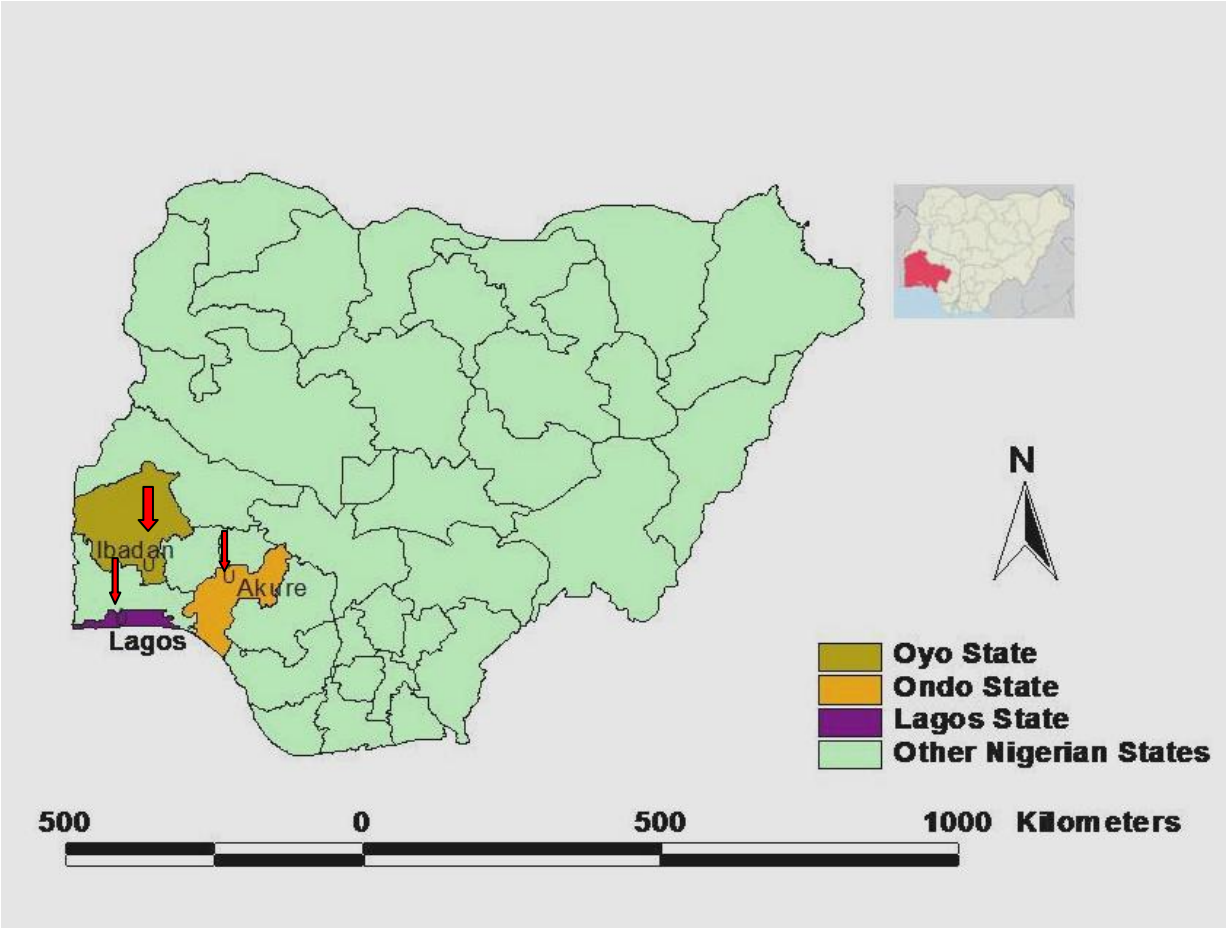


Figure 1.1 Map of Nigeria showing the study area (southwestern Nigeria)

CHAPTER TWO

LITERATURE REVIEW

2.1.1 Food Animal Production in Nigeria

Food is a basic necessity of all living things and it has been identified globally as not only a biological need but also as an economic and political weapon. It is a potential source of socio-political problems in communities and nations (Friedmann, 1998). Global food security policies are geared towards the production and supply of adequate, nutritious, high quality, safe and wholesome food to the consumers (Delgado, 1999). Before the 1970's oil boom, agricultural exports were the backbone of the Nigerian economy with livestock products accounting for a significant share of exports and had a well-developed domestic agricultural market. However, since the advent of the oil boom, despite this sound potential for growth in the domestic market, Nigeria witnessed drastic decline in agricultural production, especially in livestock and meat sectors of the industry (Osho and Asghar, 2004). Agriculture, including farming and herding, accounts for 23 percent of Nigeria's GDP and it engages 3 percent of the economically active population (Blench, 1999). The livestock sub-sector accounts for about 20% of Nigeria's agriculture and it contributes about 7% of the Gross Domestic Product (Blench, 1999). According to David-West (1983), over 6 million transhumance pastoralists derive their sole livelihood from cattle and of the small ruminant's population in the northern parts of Nigeria. Commercial poultry and piggeries are reared in peri-urban area near the consumer markets.

Although current information about the country's livestock population and herd structure are not easily obtainable, it has been observed that Nigeria has a sizeable livestock population, which account for about 95% of her current domestic livestock intakes. In 1999, the livestock population in Nigeria were estimated at 19.8, 20.5, 24.3, 4.8 and 126.0 million cattle, sheep,

goat and chicken respectively (FAO, 2000), with Nigeria taking the lead among many West African countries (Table 2.1). According to Blench (1999), livestock production operations in Nigeria can be categorised into the following systems:

- Traditional pastoralist system (migratory, nomadic);
- Integrated (mixed) crop livestock system which could be at both subsistence or slightly above subsistence level;
- Ranching (dairy/beef);
- Intensive system especially for commercial dairy, piggery and poultry production;
- Extensive, free range and backyard livestock production system.

Majority of livestock in Nigeria especially cattle are reared on extensive management system by pastoralists or nomadic herdsmen. The distributions of livestock across the different parts of Nigeria depend on several factors such as climatic conditions, disease and pest prevalence, availability of grazing vegetation. About 80% to 90% of cattle, sheep and goats in Nigeria are reared by traditional pastoralists who keep these animals as a way of life without much of economic motivation. Also, the indigenous chicken (*Gallus gallus*) constitutes about 80% of poultry population in Nigeria (Sonaiya, 1990) which are not economically viable to meet the growing protein demand of the populace. While about 20% of poultry population in Nigeria are reared on intensive commercial system most of which are located in the south western Nigeria. The south western Nigeria is the main entry point of exotic chicken and is also the location of major poultry breeders and retailers that distribute poultry round the country and beyond (Owoade *et al*., 2004). According to FAO (2009) Nigeria produced about 196,000 tonnes of chicken meat annually between 1997 and 2007. This was about 50%, 7% and 0.31% of total chicken meat produced in West Africa, Africa and the World, respectively (Table 2.1).

Table 2.1: Livestock population ('000) in West Africa (% for Nigeria) in 1999.

	Cattle	Sheep	Goats	Pigs	Chicken
Benin	1438	645	1183	470	29000
Burkina Faso	4550	6350	7950	590	21000
Chad	1330	1370	1070	1275	29000
Cote d'lvore	5582	2432	4968	23	4800
Gambia	360	190	265	14	680
Ghana	1273	2516	2739	352	17467
Guinea	2368	687	864	54	8900
Guinea-Bissau	520	280	315	340	8500
Liberia	36	210	220	120	3500
Mali	6058	5975	8525	65	24500
Mauritania	1395	6200	4133	20	4100
Niger	2174	4312	6469	39	20000
Nigeria (%)	19830(39.3)	20500(36.0)	24300(35.8)	4855(57.5)	126000(36.7)
Senegal	2960	4300	3595	330	45000
Sierra Leone	400	350	190	52	6000
Togo	223	740	1110	850	7500
Subtotal	50497	57057	67896	8449	343497

Source: FAOSTAT (2000)

Livestock production in Nigeria provides less than 30% of minimum protein requirement of Nigerians due to various socio-economic constraints and agro-climatic factors affecting distribution and production (Blench and Marriage, 1999). Livestock diseases are major limiting factors affecting productivity and profitability by increase mortality and morbidity rates, reduced rates of reproduction, weight gain and milk production. Apart from trypanosomiasis, other reported cattle diseases are anthrax, blackleg, rhinderpest, lumpy skin, brucellosis, papillomatosis, keratoconjunctivitis, foot-and-mouth diseases, contagious bovine pleuropneumonia and helminthosis (Maina, 1986). Ticks infestation is also common among Nigerian cattle, which lowers the fertility, skin quality, and milk and meat yields of the animals. Small ruminants are afflicted by a range of diseases such as *peste des petits ruminants* (PPR), contagious caprine pleuropneumonia, heartwater, sheep pox, helminthosis, coccidiosis, eye infection, caseous lymphadenitis and brucellosis. Dermatophilosis attacks and damages the skins and hides resulting in loss of productivity and death among the animals and loss in foreign exchange from the famous Moroccan leather produced by the Red Sokoto goat. Poultry diseases like coccidiosis, chronic respiratory disease, fowl typhoid, fowl cholera, fowl fox, Newcastle disease and helminthosis are enzootic in Nigeria (Maina, 1986; Sonaiya and Swan, 2004). Nigerian livestock are therefore sustainable and renewable resources that can be improved to meet the challenges of the growing animal protein needs of over 140 millions Nigerian.

2.1.2 Changing patterns of livestock production and consumption

The challenges of increasing world population and the need to cater for the human nutritional needs in term of food supply are driving force behind intensive agriculture and cardinal Millennium Development Goals (MDGs).

According to FAO (2010), agriculture including intensive livestock production will directly or indirectly facilitate the achievement of the following MDGs:

Goal 1: Eradicate extreme poverty and hunger; through pursuance of adequate structure and practice of modern agriculture for effective household food security.

Goal 2: Achieve universal primary education; through agriculture including crop, livestock and veterinary extension education on food safety.

Goal 3: Promote gender equality and empower women; by encouraging participation of women in agricultural production activities

Goal 7: Ensure environmental sustainability; good agricultural practice through irrigation, soil degradation, use of mineral fertilizers, pesticides drugs and other chemicals will ensure sustenance of agricultural environment.

Goal 8: Develop a global partnership for development; good agricultural practice including Good Practice in the Use of Veterinary Drugs (GPVD) will ensure global food trade such as WTO.

In recent years, there is increased global consumption of meat, poultry and dairy products. It is estimated that per capital consumption in developing countries of livestock products could rise by as much as 40% by 2030 (FAO, 2002). Income, population movements, education, preferences and lifestyles are critical factors affecting dietary patterns. Throughout the world, there is currently major shift from consumption of basic staple diets towards more diversified diets with milk and other livestock products, fruits vegetables and processed foods are on increasing demand (Delgado, 2003).

Meat is defined as the freshly dressed or processed tissues, mainly skeletal muscles from warm-blooded animals suitable for use for food (Alonge, 2005). Meat is a good source of

essential nutrients required for growth and development, and one of the most important sources of high quality protein, vitamins and mineral (Speedy, 2003). Pig meat is particularly rich in thiamine. Liver, and to a lesser extent kidney, are also rich in vitamin A and folic acid and in iron, riboflavin and other B-vitamins. Fish is an equally good source of protein and vitamins, including vitamins A and D in fatty fish. It also contains a well-balanced supply of minerals, including iodine, and if the bones are eaten, calcium, phosphorus and fluoride. Milk is the most complete of all foods, containing nearly all the constituents of nutritional importance to man, although it is comparatively lacking in iron and vitamins C and D. It also contains substantial amounts of lactose and protein. Eggs make a useful contribution to the daily intake of vitamin D, retinol, riboflavin, iodine, iron and protein (Speedy, 2003). Alonge, (2005) enumerated conventional sources of meat to include cattle, lambs, sheep, goat, swine, camel, rabbits, horse, game like interlopes, deer, buffalo, poultry (chicken, ducks, geese and turkeys while the unconventional sources of Nigerian meat include Guinea pigs, frogs, pigeon, pheasants, grabs turtles, termites, beetle, caterpillar, giant rats, grass cutters, porcupine, squirrel, snail, bat, rat, mice, rainbow lizard, alligator and birds. Meat consumption from different species of food animals varies from country to country; culture to culture and religious believe.

The average global per capital meat consumption is 52kg/year while the annual average of 9.5kg/year was estimated for sub-Sahara Africa as against the FAO recommended per-capital consumption of meat of 36.4kg/year (FAO, 2002). A recent statistics showed that Nigeria's per-capita meat consumption is approximately 6.4 kilograms a year compared to China's is about 23 kg, while Canadains consume an average of 65 kg a year and the citizens of the US eat 95 kg. This was described as "meatcentricity" of western society (Earth Day, 2008). Alabi and Isah, (2002) reported that the average Nigerian consume less than 61 % of the recommended minimum per capita protein requirement. In Nigeria the per capita meat

consumption declinined from 13.8 kg in 1986 to 6.4kg in 2008 (Osho and Asghar, 2004; Earth Day, 2008) due to escalating increase in meat prices is a major factor (Osho and Asghar, 2004). In Nigeria beef and chicken are popular staple livestock products and have wide consumers' acceptance (Gomna and Rana, 2007). Osho and Asghar (2004) also reported that beef and chicken are classified as luxury goods with greater proportions of these meats are being consumed in the cities (Udoh and Akintola, 2002).

According to FAOSTAT (2009), the annual beef and chicken production in Nigeria 2009 was about 1.8 million tons which accounted for 77% total meat production in the country. FAO data showed that livestock production is growing rapidly, which is interpreted to be the result of the increasing demand for animal products. Since 1960, global meat production has more than trebled, milk production has nearly doubled and egg production has increased by nearly four times. This is attributed partly to the rise in population, as well as to the increase in affluence in many countries. FAO, (2000) estimated global production and consumption of meat will continue to rise from 233 million metric tons (Mt) in the year 2000 to 300 million Mt in 2020, as will that of milk, from 568 to 700 million Mt over the same period. Egg production will also increase further by 30%. The greatest increase is in the production of poultry and pigs, as well as eggs and milk (Speedy, 2003). The demand for meat in developing countries is growing and is expected to rise rapidly, although from very low consumption levels. This will stretch the capacity of existing production and distribution systems, but will provide income growth opportunities as well (Delgado *et al.*, 1999; FAO 2009) Table 2.3 and 2.4 showed the trend of production of beef and chicken meat in countries of the world between 1999 and 2007.

Table 2.2: World Beef Production (1000 Tonnes)

Countries	1994-1996	1999-2001	2005	2006	2007
Benin	16	19	22	23	23
Burkina Faso	67	84	106	111	116
Cameroon	74	93	94	94	94
Chad	62	76	82	84	86
Côte d'Ivoire	35	33	30	31	29
Gambia	3	3	3	3	3
Ghana	21	23	25	24	24
Guinea	24	32	41	44	47
Guinea-Bissau	4	4	5	5	5
Liberia	1	1	1	1	1
Mali	56	66	108	106	112
Niger	77	119	170	177	190
Nigeria	270	285	261	284	287
Senegal	44	46	47	50	39
Sierra Leone	6	5	6	8	8
Canada	948	1,263	1,464	1,327	1,279
China	3,055	4,745	5,357	5,499	5,849
Denmark	183	155	136	129	130
Germany	1,437	1,346	1,167	1,193	1,186
Netherlands	588	450	396	384	386
World	54,068	56,304	59,493	58,758	59,852

Source: FAOSTAT, 2009

Table 2.3: World Chicken Meat Production (1000 Tonnes)

COUNTRIES	1994-1996	1999-2001	2005	2006	2007
Benin	11	12	15	16	17
Burkina Faso	23	26	31	32	33
Cameroon	22	27	30	30	30
Chad	4	5	5	5	5
Côte d'Ivoire	24	21	23	22	23
Gambia	1	1	1	1	1
Ghana	12	19	29	30	30
Guinea	3	4	6	5	6
Guinea-Bissau	1	1	1	2	2
Liberia	5	6	8	9	9
Mali	25	29	36	37	38
Niger	10	11	12	14	14
Nigeria	169	172	219	232	243
Senegal	16	23	29	32	37
Sierra Leone	9	10	11	11	11
Canada	729	908	1,000	997	1,030
China	5,783	8,695	9,964	10,164	10,617
Denmark	158	191	183	166	170
Germany	415	458	605	608	688
Netherlands	611	699	628	621	610
World	45,972	58,674	71,412	72,396	75,826

Source: FAOSTAT, 2009

2.1.3 **World Food Situation and Safety Concerns**

There is currently global food security challenge to meet the demand of the ever increasing world population as affirmed during the FAO/WHO 1996 world food summit and it is also a cardinal millennium development goal. Concerted efforts are being made by national governments to increase the quantity and quality of global food supply so as to improve the nutritional status of populations. However, millions of people are exposed to contaminated food and water without access to sufficient supplies of a variety of safe, good quality food in many countries, even where food supplies are adequate at the national level. Almost one-quarter of the world's undernourished are in sub-Saharan Africa, which is the region with the highest proportion of its population being undernourished (FAO, 1999).

At the World Food Summit, governments and international organizations arrived at a consensus on key strategies for improving food security and nutritional status. They identified the major factors in world food security as constraints on food production, population growth, urbanization rates, changing dietary patterns, conflict and instability, government policy and limited investment in agriculture and research (FAO, 1996). Feeding the world's populations require coordinated and regulated interaction of the operators in every segment of the food chain, from the point of production, through harvesting, storage, processing, preservation and marketing with shared responsibility among primary producers, food handlers and consumers (Delgado *et al. 1*999; FAO, 2000). Intensification of livestock production through commercial enterprises is very imperative to meet global animal protein demand. Incidentally the global livestock sector is undergoing changes at an unprecedented pace over the past few decades as process termed "livestock revolution" resulting from booming demand in the world's most rapidly growing economies for food derived from animals has led to large increases in livestock production (Delgado *et al*., 1999). This has to be supported by major technological innovations and structural changes in the sector (FAO,

2009) which are mostly applicable in commercial livestock production. However, millions of rural people still keep livestock in traditional production systems, where they support livelihoods and household food security. This rapid transition of the livestock sector has been taking place in an institutional void as the speed of change has often significantly outpaced the capacity of governments and societies to provide the necessary policy and regulatory framework to ensure an appropriate balance between the provision of private and public goods. FAO (2009), affirmed that several systemic risks and hazards associated with the growth and transformation of livestock industry has outpaced the capacity and willingness of governments and societies to control and regulate this sector. The control of diseases in animals is an important component of livestock production. Consequently, antibiotics and other drugs are frequently used in the production of food animals.

2.2 Antibiotics

Antimicrobial substance is any substance of natural, semi synthetic or synthetic origin that at in vivo concentrations kills or inhibits the growth of microorganisms by interacting with a specific target (Cerf *et al*., 2010).

2.2.1 Historical Overview of Antibiotics

During the second half of nineteenth century Robert Koch observed that some microorganisms could destroy others (Koch, 1876). This phenomenon was confirmed by Louis Pasteur, (1880) who believed it could be utilized in medicine. This was followed by discovery of the first "chemical cure" for a disease by Paul Ehrlich which was arsenical compound called arsphenimine (marketed as Salvarsan®) that was selectively toxic for *Treponema pallidium* (Erhlich and Hata, 1910). The period of 1920s and 1960s became the era of wonder drugs known as antibiotics; in 1935, the German bacteriologist and pharmacologist Gerhard Domagk discovered that the dye, protonsil red used to tint cloth was

effective in the treatment of streptococcal infections in mice (Domagk, 1935). When his young daughter was dying of streptococcal infection, in desperation he injected this dye into to the girl and her fever dropped almost immediately, this quick recovery at that time was termed as nothing short of miraculous. It was a Swiss-born Chemist, Daniel Bovert, who later showed that the antibacterial activity of protonsil red was caused by sulphanilamide component of the dye and by 1940 sulphanilamide was available under at least thirty three different trade names (Magner, 1992).

The discovery of penicillin by Alexander Fleming in 1928 was the starting point of modern antibiotic therapy. Alexander Fleming, an English bacteriologist returned from holiday to his laboratory at St. Mary's hospital in London by early September 1928 where he made his famous observation on an old, uncovered culture plate of bacteria. He noticed a blue-green mould that was attacking the bacteria. He identified the mould as *Penicillium notatum,* cultured it in nutrient broth, filtered it and observed in the filtrate (which he named "Penicillin") a substance that ravaged bacteria. Fleming reported this accidental discovery to the scientific world in the British Journal of Experimental Pathology (Fleming, 1929). Fleming was recognised for this landmark discovery and was awarded Nobel Lauret in Medicine in 1945 (http://nobelprize.org/medicine/laureate/1945).

The term antibiotics (from Greek words that literally means against life anti – means against, biosis – means life) first appeared in scientific literature in 1942 (Waksman *et al.*, 1942) was coined by Selman Blaksman to embrace those newly discovered antimicrobial substances such as pyocyanin, penicillin, actimycin and others, Waksman used this term to describe and define those substances of microbial origin that specifically inhibit the growth of other microorganisms. The usage for this term has now been extended to include any low molecular weight metabolites from living organisms or synthetic compounds which at low

concentrations will kill or inhibit the growth of other organisms (Greenwood and O'Grady, 1997).

According to (Lancini *et al.,* 1995), antimicrobial agents affect the growth of bacteria either by killing bacteria cells with or without the lysis or rupture does not occur are known as bacteriocidal or by inhibiting cell wall and cytoplasmic membrane synthesis thereby inhibiting their growth and are known as bacteriostatic.

Antibiotics that kill or inhibit the growth of wide groups of bacteria (both Gram-negative and Gram-positive) are called broad spectrum antibiotics while those that act only on a single group of bacteria (either Gram-negative or Gram-positive) are narrow spectrum antibiotics. Majority of antibiotics used in human and veterinary medicine are "natural product" elaborated as secondary metabolites by living organisms primarily bacteria and fungi (Bennet and Bentley, 1989; Davies, 1990; Vining, 1990). Most antibiotics are products of secondary metabolism of three main groups of microorganism: Actinomyces, Eubacteria and filamentous fungi (Greenwood, 2009). The actinomyces produce the largest number and greatest variety of known antibiotics, with more than six thousand active substance isolated from them (Greenwood, 2009).

2.2.2 Classification of Antibiotics

There are several methods used to classify and group antibiotics. They are grouped together based on their source, mechanism of action, chemical structure and spectrum of activity (Lancini, *et al.,* 1995; Greenwood and Whitney, 2009). They are also classified as broad or narrow spectrum, depending on the range of bacterial species against which they are active, or as bacteriostatic or bactericidal on the basis of their mechanism of action. An antimicrobial that exhibits a large dilution difference between inhibitory and bacteriocidal effects is considered to be a bacteriostatic drug. An antimicrobial that kills the bacterium at or near the

same drug concentration that inhibits its growth is considered to be a bactericidal drug (Greenwood and Whitney, 2009). In general, antibiotics and other chemotherapeutic agents are grouped together based either on their mechanisms of action and more usually chemical structure (Lancini, *et al.*, 1995). Figure 2.1 shows the chemical structures of some antibiotics.

2.2.3 Mode of action of Antibiotics

Since the discovery of antibiotics much knowledge has been acquired on their mode of action and it is a major basis for their choice in the treatment of man and animals (Lancini, *et al.*, 1995). Antibiotics can be classified into several groups according to their mode of action on or within bacteria (Lancini, *et al.*, 1995; Millodot, 2009):

- Inhibiting bacterial cell wall synthesis, such as bacitracin, vancomycin and the β-lactams based agents (e.g. penicillin, cephalosporins).
- Drugs affecting cytoplasmic membrane, such as polymyxin B sulfate and gramicidin.
- Drugs inhibiting bacterial protein synthesis, such as aminoglycosides (e.g. amikacin sulfate, framycetin sulfate, gentamicin, neomycin sulfate and tobramycin), tetracyclines, macrolides (e.g. erythromycin and azithromycin) and chloramphenicol.
- Drugs inhibiting the intermediate metabolism of bacteria, such as sulfonamides (e.g. sulfacetamide sodium) and trimethoprim.
- Drugs inhibiting bacterial DNA synthesis, such as nalixidic acid and fluoroquinolones (e.g. ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin and ofloxacin).
- Other antibiotics such as fusidic acid, the diamidines, such as propamidine isethionate and dibrompropamidine. *Syn.* antibacterial.

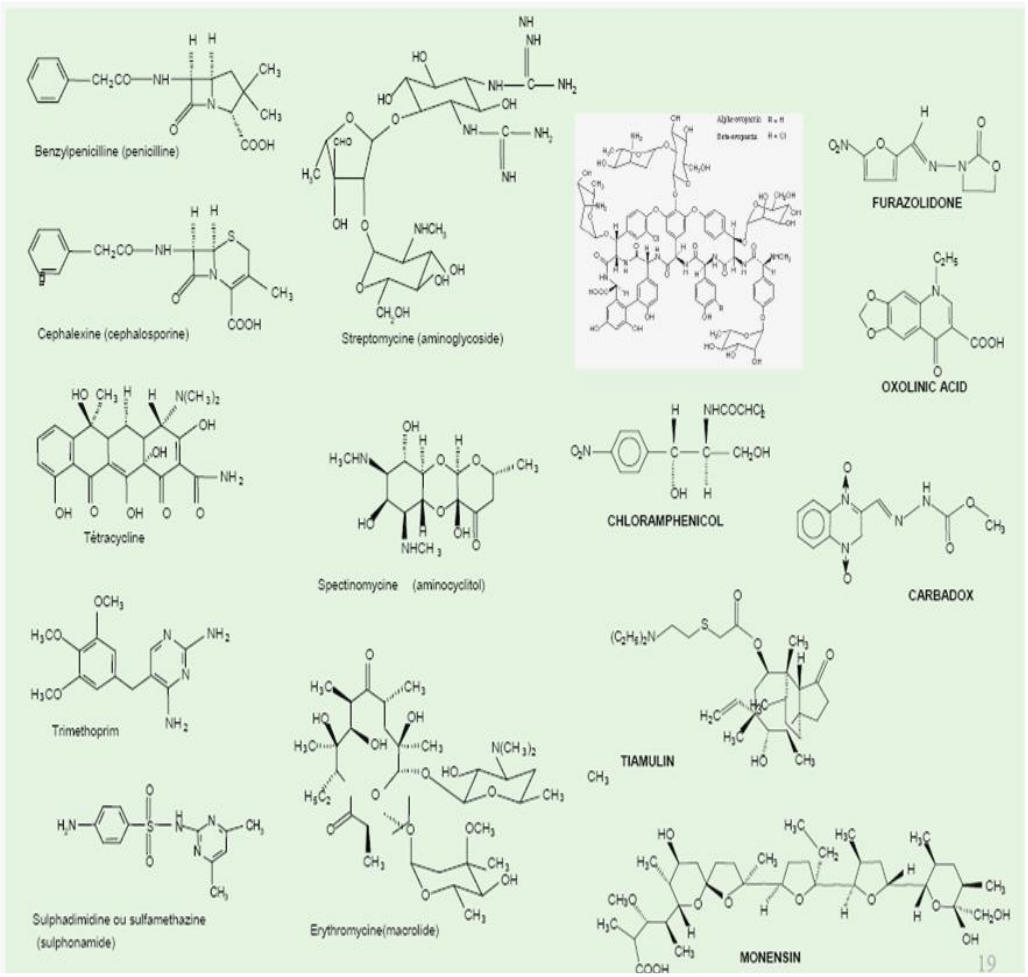


Figure 2.1: Chemical Structures of Some Antibiotics

2.2.4 Metabolism of antibiotics

Pharmacokinetics describes how the drug behaves in the body, i.e. absorption, distribution, metabolism and elimination, as reflected in the time course of drug concentrations in plasma or tissue (Chopra *et al*., 1992; Chopra and Roberts, 2001). After each administration of an antimicrobial drug, the distribution and concentrations in the body compartment depend on certain key the pharmacokinetic variables such as; the maximum plasma concentration, the peak time plasma concentration, the area under the plasma concentration time curve, the volume of distribution, and the plasma half-life. Most veterinary drugs are metabolized in the body to produce more water soluble compounds, which are more readily excreted (Aerts *et al*., 1995). For example, penicillins are eliminated almost entirely by the kidneys, which results in very high concentrations in the urine, (Prescott, 2000b). Penicillin-G is metabolized to some extent by hydrolysis of the β-lactam ring, and the metabolites are microbiologically inactive (Prescott, 2000b). Most tetracyclines are excreted unchanged in urine and, to a lesser extent, bile (Prescott, 2000b). Oxytetracycline is not metabolized to a significant extent in the body; it is eliminated in the urine and faeces (Spoo, 1995). High concentrations of fluoroquinolones are found in organs of excretion Aminoglycosides are eliminated unchanged by renal excretion (Prescott, 2000a). Chloramphenicol is excreted mainly in the form of a microbiologically inactive glucuronic acid conjugate (Glazko *et al*., 1977).

The highest free drug concentrations of most antimicrobials are found in kidney and renal pelvis (Aerts *et al*., 1995). After intramuscular administration of oxytetracycline to cattle, the injection sites and kidneys contained the highest residue concentrations followed by the liver, hence oxytetracycline concentrations in edible tissues could be predicted from kidney cortex concentrations, whereas renal penicillin-G concentrations did not correlate with muscular drug concentrations (Black and Gentry, 1984). Aminoglycosides are usually found in tissues in low concentrations and unbound, except in the renal cortex where they tend to concentrate

(Isoherranen and Soback, 1999). Incidence of false positive reactions and varying ratios of residue concentrations in kidney and muscle of healthy and diseased animals (Nouws, 1984; Engel *et al*., 1983) complicate the use of kidney tissue as the test matrix.

2.2.5 Antibiotic Resistance

The term antibiotic resistance is often used in a general sense to signify the lack of effect of antibiotic agent on a bacteria cell. The phenomenon antibiotic resistance occur when bacteria strains derived from species that were susceptible to certain antibiotics are no longer inhibited by the minimal concentration of the antibiotic that inhibit, the growth of typical strains of that species (Greenwood, 1995). The resulting resistance is dependent on the interaction among a given bacterial strain, the particular antibiotic and the concentration of the antibiotic (Lancini *et al*, 1995). A bacterial strain is considered truly resistant to a given antibiotic only if it can grow in the presence of a concentration equal to or greater than that which antibiotic can reach in the serum or tissue (Drlica, 2001). Antibiotic resistance in clinical bacteriology has been recorded since these agents first came into use. The first antibiotic resistance mechanism was identified in 1940 when Abraham and Chain described the presence of a penicillinase, an enzyme that inactivates penicillin, in resistant *Escherichia coli* (Abraham and Chain, 1940) and a similar mechanism penicillin resistance was reported in 1944 in an isolate of *Staphyloccocus aereus* (Kirby, 1994). The enormity and complexity of the current problem of antibiotic resistance have earlier been fore told in the early days of antibiotic discovery when Alexandar Flemming recognized the threat and factors that would promote resistance (Fleming, 1929). Over the last three decades there has been concern over the problem of antibiotic resistance pathogens, evidence is mounting that antibiotic-resistant enteric bacteria (for example, *Escherichia coli, Salmonella, Campylobacter* and *Enterococci*) can transfer from animals to man via the food chain or by direct contact, leading to the establishment of a community reservoir of resistance genes (Van den Bogaard and Stobberingh, 1999). There

are increasing reported numbers of bacterial infections that fail to respond to antibiotic treatment (Levy, 1992). WHO, CDC, FDA, EUCVM and many public health agencies are concerned that antibiotic resistance is "a growing menace to all people," noting that continued spread of resistance means that treatments for common infections "will become increasingly limited and expensive and, in some cases, non-existent."

Bacteria reproduce rapidly, sometimes in as little as 20 minutes hence, it does not take long for antibiotic-resistant bacteria to spread (Levy, 1997). Microorganisms display amazing versatility in terms of their ability to avoid, withstand, or repel the antibiotic onslaught (Neu, 1992; Courvalin, 1994). Often, the use of antibiotics disrupts the delicate bacterial ecology within the body of humans and animals thereby allowing the proliferation of resistant species and sometimes initiating new infections that are worse than the one originally treated (Levy, 1997). The historical cycle that has been witnessed in the last fifty years is that as drugs were discovered and diseases successfully treated with certain antibiotics soon the organisms develop resistance (Levy, 1992). Antibiotic resistance makes diseases like cholera, bacterial meningitis, tuberculosis, pneumonia, and even plagues to reemerge a renewal vengeance (Neu, 1992; Caputo *et al*, 1993; Doern *et al.*, 1996).

2.2.5.1: Resistance Mechanisms, Cross-Resistance and Co-Selection

Resistance to an antibiotic may be an inherent property of the infecting organism or it may be acquired. The intrinsic resistance is a natural property of the bacteria resulting from mutation or acquisition of new genetic material (Kathleen *et al.*, 2003). Intrinsic resistance refers to bacteria in their natural state are insensitive to an antibiotic without acquiring resistance factors. Mutations that result in antibiotic resistance are spontaneous events involving changes in chromosomal nucleotide sequences. The development of mutational resistance is favoured by low and intermittent drug dosage (Prescott and Baggot, 1993). Acquired

resistance may result from mutation or from transfer of an extrachromosomal genetic material followed by selection of resistant organisms during therapy (Neu, 1992).

An acquired resistance gene induces a resistance mechanism if this gene is expressed. Resistance mechanisms can be either constitutive or inducible, depending on whether the resistance gene is can be expressed in the presence of the antimicrobial drug (Catry *et al.*, 2003). The different resistance mechanisms are related to the mechanisms of action of different antimicrobial drugs on the bacterial cell, but more than one resistance mechanism can be present in a given bacteria.

The most important resistance mechanisms are antimicrobial drug modification, reduced intracellular accumulation, and modification of the target site (Livermore, 1995). Antimicrobial drug modification consists of an enzymatic modification or inactivation of the antimicrobial drug that prevents the molecule from reaching the target site. The inactivation of penicillins and cephalosporins by β-lactamases (Livermore, 1995) and of aminoglycosides by acetyl-, adenyl- and phospho-transferases (Davies, 1994) in both Gram-positive and negative species are examples of enzymatic inactivation. The second mechanism arises from an alteration in bacterial cell-wall porins, resulting in either a decreased uptake or an increased removal of the antimicrobial drug in the cell. The reduced intracellular accumulation inhibits the antimicrobial drug from proceeding with its normal intracellular action. An increased removal of tetracyclines, for example, can be caused by the presence of tetracycline efflux genes, e.g. tet (A-E), which are found in a wide variety of bacteria (Roberts, 1996). The third resistance mechanism results from a chemical modification of the target site. For instance, mutations in the genes for DNA gyrase and topoisomerase can cause structural changes that inhibit fluoroquinolones from binding to these target sites (Webber and Piddock, 2001). Finally, a fourth but less prevalent mechanism is based on a bypass effect that prevents the inhibitory action of the antimicrobial drug. This is a well-known

resistance mechanism for the combination of sulphonamides with trimethoprim. In this case an alternative synthetic pathway for folic acid production makes the microorganism unsusceptible to the antimicrobial drug combination.

The most common and important process of resistance transfer is conjugation in which two organisms exchange R-plasmids by contact through sex pilus. R-factors may also be released by one bacterium and take in through the cell wall of another (transformation). R-factors, therefore, can circulate in humans, in animals and in the environment and possibly between animals and humans (Landicho, 1996). Transmission of antibiotic resistance in pathogenic bacteria may cause zoonotic infections, while resistant non-infectious bacteria may serve as a reservoir of R-plasmids for other virulent organisms. R-factors encode at least four different biochemical mechanisms: enzymatic degradation; alteration of the antibiotic by the cell, alteration of the target site of the antibiotic and synthesis of a resistant form of an essential metabolic enzyme that is normally sensitive to different antibiotics (Timoney *et al*., 1988).

Cross-resistance arises from a resistance mechanism that makes bacteria resistant to more than one antimicrobial drug. This is often true for different molecules of a given class of antimicrobial drugs, such as tetracyclines, because they are structural analogues. For instance, tet (M) genes induce resistance to oxytetracycline, doxycycline, chlortetracycline and minocycline (Roberts, 1996). In contrast, multi-drug efflux pumps (mex) genes can cause cross-resistance to a wide range of structurally heterogeneous toxic compounds, including antimicrobial agents (Paulsen, 2003). Cross-resistance can also occur if analogue antimicrobial drugs act on the same target site of a microorganism. The presence of one erm (X) gene causes resistance against macrolides, lincosamides and B-compounds of the streptogramins. In livestock this gene has been found in streptococci of the bovine udder and pneumonic lungs from swine, and in faecal enterococci of different animal species (Butaye, 1998; Martel *et al*., 2001). Such solitary genes causing cross-resistance, or different

resistance genes gathered on a single mobile genetic element (transposons or plasmids), may cause co-selection (Schwarz *et al*., 2001). With co-selection, resistance to several different antimicrobial drugs emerges while only one antimicrobial drug is used. Thus the importance of co-selection is substantial. Levy *et al*. (1976) clearly demonstrated co-selection in caecal coliforms of poultry. Tetracyclines given orally made the bacterial caecal flora less susceptible not only to tetracyclines, but also to ampicillin, streptomycin and sulphonamides. Bager *et al*. (1999) also observed a significant difference between Danish swine and poultry in the persistence of glycopeptide-resistant enterococci (GRE) after the ban on avoparcin (glycopeptide) as a growth promoter. In poultry, the level of GRE decreased from 80 to 10%, while during the same period the percentage of GRE in swine declined from 25 to 20%. The vanA gene, selected by the use of tylosin because of linkage with the ermB gene, maintained the glycopeptide resistance level (Aarestrup, 2000).

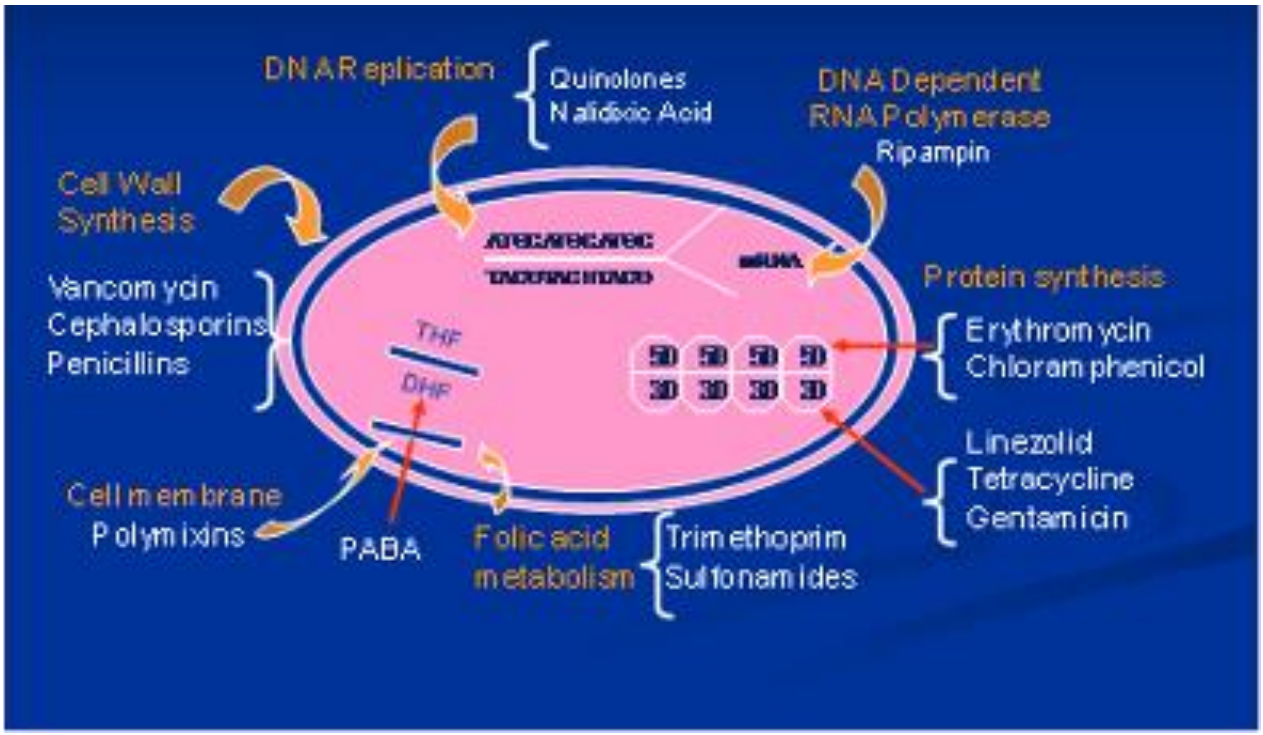


Figure 2.2: Mechanisms of Action of Antibiotics

2.2.5.2 Vectors of Antibiotic Resistance

Bacteria have evolved very sophisticated means of exchanging DNA, both within their own genus and species and across themselves. Foreign genes responsible for antibiotics resistance reside on mobile genetic elements such as plasmids, transposons and integrons.

2.2.5.2.1 Plasmids

Plasmids are extra-chromosomal resistance circular self replicating DNA molecules that are produced in bacterial cells (Thomas, 2000) they can replicate independent of the bacterial chromosomal DNA. Plasmids are one of the key players in the team of mobile genetic elements that fuel bacterial adaptability and diversity. Plasmids are capable of promoting the rapid speed of antibiotic resistance genes. They can exist in the autonomous extra-chromosomal state or can be inserted into the bacterial chromosome and then be carried as part of it (Thomas, 2000). Plasmids can be divided into two macro groups: narrow and broad host range or promiscuous plasmids. Broad host range plasmids are defined as those for which replication is not restricted to one particular species of bacteria but can replicate in many of a selected group of host species (Valla, 1998). Broad host range plasmids may further be subdivided into two subgroups: conjugative or self-transmissible and non conjugative but mobile. The self-transimisible, broad host range plasmids are the most active vehicle for a potential “horizontal gene pool” of antibiotic resistance genes that are available to many bacteria (Thomas, 2000).

2.2.5.2.2 Transposons

Transposon is a genetic element capable of moving from one DNA molecule to another, independent from the normal reaA-dependent type of combination. Transposons are linear pieces of DNA that range in size from 2.5 to 23 kilobase pairs (kb) and contain two identical

insertion sequences (Iselements), one at each end of the molecule. Following the discovery R-factors, many antibiotic resistance genes were found to be located on small pieces of DNA that can independently move around (Hedges and Jacob, 1971). Transposons appear to be ubiquitous in nature and have been identified in many types of organisms including plants and animals, as well as bacteria (Berg and Howe, 1989; Sherrat, 1989). There are several classes of transposons, grouped according to their general structural and functional organization, using features such as size, conserved DNA regions, number of open reading frames (ORFs), presence of the host genes and particularly the transpositional pathway (Nield *et al.*, 2001). Some transposons, such as Tn10, which encode resistance to tetracycline, move in a "conservative" manner, without replication (Kleckner, 1989). Conjugation transposons, found in many gram-positive and a few gram-negative bacteria, are integrated DNA segments that excise from the chromosome to form a circular intermediate which then transfers itself to a recipient, where it integrates once again into the chromosome (Salyes and Shoemaker, 1997). Conjugation transposons resemble plasmids in that they are transferred by conjugation and have a circular intermediate, but do not replicate (Salyers and Shoemaker, 1997).

2.2.5.2.3 Integrons

The term integron was originally coined to describe the group of apparently mobile elements that contain one or more antibiotic resistance genes located at a specific site as well as contain the determinants of the site-specific or recombination system responsible for insertion of the resistance genes (Stoke and Hall, 1989). Integrons are "nature" vectors gene-capture and expression systems (gene cassettes) for exogenous DNA that incorporate open reading frames (OFR) and convert them into functional gene (Ploy *et al.*, 2000). They possess conserved region that codes an integrase (Int) and a variable region (5´CS-3´CS) where various resistance cassettes can be integrated themselves (Rodriguez, 2006). More than sixty different antibiotic resistance genes to most commonly used antimicrobial drugs have been

characterized in cassette structure (Mazel and Davies, 1998). Carattoli (2001), reported that integrons are found among the genetic element and contributed to the development of antimicrobial multi-resistance in Gram-negative bacteria.

2.2.6 Determination of Antibiotic Resistance

Antimicrobial resistance have been conventionally determined using cultural broth- and agar-based antimicrobial susceptibility testing methods to provide a phenotypic profile of the response of microbial pathogens to an array of antimicrobial agents (Pfaller, *et al*., 2001). Antimicrobial susceptibility is to guide the clinician in the choice of appropriate agents for therapy or used to evaluate the *in vitro* activity of new agents.

The three phenotypic methods commonly used are, disc diffusion, minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MIB) determination and Breakpoint methods (Jones and Pfaller, 1998). The protocols based on microbial cultural technique are described and standardised by National Committee for Clinical Laboratory Standards (NCCLS, 2004). Antimicrobial susceptibility are usually reported qualitatively as: sensitive (S), which indicates that the standard dose of the agent should be appropriate for treating the patient infected with the strain tested; resistant (R), which indicates that an infection caused by the isolate tested is unlikely to respond to treatment with that antimicrobial agent; and intermediate (I), where the strains are moderately resistant or moderately susceptible and indicates that the strain may be inhibited by larger doses of the agent or may be inhibited in sites where the agent is concentrated (e.g. urine). Quantitative testing determines the MIC, which is the lowest concentration of the agent that will prevent a microbial population from growing. The minimum bactericidal concentration (MBC) is the lowest concentration that kills 99.9% of the population.

However, due to the rapid emergence, dissemination and global threat of bacterial resistance there has been need to pursue molecular typing of resistant strain of these bacteria. This is a critical tool for effective surveillance and intervention against the menace of resistance (Diekema, *et al*., 2000). Comprehensive molecular typing of organisms collected in a global surveillance program will helps to provide information regarding the emergence and distribution of specific pathogenic strains as well as the spread of resistance determinants. Table 2.5 shows the some of the molecular methods used in antimicrobial active surveillance program (Pfaller *et al*., 2001).

Table 2.4: Molecular typing antibiotic resistance methods.

Method	Comments	Examples
Plasmid analysis	Plasmids may be digested with restriction endonuclease enzymes; useful in tracking spread of resistance genes	Staphylococci, Enterobacteriaceae
Restriction endonuclease analysis of chromosomal DNA with conventional electrophoresis	Large number of bands; difficult to interpret; not amenable to computer analysis; a comparative typing method only	Enterococci, Staphylococcus aureus, Clostridium difficile, Candida species
Genome restriction fragment-length poly- morphism analysis: ribotyping, insertion sequence probe fingerprinting	Fewer bands; amenable to automation and computer analysis; sequence based profiles; library typing methods	Enterobacteriaceae, staphylococci, and Pseudomonas aeruginosa, Candida species
PCR-based methods: repetitive-elements PCR spacer typing, selective amplification of genome restriction fragments,multilocus allelic sequence-based typing	Crude nucleic acid extracts and small amounts of DNA may suffice; amenable to computer analysis	Enterobacteriaceae, Acinetobacter species, staphylococci
Pulsed-field gel electrophoresis (PFGE)	Fewer bands; amenable to automation and computer analysis; sequence based profiles; library typing methods	Enterobacteriaceae, staphylococci, enterococci, Candida species

2.2.6 Antibiotic Residues in Food Animals

Consumers of animals and animal products (meat, milk, eggs, etc.) are liable to ingest whatever chemicals the animal has consumed or been exposed to veterinary drugs, and also insecticides used on the animal, herbicides and fertilizers used on pastures and chemical additives used in its feed. Some of these substances are toxic (in particular, pesticides and herbicides), and some are undesirable in other ways for use on animals whose products are consumed by humans. Residues of veterinary drugs may exert adverse effects on consumers in a number of ways, such as; chronic toxicological adverse effects, acute pharmacological effects on consumers and on the microflora of the gastrointestinal tract of consumers and allergic reactions (Sundlof *et al.,* 2000; Berends *et al*., 2001; Doyle, 2006). The two main major substances of public health concern on drug use in food animal production are hormones and antibiotics. Several concerns regarding antibiotics use in animals result from the development of bacterial resistance genes that are transferable to human beings, the effect on human intestine microflora composition, potential allergic reactions in sensitized individuals, direct toxicity and technological problems of their residues in meat and milk products (Stark, 2000).

2.2.6.1 Tetracyclines Residue

Tetracyclines are broad-spectrum antibiotics discovered in the 1940s and are used to treat a variety of bacterial, protozoan and rickettsiae infections, also as growth promoter in animals (Chopra and Roberts, 2001). In studies with humans, about 60% of an ingested dose of oxytetracycline was absorbed from the gastrointestinal tract and was then widely distributed in the body, particularly to liver, kidney, bones and teeth (Agwuh and MacGowan, 2006). There appeared to be little, if any, metabolism of this drug in humans or animals and it was primarily excreted in the urine (Agwuh and MacGowan, 2006; JECFA, 2003). At therapeutic

doses, tetracyclines are occasionally associated with discoloured teeth, allergic reactions, or peripheral blood changes (Waltner-Toews and McEwen, 1994). Subtherapeutic dosages as low as 2mg/l of oxytetracycline administered over seven days resulted in increased drug-resistant enterobacteriaceae (Corpet, *et al.*, 1996). A no-observed-effect level residue of tetracycline of 2 mg/person/day was observed. There have been reports of allergic reactions to tetracyclines (Waltner-Toews and McEwen 1994). The CAC recommended MRLs of tetracyclines in kidney, liver, egg, muscle and milk of food animals destined for human consumption are 1200, 600, 400, 200, and 100 μg/kg respectively (CAC, 2009). The ADI of 0-30 μg/kg bw for tetracycline, oxytetracycline and chlortetracycline (group ADI) was established (JECFA, 2003). Oxytetracycline is used by, and JECFA (2003) as the marker residue to determine acceptable levels of intake because it is found to induce antibiotic resistance among the coliform bacteria in the human intestine. Liquid chromatographic techniques are the official methods widely recommended for confirmation of tetracycline residue levels in these food items (MacNeil *et al*., 1996; Posyniak *et al*., 1999; CAC, 2009).

2.2.6.2 Causes of antimicrobial residues in meat

The major reported cause of violative levels of veterinary drugs in food is failure to observe and adhere to the recommended withdrawal periods (van Dresser and Wilke, 1989; Kukanich *et al*., 2005). Improper maintenance of treatment records or failure to identify treated animals adequately can also lead to their omission (Sundlof, 2000). Results by FSIS National Residue Monitoring Plan indicated that penicillin and sulfonamide drugs, Neomycin and gentamicin were commonly detected at violative levels in cattle, swine and poultry (FSIS, 2006). Other drugs detected in cattle and swine included tilmicosin, flunixin, and tetracyclines. Faecal recycling, where the drug excreted in faeces of treated animals contaminates the feed of untreated animals, can be the cause of residues of certain antimicrobial groups (McCaughey

et al., 1990). Housing of un-medicated pigs in boxes where pigs had previously been treated orally with sulfamethazine resulted in residues in urine, kidney and diaphragm (Elliott *et al*., 1994). Violative drug residues can also occur as a result of improper use of a licensed product or through the illegal use of an unlicensed substance or extralabel dosages and use (Kaneene and Miller, 1997; Higgins *et al*., 1999). Residues can also occur in calves fed milk and/or colostrum from cows receiving antimicrobials (Guest and Paige, 1991). In most countries β-lactams are widely applied in mastitis therapy and are consequently the major reason for the presence of inhibitory substances in milk (Sternesjö and Johnsson, 1998).

The disease status of an animal and the way in which drugs are administered also influence the potential for residues as they affect the pharmacokinetics of the drugs, metabolism, or the presence of infection and/or inflammation may cause the drug to accumulate in affected tissues (Kaneene and Miller, 1997). Subcutaneous and intramuscular administrations increase the potential for residues at the injection sites (Kaneene and Miller, 1997; Berends *et al*., 2001). Secondary drug concentration peaks in plasma have been detected after subcutaneous injections of benzathine procaine penicillin.

Contamination of feeding stuffs could also be an important source of unintended application of antimicrobials (McEvoy, 2002). In a survey carried out in Northern Ireland antimicrobials were detected in 44% of feeds declared by the manufacturers to be free of medication (Lynas *et al*., 1998). Residual quantities of medicated feed may be retained at various points along the production line, contaminating subsequent batches of feed as they are processed (Kennedy *et al*., 2000). Data from a sulfamethazine residue programme suggested that 25% of violations were due to inadequate cleaning of feed mixers (Guest and Paige, 1991).

2.2.6.3 Public health risks of antimicrobial residues

Antimicrobial residues in foods of animal origin may cause problems for several reasons. In addition to toxic effects, effects on intestinal microbiota and the immune system are important (Perrin-Guyomard *et al*., 2001; Doyle, 2006). Microbiological endpoints are considered more valid and sensitive in the safety evaluation of antimicrobial residues in production animals than standard toxicological endpoints (Boisseau, 1993).

2.2.6.4 Effects of residues on gut microbata

The microbiota in gastrointestinal tract of man and animals are extremely complex, yet relatively stable, ecological community, containing more than 400 bacterial species (Carman *et al*., 1993). The concentration of anaerobic microbiota is 1011-1012 CFU g^{-1} faeces, and the concentration of aerobic microbiota much lower i.e. less than 0.1% of the normal microbiota consists of aerobes (Vollaard and Claesner, 1994). In addition to the resident dominant anaerobic microbiota, the microbiota consists of a subdominant microbiota, a resident minority microbiota and a variable microbiota composed of bacteria which may be present for a variable period of time (Boisseau, 1993). Colonisation resistance means the natural defence by normal microbiota against colonisation and translocation by exogenous potentially pathogenic microbes or against the overgrowth of indigenous opportunistics (Corpet, 1993; Berends, *et al*., 2001). Administration of antimicrobial agents may cause disturbances in these functions (Nord and Edlund, 1990). The extent of disturbances in the ecological balance between host and microorganisms occur depends on the spectrum of the antimicrobial agent, the dose, pharmacokinetic and pharmacodynamic properties, and in-vivo inactivation of the agent (Wegener *et al*., 1999; Sullivan *et al*., 2001).

Four microbiological endpoints have been identified that could be of public health concern: modification of the metabolic activity of microbiota, changes in bacterial populations,

selection of resistant bacteria, and perturbation of the barrier effect (Boisseau, 1993; Gorbach, 1993; Sundlof *et al*., 2000; Perrin-Guyomard *et al*., 2001). In cases of reduced colonisation resistance not only are the minimal infectious or colonisation doses of pathogenic or resistant bacteria considerably lower, but animals also excrete these bacteria in higher numbers and over a longer period of time compared to animals with an intact colonisation resistance (van den Bogaard and Stobberingh, 1999; 2000). Low doses of antimicrobial drugs could alter intestinal enzyme activity and have an effect on microbiota ecology (Gorbach, 1993).

2.2.6.5 Hypersensitivity effects of antibiotic residues

Antimicrobial drug residues in animal tissues may cause hypersensitivity reactions in humans. Drug hypersensitivity is defined as an immune-mediated response to a drug agent in a sensitized patient, and drug allergy is restricted to a reaction mediated by IgE (Riedl and Casillas, 2003; Gomes and Demoly, 2005). Drugs are foreign molecules, but their molecular weight is usually too small to be immunogenic. For drugs to be immunogenic, they must act as haptens, which must combine with carrier proteins to be immunogenic and elicit antibody formation (Dewdney *et al*., 1991). An allergic reaction may be triggered by antimicrobial residues in a previously sensitized individual. In relation to primary sensitization, it is unlikely that residues could contribute to the overall immune response in view of the very low concentrations that are likely to be encountered. More so, the duration of exposure is also short (Dewdney *et al*., 1991; Sundlof *et al*., 2000). All antibiotics have the potential to cause allergic reactions; penicillins are most commonly implicated, affecting up to 10% of people receiving these drugs therapeutically (Dayan, 1993). Notwithstanding their non-toxic nature, β-lactams appear to be responsible for most of the reported human allergic reactions to antimicrobials (WHO, 1991; Fein *et al*., 1995). Angioneurotic oedema and tightness of chest due to penicillin residues in meat was reported by Schwartz and Sher (1984). Also, in one

study of 15 people known to be very sensitive to penicillin found that three reacted after drinking milk containing a total of 2.5 μg penicillin, and two sensitive volunteers developed rashes after eating pork containing 0.02–0.04 ppm penicillin (Dayan, 1993). Anaphylactic reactions to penicillin in pork and beef have also been reported (Kanny *et al*., 1994; Raison-Peyron *et al*., 2001). Also one case of anaphylaxis was possibly caused by streptomycin residue was reported by Tinkelman and Bock, (1984). Aminoglycosides, sulphonamides and tetracyclines may also cause allergic reactions (Paige *et al*., 1997). Certain macrolides may be responsible for liver injuries, caused by a specific allergic response to macrolide metabolite-modified hepatic cells (Dewdney *et al*., 1991). Adverse reactions to antibiotics have been linked to hypersensitivity (Woodward, 1991) and cases of chronic urticaria (Dayan, 1993). Minor reactions such as urticaria, due to exposure to allergenic residues are often not usually associated and reported (Woodward, 1991).

2.2.6.6 Other harmful effects of antibiotic residues

Hazards of chloramphenicol include non-dose related aplastic anemia, reversible suppression of the bone marrow, gray baby syndrome (causing circulatory collapse in children less than 30 days) and irreversible, idiosyncratic (Schmid, 1983; WHO, 1988; Waltner-Toews and McEwen, 1994). Aplastic anemia (fatal blood dyscrasia) can occur in sensitised individuals exposed to concentrations of chloramphenicol that might remain as residues in edible tissues of chloramphenicol-treated animals (Settepani, 1984). Aminoglycosides residue can produce damage in urinary, vestibular and auditory functions (Shaikh and Allen, 1985). Also the residue of furazolidone and its metabolites have been shown to be highly carcinogenic hence, its FDA approval was withdrawn in 1991 and NFDAC placed a ban on it use in Nigerian livestock since 1996 (NAFDAC, 1996)

2.2.7 Safety factors for the evaluation of antimicrobial drug residues

To assess the safety of ingested antimicrobial residues national and international committees evaluate data on chemical, pharmacological, toxicological and other, e.g. antimicrobial, properties of the drugs derived from studies of experimental animals and observations in humans (Woodward, 1998). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) of Codex Alimentarius Commission undertakes the safety evaluation of residues of veterinary medicines. In the safety evaluation of veterinary drugs tests undertaken to demonstrate the safety of the substance are performed in order to determine a no observed adverse effect level (NOAEL). This level is the basis for calculating an acceptable daily intake (ADI). After an ADI has been determined, maximum residue limits (MRLs) are determined for various food commodities so that overall residue intake remains below the set ADI in a standard food basket. In order to ensure that drug residues have declined to a safe concentration in various tissues, a specified period of drug withdrawal is set for a veterinary medicinal product. In the European Union (EU) maximum residue limits (MRLs) must be established for all pharmacologically active substances for the concerned animal species and relevant tissues or products. These MRLs are the basis for the determination of limits of detection (LODs) of various analytical methods used in residue surveillance. In the EU safety evaluation of residue is performed by the Committee for Veterinary Medicinal Products (CVMP) of the European Agency for the Evaluation of Medicinal Products (EMEA), while in the USA by Food Safety and Inspection Service and Food and Drug Administration of the United States Department of Agriculture (USDA), and United States Department of Health and Human Services. The FDA and FSIS are the Food Safety Working Group that share authority for helping to ensure the safety of America food supply. Each agency investigates foodborne illness outbreaks and other foodborne risks (including drug residues) associated with the products they regulate. These investigations, conducted in close cooperation with the

U.S. Centers of Disease Control and Prevention and state and local health and agriculture Departments, often involve tracing backward or forward in the supply chain the distribution of food products and ingredients associated with risk to consumer health (FDA-FSIS, 2009). FSIS release monthly list of individuals or firms responsible for repeat drug, pesticide, or other chemical residue violations in animals presented for slaughter.

2.2.7.1 Acceptable daily intake (ADI)

The ADI is an estimate of the residue, expressed on a body weight basis that can be ingested daily over a lifetime without any appreciable health risk (EC, 2001). The ADI approach was originally developed to take account of effects based on classical toxicology, and it was applied to the results of standard toxicity studies. These studies were used to derive a NOAEL. The ADI was calculated by dividing this by a suitable safety factor, usually 100, which assumes that humans are 10 times more sensitive than animals and that within the human population there is a 10-fold range of sensitivity (IPSC, 1987; Woodward, 1998). Standard toxicological studies are inadequate in evaluating adverse effects of antimicrobials, thus the determination on microbiological endpoints is necessary (Perrin-Guyomard *et al*., 2001). Microbiological properties of residues are included in the safety evaluation at national and international levels (WHO, 1991; US FDA, 1996; CVMP, 2001a and 2001b).

In the EU, the classical toxicology tests required include single dose toxicity, repeated dose toxicity, tolerance in the target species, reproductive toxicity, mutagenicity and carcinogenicity. Studies on other effects include immunotoxicity, microbiological properties of residues, observations in humans, and neurotoxicity (EC, 2001). In the determination of a microbiological ADI, various *in vivo* and *in vitro* methods have been developed for testing the effects of the drug residues on human intestinal microbiota. Changes in microbiota can be measured by selectively culturing and identifying the predominant species by traditional

bacteriological methods, by enumerating the enterobacteria as an indication of colonization resistance status, by measuring the biochemical activities of the bacterial enzymes in human fecal samples and by monitoring the susceptibility of the microbiota to colonization by a challenging organism. Studies *in vitro*, such as those to determine the MIC, are relatively simple to carry out and inexpensive, but are not always representative of the relevant bacteria, and may not take into account factors such as pH, anaerobiosis and the barrier effect (Boisseau, 1993; Cerniglia and Kotarski, 1999).

The US FDA does not accept the use of *in vitro* MIC data for establishing a microbiological no-effect level. The human-microbiota-associated rodent model may have high relevance in determining the effects of low concentrations of antimicrobials on human microbiota (Boisseau, 1993). An *in vitro* gut simulation model (McConville *et al*., 1995) has also been considered as a good means for testing the effects. Studies in human volunteers enable the establishment of a no-effect level in conditions which most closely mimic the conditions of use. However, they are less favorable from a practical and ethical point of view (Boisseau, 1993). According to US FDA, (1996) the model system used to establish the microbiological NOEL evaluates changes in metabolism of intestinal microbiota, the selection of antimicrobial resistant organisms, and/or changes in colonization resistance to potentially pathogenic microorganisms (Greenless, 2003).

2.2.7.2 Maximum Residue Limit (MRL)

According to Council Regulation 2377/90 (EEC, 1990) maximum residue limit is the maximum concentration of residue of both the parent drug and/or the metabolites resulting from the use of a veterinary medicinal product which may be legally permitted or recognized as acceptable in or on a food. It is based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the ADI. In calculating

MRL and ADI, the residue depletion patterns of a compound in the edible tissues of a particular food-producing animal and the theoretical food intakes are taken into account. Possible persistence of residues in organs or at the injection sites is also considered (Fitzpatrick *et al*., 1995; EC, 2001). Once the process of safety evaluation is complete and MRLs have been derived for a particular substance, consideration is given to the likely level of residue which may be expected to remain after the use of the substance in accordance with good veterinary practice, and to the availability of analytical detection methods suitable for use for routine monitoring purposes. Internationally the MRLs and ADIs are developed and recommended as Codex MRLs (i.e. international standards) after the risk assessment has been performed by Joint FAO/WHO Expert Committee on Food Additives JECFA of the Codex Alimentarius Commission. The MRLs may be further reduced to take safety factors into account (EEC, 2001).

2.2.7.3 Withdrawal period

Following the use of a veterinary drug in food animals, a specified period of drug withdrawal is set to be observed prior to providing any products for human consumption. This period will ensure that the drug residues have declined to a safe concentration in the edible portions of the animals It is the time which passes between the last dose given to the animal and the time when the concentration of residues in the tissues: muscle, liver, kidney, skin/fat or products milk, eggs, honey is lower than or equal to the MRL (Jackson, 1980). The CVMP recommends the use of a statistical method in the assessment of a withdrawal period (CVMP, 1995) whenever possible, and particularly for products containing new chemical entities. Linear regression technique is recommended. A withdrawal period is determined at the time when the upper one-sided tolerance limit with a given confidence when the residues in all tissues of all observed animals have fallen below the respective MRLs (Concordet and Toutain, 1997). Failure to adhere to these recommended periods is reported to be the primary

cause of violative levels of veterinary drugs in food (Kabir *et. al*., 2004; Kukanich *et al*., 2005). The maximum residue limit for veterinary drugs (MRLVD) can be assured with good practice in the use of veterinary drugs which implies the official recommended or authorized usage including withdrawal periods, approved by national authorities, of veterinary drugs under practical conditions.

2.2.8 Detection and Analysis of Antimicrobial Residues

The effectiveness and accuracy of the residue monitoring and surveillance program depend on the proper choice of the antibiotic screening and analytical tests. These tests designed for use by regulators and producers are used for the detection of antibiotic contamination in food to reduce or prevent the introduction of contaminated meat and milk into food chain (Popelka *et al*., 2005). Since residues are present in very low levels in foods very sensitive methods are used for their detection, also due to the diversity of their chemical structures, the methods of choice are expected to be discriminatory i.e. sensitive and specific. In recent time the methods for detection and characterisation of chemical and veterinary drug residues in foods of animal origin has been a dynamic area in food processing and is experiencing important developments mainly from the standpoint of food safety undergoing (Toldra and Reig, 2006). Different nationals and food safety regulators are developing and adopting varieties of techniques; however, CAC reviews and recommends both the qualitative and quantitative approaches for international equivalence in food trade.

Several methods have been developed for the detection and quantitative analysis of drugs residues in animal tissues (Crosby, 1991). The analysis of antibiotic residues in animal tissues is generally grouped into qualitative (screening) and quantitative (confirmatory or physico-chemical) methods. The methods involved include microbiological assay, immunoassays and several forms of chromatographic analysis such as thin - layer chromatography (TLC), high

performance TLC, gas - liquid chromatography (GLC) and high performance liquid chromatography (HPLC) (Okerman *et al*., 1998a). An essential tool in assuring the safety of food products is the availability of a simple and reliable screening system for the detection of antibiotics (Popelka *et al*., 2005). Regulatory monitoring programmes involve analysis of large samples which may be prohibitive in term of cost (Fletouris *et al.*, 1990). Hence the common approach is the use cheap, sensitive, simple and rapid screening procedure initially to reduce numbers of samples to the suspected cases which are further confirmed by chemical methods. Thus a screening method is the first-hand analysis of the sample to establish the presence or absence of residues (Aerts *et al*., 1995). Screening tests allow a large number of samples to be analyzed in relatively short time, and are designed to minimize the number of false negatives. When a positive sample is found by a screening test, a confirmatory test is carried out, which normally involves a more sophisticated testing method that provides full or complementary information, enabling the substance to be identified precisely (Popleka, 2005).

2.2.8.1 Screening Methods

2.2.8.1.1 Microbiological Assay

The traditional methods of residue analysis in animal tissues, eggs and milk have been microbiological procedures. An appropriate microbiological method should be a low-cost and high-sample throughput method, optimised to prevent false-negative results and to have an acceptable number of false-positive results (Heitzman, 1994; Korsrud, and MacNeil, 1987). Microbiological assays depend on the ability of antibiotics to inhibit the growth of sensitive bacterial test organisms. This growth inhibition can either be manifested as failure to form colonies whereby a clear zone of inhibition is observed, or by the suppression of the organism's normal metabolic processes which results in the production of acid or gas

(Crosby, 1991). Traditionally the methods require overnight incubation and are considered as multi-residue screening tests for antibiotics in milk, meat and other animal tissues. The size of the inhibition zone is determined by the interaction between the growth of the test organism and the diffusion of the antibiotic from its point of application in the test agar (Crosby, 1991). Methods based on inhibition are useful for the detection of an antibiotic or a group of antibiotics. For these tests, the micro-organism Bacillus spp. is often used. However, inhibition tests may be of high sensitivity but they lack specificity. They cannot distinguish among different forms antibiotics, therefore every inhibition test can only be used as a tool for screening. Some of the microbiological methods may be very time-consuming as they may require 24 hours incubation. Several microbiological assays have been described for the detection of antibiotic residues in meat.

(i) The four-plate test (FPT)

The FPT was described in the Manual of Reference Materials and Methods to Detect Veterinary Drug Residues (Heitzman, 1994), originally, the FPT was developed to identify five different groups of antibiotics: beta-lactam antibiotics, tetracyclines, sulphonamides, aminoglycosides and macrolides. The method does not detect sulphonamides, and is not reliable for detection of aminoglycosides or macrolides in meat (Okerman *et al*., 1998b). The method involves incubation of thin slices of meat on agar plate inoculated with *Bacillus subtilis* spores at pH values 6, 7.2, and 8, together with a further plate inoculated with *Micrococcus luteus* at pH 8. The formation of annular zones of inhibition of at least 2mm wide to one or both microorganisms indicates the presence of an antibacterial substance. The FPT is based on the inhibition of growth of the micro-organism, which is included in the test. This inhibition becomes visible as a clear zone around the sample; in the case antibiotic substances are present at or above the limit of detection (LOD) of the plate. The size of this zone is dependent on the antibiotic concentration present in the meat sample.

(ii) The swab test on premises (STOP)

STOP is another microbiological method of detecting antimicrobial residues developed in the USA. This assay was specifically designed to be used by non-specialist inspectors at the slaughterhouses for the routine Control Of antibiotic residues in meat. In this method, slits are cut into the carcass and sterile cotton swabs are inserted for 30 minutes to soak up the exudate. The swabs are then laid on prepared agar plates seeded with *Bacillus subtilis* and incubated overnight at $29^{O}C$, after which the plate is observed for inhibition zones.

Microbiological methods are suitable for large scale screening because of their convenience and broad spectrum characteristics (Aerts, 1995). In the search for rapid methods for determining the interaction of antimicrobial agents and organisms, intermediate and end products of bacterial metabolism, as well as the interaction of the organism with various energy sources have been examined (Amsterdam, 1996). Currently, most microbiological methods used in antimicrobial residue analysis are based on agar diffusion, which have been developed to rapid screening kits.

2.2.8.1.2 Immunological Techniques

Antigen and antibody reaction has been used for many years to detect a wide variety of food constituents including substances responsible for adulterations and contaminations. The basis of immunoassays is an interaction between an antibody and a corresponding antigen, and the detection of the interaction. In the immunoassay analysis of antibiotic residues in meat, the antigen is the antibiotic, but because most of the antibiotics have low molecular weights, they are by themselves not immunogenic, and have to be coupled with another molecule to form hapten, which are then rendered immunogenic by the attachment of a large macromolecular substance such as albumin (Heitzman, 1994). The detection of the antibody-antigen interaction is facilitated by the use of labels. These labels, which can either be attached to the

antigen or the antibody may be radioactive atom in radio immunoassay (RIA), an enzyme in enzyme immunoassay (EIA) or a fluorescent substance in fluorescence immunoassay (FIA) (Heitzman, 1994). The interaction antigen–antibody is very specific and useful for the detection of residues of chemical and veterinary drugs in animal foods. They are relatively easy to perform and capable of detecting very low levels of residues even if the residues are covalently bound to proteins. However, their application is limited by the availability of suitable antibodies. In addition, immunoassays frequently lack specificity, since any compound, part of which is identical with or closely similar to the antigenic determinant of the analyte can compete for antibody binding sites. The most usual technique consists in the enzyme-linked-immunosorbent assay (ELISA) and the detection system is usually based on enzyme-labelled reagents. ELISA kits have been developed, they showed good performance for analysing antibiotic residues like tylosin and tetracycline in meat (Draisci *et al*., 2001), chloramphenicol in milk and meat (Gaudin *et al*., 2004). Delwiche *et al*., (2000) developed an enzyme immunoassay to detect penicillin residues in meat and dairy products. Radioimmunoassay (RIA) is another technique which involves measurement of radioactivity of immunological complex using a counter (Delwiche *et al.,* 1991). A preliminary purification step is often necessary whereas some require the use of bio-hazardous radioactive isotopes which have a relatively short shelf life (Katz, 1982).

2.2.8.1.3 Antibiotics Residues Rapid Screening Kits

Several commercially immunological (Charm II test) or ELISA and microbial inhibition (Delvotest SP and Premi®Test) test kits have been developed for rapid screening and detection of antibiotics residues in food.

(i) Charm II Test

The Charm II test produced by Charm Sciences Inc., USA is a screening test, used in different food items such as meat, milk and testing. It is based on the specific binding of antibiotics to receptors. The quantitation is determined by measuring of radioactivity: H^3 r C. The test has been shown to detect the several groups of antibiotics in food sulfonamides, tetracyclines, beta-lactams, macrolides, amphenicols and aminoglycosides of the streptomycin type. The screening by the Charm test will exclude the negative samples with low rate of false positive results (Edder and Corvi, 2001).

(ii) Premitest

Premi®test (DSM food industries, Netherlands), is commercially available in kit form and has been shown to be responsive to wide variety of antibiotics (Stead *et al*., 2004). It provides a simple yes/no response to presence of antibiotics using *Bacillus stearothermophilus*, a thermophilic bacteria, which is responsive to all of the most commonly used antibiotics and provides a measurable effect when exposed to samples of contaminated meat or other substrates (Reybroeck 2000). The Premi®Test is based on the inhibition of growth of *Bacillus stearothermophilus*, a thermophilic bacterium sensitive to many antibiotics (Stead *et al*., 2004). The microbiological inhibition by this kit is obtained within 3hours and has been validated to detect residues of some antibiotics below MRLs (Popleka *et al.*, 2005). Premi®Test is simple, cheap, and practical in routine residue testing. The great advantage of the Premi®Test is the short time of the analysis within 4 hours. Premi®Test involves simple equipments and techniques that can relief the chemical analytical resources of food inspection laboratories (Popleka, 2005).

2.2.8.2 Physico-chemical (Quantitative) Residue Analysis Methods

According to the Codex Guidelines for the Establishment of a Regulatory Programme for Control of Veterinary Drug Residues (CCFH, 1993), methods that are suitable for determining compliance with MRLs are those that have successfully completed an extensive, multi-laboratory study for specific tissue and species combination. In some cases, these methods may be considered reference methods. Chromatographic methods are commonly employed in the confirmatory quantitative analysis of drugs and pesticides residues in different food and tissue matrices.

2.2.8.2.1 Chromatographic Techniques

Chromatography is a common name for techniques based on the partition of the molecules to be analyzed between a mobile and a stationary phase. Separation is the result of different partitions of molecules between the two phases. Because of the high sensitivity, selectivity, and reproducibility of chromatographic methods have been extensively exploited in food and nutrition science and technology (De Wasch *et al*., 1998). Chromatography is a method of analysis in which the flow of solvent or gas promotes the separation of substances by differential migration from a narrow initial zone in a porous sorptive medium. Chromatography method enables the qualitative and quantitative analysis of residues of pharmacologically active substances in food products of animal origin. Chromatographic techniques include high performance liquid chromatography (HPLC), gas chromatography (GC) and thin layer chromatography (TLC). Thin layer chromatography are useful for handling many samples simultaneously and may greatly assist in residue identification through the use of derivatizing reagents specific for each functional group but precision cannot be compared with that of gas chromatography or high performance liquid chromatography (Aerts *et al*., 1995; McCracken *et al*., 2000).

Gas chromatography (GC) is the common name for chromatographic methods in which the mobile phase is gas, and the stationary phase is solid or liquid (gas– solid chromatography (GSC) or gas–liquid chromatography (GLC)). A compound needs to have an appreciable vapour pressure at temperatures below 350–400^{o}C and has to be vaporized rapidly without decomposing or reacting with the components of the stationary and mobile phase. Gas chromatographic technique has very limited application to the determination of antibiotic residues in animal tissues (Shaikh and Moats, 1993; Botsoglou and Fletouris 2001) because most antimicrobial agents are non-volatile and will required to be made volatile by derivatization. Most antimicrobials are either insufficiently volatile and are thermally unstable to permit their analysis using GC (Oka *et al*., 2000). Losses of residues can occur during derivatization. Since residues occur at low concentrations, such losses may significantly affect the reliability of the analysis (Shaikh and Moats, 1993; Kennedy *et al*., 1998).

High performance liquid chromatography offers many advantages in residue analysis over other analytical methods (Botsoglou and Fletouris 2001). It is a very useful technique for the separation of compounds which are not easily separable by other means and gives the best separation efficiency (Oka *et al*., 2000). High performance liquid chromatography (HPLC) has been applied successfully for the qualitative and quantitative detection of multi-residues in food samples. HPLC is getting expanded use in control laboratories due to the possibility to analyse simultaneously multiple residues in a sample in relatively short time and is increasingly used in the field of residue analysis. The popularity of the method is largely due to the variety of mobile phases, the extensive library of column packing, and the variation in modes of operation e.g. normal - phases (partition) ion exchange. Recent developments of high speed HPLC can reduce sample treatment and analysis time. In addition, this technology is fully automated (injection, elution, washing of column, detection) and computer-controlled,

facilitating its use as a screening technique. The basic isocratic high performance liquid chromatography can be considered to consist of an injection loop, high-pressure pulse – pump, column with appropriate packing and a detector. A typical HPLC analytical scheme involves sample preparations (extraction and clean-up), chromatographic separation (elution), resolution and detection.

(i) Sample Preparation

Sample pre-treatment before chromatographic separation involve extraction and clean-up. In the analysis of residues from edible animal products, the sample often has a very low content of the residues but much higher concentration of endogenous interfering components. Physico-chemical methods usually proceed with a preliminary extraction in order to isolate the drugs of interest from the biological matrix. The main objectives of sample treatment are removal of macromolecules and other matrix constituents that may either adversely affect the chromatographic system or interfere with the detection, and enrichment of the analytes in order to achieve the required low limits of detection (Aerts *et al*., 1995).

(a) Extraction

According to Botsoglou and Fletouris (2001), the first step in residue analysis of food of animal origin is sample deproteinization. This may be accomplished by the addition of mineral or organic acids such as hypochloric or trichloroacetic acid and/or water-miscible organic solvents such as acetonitrile, acetone or methanol which precipitate the protein to allow their removal by centrifugation. Organic solvents such as dichloromethane or chloroform, although less efficient precipitants can be used but much higher volumes are required. Sample deproteinization not only protects the HPLC column from irreversible contamination, but may also be effective in releasing protein bound residues (Shaikh and Moats, 1993). Extraction efficiency is generally determined by the polarity of the extracting

solvent, the PH of the sample/solvent system, the sample to solvent volume ratio and the method of contacting sample and solvent.

(b) Clean-up

The extract obtained from the extraction process most often contains too much co-extractive, which might interfere with antibiotic detection. This necessitates the need for sample clean-up. Clean-up refers to the separation of the analyte from co-extracted matrix constituents. Several methods are used for this process; the easiest method is the liquid-liquid partitioning between immiscible solvents allowing the analyte to be selectively partitioned in one of the two phases and most of the interfering components in the other phase. In this method the extract and an immiscible solvent are manually or mechanically shaken and allowed to separate in a funnel. Organic solvents are usually used as extracting agents, they are better than water because of their power to denature proteins which permits not only the extraction of free residues but also of non-covalently bound residues (Botsoglou and Fletouris (2001).

Solid phase extraction SPE also known as liquid/solid extraction method involves the use of a solid surface (usually based on a powdered silica to which organic functional groups have been bonded) as the extracting phase, on which the compounds to be extracted can be retained. The type of SPE to be used is chosen based on the nature of the compounds and the solution from which the compound is extracted. This clean-up method is rapid and requires lesser amount of solvents than Liquid-solid extraction or solid phase extraction (SPE) procedures on the other hand, offer an important alternative to solvent partition to eliminate problems associated with the separation of the two liquid phases (Oka *et al*., 2000). Disposable SPE columns or cartridges containing a wide range of adsorbent including silica, alkyl-bonded silica or ion exchange materials are commercially available.

(ii) Chromatographic separation

HPLC analysis of antimicrobial residues can be performed in either the normal or reverse-phase mode, and in the ion - exchange mode. The choice is governed by the polarity and the ionisable groups of the analytes. However, simultaneous separation of both ionised and non-ionized compounds cannot be carried out. In the reverse-phase HPLC, many parameters can influence both the resolution of the compounds and column efficiency (Botsoglou and Fletouris (2001). A combination of the appropriate stationary/mobile phase system and mode of elution (isocratic or gradient) help to obtain best results.

(iii) Resolution and Detection

After the clean-up process, the purified extract is analysed by a suitable chromatographic method in order to detect, identify and to measure quantitatively the analyte. Most antimicrobials have relatively high molar absorbtivity within the UV absorption range, such that UV detection permits quantitation to be made with high sensitivity. Single/variable wavelength UV detectors or UV with spectrophotometers equipped with stop-flow scanning capabilities and photodiode array UV spectrophotometers have all been widely used for quantitative purposes. Antimicrobials that strongly fluoresce can be detected by fluorescence spectroscopy. The elute compounds are usually identified by comparing their retention time with those of reference analytical standards processed in an identical manner.

2.9 Antimicrobials use in Livestock

Veterinary use of antibiotics started soon after it became available for the treatment of human diseases in mid 1940s. Penicillin was used to treat mastitis before World War II. In food animals, antimicrobials are used for the control (prophylaxis) and treatment (therapeutic) of bacterial associated infectious diseases as well as for growth promotion purposes (Phillips *et*

al., 2004). The use of drug in food animals are supposed to the principle of Good Veterinary Practice (Nouws, 1990). In relation to pharmacotherapy GVP is defined as; The selective use (in accordance with directions for users) of veterinary drugs registered by the authorities in those indications in which they are permitted when the diagnosis has been established and in which the problem of residues in foods of animal origin on using these agents has been taken into account (van Miert, 1990). Farmers and veterinarians have a key role to play in the avoidance of residues by ensuring safe and prudent use of veterinary medicines such as the need to follow label instructions and observe withdrawal periods.

The accurate figures on the antibiotic use in humans or animals are usually difficult to obtain since the use are affected by various socio-cultural, economic and political factors (Okeke *et al*., 2007). Antimicrobials represent the largest proportion of pharmaceutical sales both in volume and money value of any drugs used in animal production (Kaneene and Miller, 1997). It has been estimated that as much as 50% of total antibiotic production (by weight) is used in animals and plants, with 50–80% used in some countries for growth promotion or disease prophylaxis and the rest used for therapeutic purposes (WHO, 2000). In the United States alone some 15 million pounds of antibiotics are administered to farm animals annually (Walter, 2005), while Mellon, *et al*., (2001), estimated annual use of antibiotics by livestock producers in the United States as 12.5 million kilograms of antimicrobials for non-therapeutic purposes. Most data on antimicrobial use in humans and animals are based on the monetary value of sales of antibiotics per annum (Kaneene and Miller, 1997). However, antibiotic use in humans is more expensive per gram of active compound than therapeutic and growth promoter antibiotic use in veterinary medicine and animal husbandry (Gustafon and Bowen, 1997; van den Bogaard and Strobberingh, 1999). They concluded that consumption of antibiotics by animals might be higher, because the lifespan of food animals is short (approximately 7 weeks for broilers, and 6 months for fattening pigs). More so, that the

dosage per kg bodyweight in small animals like poultry, is much higher than that in humans. Prescott (1997) and Levy (1998) also estimated about 55 to 60% of benzylpenicillin (penicillin G) and tetracyclines used in US were used in food animal feeds mainly at subtherapeutic doses mixed in animal feeds. In the EU and Switzerland in 1997, 10493 tons of antibiotics (active ingredient at 100% purity) were used and of this amount nearly 50% was used in animals where 3474 tons (33%) were used in animals for therapy and prevention, and 1590 tons (15%) as growth promoters. According to the European Federation of Animal Health Industries (FEDESA), the World Animal Health Product Market was, at manufacturers' prices, 11 billion Euros in 1995, of which 44%were therapeutic pharmaceutical products and 41% feed additives (EU, 2002).

2.9.1 Antibiotics as Growth Promoters

Antibiotics are used in food animals both therapeutically to treat disease and subtherapeutically, usually over long periods, to improve their rate of growth and feed conversion efficiency. The mechanisms responsible for growth promotion have not been fully elucidated but appear to include enhancement of vitamin production by gastrointestinal microorganisms, elimination of subclinical populations of pathogenic organisms, and increased intestinal absorption of nutrients (Committee on Drug Use in Food Animals, 1999). The use of antibiotics as feed additives especially in broilers and fatteners has been shown to improved growth performance and improve feed efficiency 17% in beef cattle, 10% in lambs, 15% in poultry and 15% in swine (Nisha, 2008). They may produce improved growth rate by the thinning of mucous membrane of the gut, facilitating better absorption, altering gut motility to enhance better assimilation, producing favourable conditions to beneficial microbes in the gut of animal by destroying harmful bacteria and partitioning proteins to muscle accretion by suppressing monokines. Antibiotics also favour growth by decreasing degree of activity of the immune system, reduced waste of nutrients and reduce toxin

formation. Indiscriminating use of antibiotics in all cases of pyrexia, inflammation, wounds and viral diseases have widespread residual effects on edible tissues (Nisha, 2008).

Most livestock producers indiscriminately feed antibiotics to healthy farm animals to promote growth and compensate for unhygienic husbandry. Van den Bogaard, (1998) concluded that the selection pressure exerted by the veterinary use of antibiotics on the animal bacterial population selection pressure could be more than doubled in intensively reared animals than by human use due to their use as growth promoters along with prophylactic and therapeutic uses. The use of antimicrobial growth promoters in particular, has being receiving global attention and scrutiny as most antibiotics used in animals are also used in human medicine. The use of antimicrobial growth promoters have direct association with the resistance observed in indicator bacteria isolated from livestock animals (Bates et al., 1994; van den Bogaard and Stobberingh, 2000; Swartz, 2002). These have led to regulatory precautionary measures taken against the use of certain antimicrobial agents (Swartz, 2002). In the European Union, the only four agents are acceptable for use as AMGP are monensin-Na, salinomycin-Na, flavophospolipol, and avilamycin (Butaye, 2000). These antimicrobial agents are considered unlikely to exhibit cross-resistance with therapeutic compounds used in human and veterinary medicine (Schwarz *et al*., 2001).

2.9.2 Antimicrobials Use in Animal and Resistant Bacteria Transfer to Man

There is a casual relationship between increased use of antibiotics and increased prevalence of resistant bacteria has been demonstrated (Holmberg *et al*., 1987). The question of whether antibiotic use and antibiotic-resistant isolates of bacteria from animals have an impact on human health has been under scrutiny since the Swann (1969) report was published. WHO has recognized the global public health threat of increasing antimicrobial resistance as ominous and reported that the window of opportunity for the control and elimination many of the infectious diseases, including those caused by antibiotic resistant bacteria is closing

(WHO, 2000). It is generally accepted that antimicrobial resistance in veterinary medicine could form a potential public health hazard. Indeed, the commensal gastrointestinal flora (indicator bacteria) of healthy animals harbour reservoir of resistance genes that are transferable to human being through the food chain or by direct contact (van den Bogaard and Stobberingh, 1999). This is more important because livestock are carriers of food-borne pathogens such as Salmonella and Campylobacter species that constitute zoonotic pathogens undergoing selection pressure due to the use of antimicrobial drugs resulting in clonal and horizontal transfer of resistance genes. These are transferable to humans through the food chain and/or through direct contact during processing (Kruse *et al*., 1998).

The wide spread use of antibiotic in human and animals has been followed by the increased emergence of bacteria resistance to these antibiotics, particularly in enterobacteriaceae (Prescott and Baggot, 1993). Most concern about antibiotic resistance in animal isolates of bacteria is directed towards the enteric bacteria, *E. coli, Salmonella,* thermophilic *Campylobacter* and enterococci. There is considerable information on antibiotic resistance in *E. coli and Salmonella,* as these bacteria are recognised pathogens in animals, but there is relatively little information about antibiotic resistance in thermophilic campylobacters and enterococci, as they are regarded as commensal enteric organisms rather than animal pathogens. Antibiotic resistance was detected in isolates of *E. coli* from animals soon after antibiotics were incorporated into animal feeds (Smith 1967). Studies in the UK found that, in the late 1950s, tetracycline resistance was already detectable in *E. coli* isolates from chickens and pigs fed rations containing less than 100 g tetracycline/ton (Smith, 1967). Resistance to other antibiotics was detected as new agents were introduced for therapeutic and growth-promotant purposes (Smith, 1967; Anderson, 1968). Some workers (Linton *et al*., 1985; Lee *et al*., 1993) also noted the occurrence of tetracycline resistance in some piggeries, even though tetracyclines had not been used in those piggeries. A common finding has been that

resistance persists after antibiotics are withdrawn (Smith, 1973; Hinton *et al*., 1985). Feeding oxytetracycline to recently-weaned pigs was found to lead to a rapid increase in the incidence of tetracycline resistance, which was widely distributed among all strains of E. coli present, rather than being restricted to a few selected clones (Hinton *et al*., 1985). The feeding of low doses of ampicillin to chickens was shown to select for high levels of resistance to that antibiotic (El-Sam *et al*., 1993). Marshall *et al*., (1990) described an elegant experiment that demonstrated that resistant strains of *E. coli* spread among animals (and to other species such as mice), even in the absence of ongoing antibiotic treatment. It is clear that resistance to antibiotics has become very common in *E. coli* over the 50 years of use of in-feed antibiotics (Aalback *et al*., 1991; Adesiyun and Kaminjolo, 1992; Nijsten *et al*., 1993; Dunlop *et al*., 1998a; Orden *et al*., 1999; Lambie *et al*., 2000). Widespread resistance was seen in herds and flocks treated with tetracycline, aminoglycoside and sulphonamide, (Dunlop *et al*., 1998a; Sunde *et al*., 2006). However, resistance to other antibiotics such as ampicillin and olaquindox was less widespread (Linton *et al*., 1988; Dunlop *et al*., 1998). Resistance to fluoroquinolones have also been reported (Blanco *et al*., 1997). Resistance to more than one class of antibiotics was the rule rather than the exception in these published studies.

Antibiotic resistance in *salmonella* was also reported soon after antibiotics began to be fed at subtherapeutic levels to animals (Anderson, 1968). As *salmonella* is a recognised food-borne pathogen, a number of the published reports of resistance patterns in animal isolates have been linked with studies of human isolates (Threlfall *et al.*, 2000; Seyfarth *et al.*, 1997). Some countries have carried out surveys of resistance in animal isolates of *salmonella* or have ongoing antibiotic-resistance surveillance programmes (Wray *et al*., 1996; Seyfarth *et al*., 1997; Davis *et al*., 1999). The results were not easy to interpret because some reports do not distinguish between different serovars of *Salmonella* enterica and it is recognised that some serovars such as *Typhimurium* are much more likely to be resistant than other serovars.

Resistance to apramycin was reported in both *E. coli* and *Salmonella* within 3 years of its licensing for use in the UK (Wray *et al*., 1997) and fluoroquinolone resistance within a few years of enrofloxacin first being used (Griggs *et al*., 1996).

There are few reports on antibiotic resistance Thermophilic *Campylobacters* which are mostly commensal enteric commensal of farm animals. Moore *et al*., (1999) reported resistance *Campylobacter coli* isolates. Aarestrup *et al*., (1997) found that tetracycline resistance was more common in human isolates than in pig or poultry isolates, and that there was more macrolide and streptomycin resistance in isolates from pigs than in human and poultry isolates. Resistance to ampicillin, spectinomycin, streptomycin, sulphonamides and nalidixic acid (but not fluoroquinolones) was detected in campylobacter isolates in Ireland (Lucey *et al*., 2000), whereas a study in Spain (Saenz *et al*., 2004) reported very high levels of ciprofloxacin resistance in pig and poultry isolates. In addition, there were high levels of resistance to erythromycin, ampicillin, gentamicin and amikacin in pig isolates and high levels of ampicillin and gentamicin resistance in poultry isolates. The difference between these two studies presumably reflects differences in antibiotic usage in the two countries.

According to Witte, (1998) antibiotic resistance is a complex ecological phenomenon, resistant genes and bacteria can be transmitted in several ways such as via food, water, occupational exposure by farmers, hospital workers butchers and food processors. Also the local water ways and ground water get contaminated with these bacteria via animal manure used in agriculture (figure 2.2).

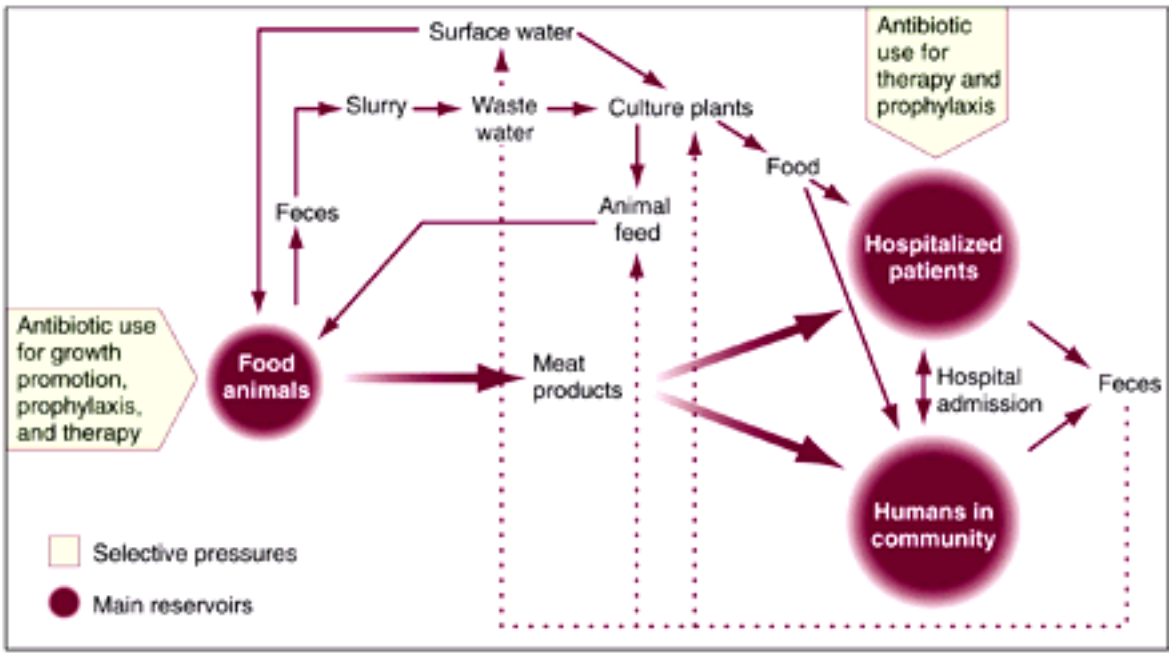


Figure 2.3: Ecological relationships between antibiotic-resistant bacteria and resistance genes: selective pressures, main reservoirs, and routes of transmission (Witte, 1998)

2.9.3 Regulatory Control of Veterinary Drugs Use and Residues in Food Animals

Food safety and quality have become increasingly important world-wide in recent years, not only in terms of protecting the health of the consumers, but also to meet requirements for international trade. Application of necessary analytical capacity to detect and monitor food contaminants such as pesticides and veterinary drugs during the production process and in finished food products is critical to assurance of chemical safety of the agricultural products (SOFA, 2009). The ability to demonstrate the origin and the authenticity of food products is also a major concern to food safety regulators and to trading partners due to increasing mobility and cross-border transportation of food commodities. Modern food production systems are supposed to be designed and managed to ensure that the exposure of food producing animals to veterinary drugs does not pose a risk to human health. Veterinary drugs are regulated in many countries for a variety of reasons, such as animal health, consumer protection, animal welfare and protection of the environment (CAC, 2009). Competent authorities responsible for providing consumer assurances of food safety and are supposed to ensure that it has sufficient knowledge of and control over veterinary drugs that are being sold and used within the production systems and have sufficient knowledge of food safety (CAC, 2009).

2.10 FAO/WHO Residue Control and World Trade Organisation (WTO)

The control of residues of veterinary drugs in animal products intended for human consumption is a question related to human health which falls as such under the purview of the Agreement of Sanitary and Phytosanitary Measures (SPS). The agreement stipulates that any SPS measure applied by a government to protect should conform to general WTO and more specific SPS rules if it affects products traded between two WTO members. The relevant definition of scope of SPS measure “human and animal life or health from risks from

food additives, contaminants, toxins, disease causing organisms in foods, beverages or feedstuffs" includes also measures that address residues of veterinary drugs (SPS Agreement). Such measures may be laid down in laws, decrees, regulations, requirements or procedures related to the various actions that control or affect the putting into circulation of a product. Among the measures listed by the SPS agreement some of the following may be relevant to the question to be studied: end product criteria, production methods, testing, approval procedures, sampling procedures or risk assessment. The SPS Agreement explicitly recognizes the Codex Alimentarius and therewith the work of the Codex Alimentarius Commission which means that a country implementing a Codex standards fulfils the obligation resulting from its membership in WTO and with the relevant provisions of the SPS Agreement.

2.10.1 Codex Alimentarius Commission (CAC)

At the beginning of the 1960s the FAO Conference and the World Health Assembly decided to launch a joint effort in the area of food which should protect the health of the consumers and ensure fair practices in the food trade. This activity was and continues to be called the Joint FAO/WHO Food Standards Programme. The Codex Alimentarius Commission (from Latin words 'Food Code'), a subsidiary organ of FAO and WHO, develops the internationally-recognised food safety standards including veterinary drugs and pesticide residues in food. The CAC was jointly established by FAO and WHO in 1963 to promote fair trade while considering the global economic and personal health of the consumer. Currently, there are 175 member countries subscribing to the international standards, codes of practice and guidelines to facilitate international trade of food products. CAC also provides information on innovative food safety systems, new technology and trade practices (Moulin and Lambert, 2008). The Codex Alimentarius Commission has the objective of protecting the health of the consumers and ensuring fair practices in the food trade by developing food

standards and other texts related to food safety and quality. With respect to veterinary drugs residues, the Commission recognized that "the occurrence and safety of residues of veterinary drugs in foods of animal origin was of significance to public health and consumer concern". In 1985 it established the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), which was tasked to:

- determine priorities for the consideration of residues of veterinary drugs in foods;
- recommend maximum limits of such substances;
- develop codes of practice as may be required;
- consider methods of sampling and analysis for the determination of veterinary drug residues in foods.

The Codex Committee on Residues of Veterinary Drugs determines priorities for the consideration of residues of veterinary drugs in foods and recommends Maximum Residue Limits (MRLs) for veterinary drugs. A Codex Maximum Limit for Residues of Veterinary Drugs (MRLVD) is the maximum concentration or residue resulting from the use of a veterinary drug (expressed in mg/kg or µg/kg on a fresh weight basis) that is recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in or on a food. An MRLVD is based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the Acceptable Daily Intake (ADI), or on the basis of a temporary ADI that utilizes an additional safety factor. An MRLVD also takes into account other relevant public health risks as well as food technological aspects.

MRLs and ADIs are developed and adopted as Codex MRLs (i.e. international standards) after the risk assessment has been performed by Joint FAO/WHO Expert Committee on Food Additives (JECFA). The recommended MRLs are achievable under Good Practice in the Use of Veterinary Drugs (GPVD) and a suitable analytical method is available. The evaluation process depends on the commitment of a sponsor to provide the data and to submit a dossier. Over two decades Codex has adopted MRLs for residues of approximately 50 veterinary drugs.

All Codex food standards and other documents that intend to provide a basis for any regulation along the food chain are based on the principle of sound scientific analysis and evidence, involving a thorough review of all relevant information, in order that the standards assure the quality and safety of the food supply. As described above WTO requires for SPS measures the use of internationally accepted risk assessment techniques which has led to some basic food safety definitions as shown below. The definitions adopted by the Codex Alimentarius Commission (CAC, 2006) were elaborated by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). The definitions were established and adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and have been modified by the Codex Committee on Residues of Veterinary Drugs in Foods).

2.10.2 Control of antimicrobial use and residues in different countries

Drug manufacturers, the regulatory agency, the veterinarians, the producers, and the producer's employees involved in administering the feed additive have collective responsibility for controlling unintended public health impacts of antibiotic use in food producing animals. The regulatory monitoring and control of antibiotics use, resistance and residue vary from country to country. In the UK and other EU countries, antibiotics are authorised as either veterinary medicinal products or zootechnical feed additives. Veterinary

medicinal products and growth promoters are subject to assessment for safety, including residues (veterinary medicines) and the risk of emergence of antibiotic resistance, cross-resistance to therapeutic antibiotics and selection for transferable resistance (Rutter, 1997). EU Member States monitor a set proportion of the total annual production of different food commodities of animal origin for residues, veterinary drugs are monitored for MRL compliance according to Council Directive 96/23/EC EC (1996). The EU has banned the use of avoparcin, tylosin, spiramycin, bacitracin and virginiamycin as animal growth-promoter (AGP) in all member states because they belonged to classes of anti-microbial drugs also used in human medicine and the use of hormonal growth promoter (HGPs) on cattle. The directive establishes the groups of substances to be controlled for each food commodity. National residue control program is carried out in accordance with both national and EU legislation. Meat inspection involves bacteriological examination and testing for antimicrobial residues if there is any reason to suspect that a carcass could contain antimicrobial residues (MAF, 2003). In the EU, maximum residues limits based on microbiological effects have been set for e.g. cephalexin, CTC, doxicycline, EF, erythromycin, florfenicol, gentamicin, kanamycin, lincomycin, marbofloxacin, nafcillin, novobiocin, oxytetracycline, pirlimycin, sarafloxacin, spiramycin and tetracycline (EMEA, 2004). An ADI not based on microbiological effects has been set only for a few antimicrobials, e.g. for danofloxacin, difloxacin, dihydrostreptomycin, neomycin, streptomycin and tiamulin. MRLs for penicillins are based on immunological effects and on the sensitivity of bacteria used in the dairy industry. Because of the impossibility to find a threshold concentration for chloramphenicol in the induction of aplastic anaemia in humans, due among other things to a lack of results from residue and toxicity test studies, no ADI could be set, and chloramphenicol was entered in Annex IV of the Council Regulation 2377/90 (EEC, 1990). Annex IV includes substances for which no maximum residue limit

can be established because residues of these substances, at whatever limits in foodstuffs of animal origin constitute a hazard to the health of the consumer. So, their use is prohibited (forbidden) for production animals. Annex I of the regulation includes substances for which final MRLs have been established, and Annex II substances for which it is not necessary for the protection of public health to establish MRL values. Annex III includes substances with provisional MRLs.

China has regulated the use of antibiotics in animal feeds since 1989 and only non-medical antibiotics such as monensin, salinomycin, destomycin, bacitracin, colistin, kitasamycin, enramycin and virginiamycin are permitted as feed additives. While Russia also restricts feed antibiotics to non-medical drugs; bacitracin, grizin, flavomycin and virginiamycin are registered for use in this way (Panin *et al*., 1997).

Agricultural use of antibiotics in the USA and Canada is also strictly regulated. In USA, antibiotics for animal production are approved by the Food and Drug Administration (FDA) after rigorous evaluation for safety against major risk factors with respect to the animal, the consumer, and the environment and their use is regulated to avoid unintended consequences. There are three categories of use: as feed antibiotics; as over-the-counter drugs; as veterinary prescription drugs. Feed antibiotics include antibiotics used as growth promotants and those used for subtherapeutic (including prophylactic and some growth-promotant use) and therapeutic purposes (Prescott, 1997). But AGPs are still widely used in the United States, However, the Food Safety Enhancement Act of 2009 was recently passed in the US towards securing effective and meaningful food safety regulation. In addition the US House is already considering HR 1549 (Preservation of Antibiotics for Medical Treatment Act of 2009). Feed antibiotics are licensed for specific uses such as for meat chickens or young pigs or calves or feedlot cattle. In the USA preregistration assessment specifically addresses human health issues relating to antibiotic resistance in enteric coliforms, *salmonella* excretion and increased

resistance in *salmonella*, increased virulence and pathogenicity of bacteria, animal disease that is difficult to treat, and residues and risk of hypersensitivity in consumers (Sundlof *et al*., 2000). In the USA, National Residue Monitoring and Surveillance Program of FSIS conducts residue testing. Under the monitoring programme, statistically based selections of random samples from normal animal population is collected and monitor drug residues in their tissues (Sundlof *et al*., 2000).

The Food Standards Australia New Zealand (FSANZ) protects the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply and setting standards MRLs and ADIs (Brent, 2004). There are three points of control of antibiotic use in animals in Australia. First, all importations are controlled by a permit system. Second, at the registration level, there are strict regulatory guidelines over which antibiotics can be used in food-producing animals. Since 1970, antibiotics intended for animal use have been assessed for their potential to compromise human health. As a result, flouoroquinolones, amphenicols, colistin and gentamicin have not been registered for use in food-producing animals because of concerns about antibiotic resistance, and the registration of carbadox was withdrawn in the late 1980s and of nitrofurans in 1992 because of concerns about carcinogenicity (Joint Expert Technical Advisory Committee on Antibiotic Resistance, 1999).

2.10.3 Veterinary Drug Use and Control in Developing Countries

There is a trend toward global increased consumption of meat, dairy and poultry products. It is estimated that the *per capita* consumption in developing countries of livestock products could rise by as much as 44% by 2030 (FAO, 2002). To meet this demand, commercial livestock production by intensive or semi-intensive peri-urban production systems rather than traditional mixed farming systems are being employed. There is a consequent increase in the

use of pharmaco-active compounds such as antibiotics, coccidiostats and growth promoting feed additive to combat challenges of infections and enhance productivity. The use and control of veterinary drugs in developing countries vary between and within regions. Antibiotics are widely used in developing countries, due to the high incidence of infectious diseases in many areas and poor animal husbandry (Mitema *et al.*, 2001; Al Mustafa and Al Ghamdi, 2002). Anthelmintics and trypanocides are also commonly used in of sub-Saharan Africa (Roderick et *al*., 2000). Many of the least developed countries lack the resources to assure food safety, and issues such as drug residues have a very low priority. However, as public awareness of food safety and quality issues grows, there is increased pressure on governments to address these issues even in low income countries. More so as member countries of World Trade Organisation (WTO), there are urge to participate in international livestock trade.

In most developing countries there are no stringent regulatory legislation or mechanisms on the use of veterinary drugs or for residues monitoring in food animals, thus occurrence of residues are usually expected (Cannavan, 2005). Veterinary drugs, including unapproved and unregulated compounds that may have no ADI, can often be easily purchased from village shops and informal vendors (Dina and Arowolo, 1991; Roderick 2000; Keyyu *et al*., 2003; Bett *et al.*, 2004). There are no quality control of the substances supplied and concentrations and dosage rates may be incorrectly recorded (Bett *et al.*, 2004). Antibiotics are also widely used in developing countries, due to the high incidence of infectious diseases in many areas and poor animal husbandry (Mitema *et al*., 2001; Al Mustafa and Al Ghamdi, 2002). However, the quantity used in this area is difficult to obtain due to lack of regulatory control of their uses. In developing countries livestock production are characterised by indiscriminate use of veterinary drugs and easy access to over the counter (Dina and Arowolo, 1991; Mitema *et al.,* 2001). There is often a lack of veterinary advice regarding withdrawal periods

and this may be compounded by illiteracy, rendering labelling and printed instructions for drugs of limited use. Drugs are frequently administered by unqualified farmers or para-veterinary field staff and extended usage or excessive or multiple dosages of the compounds are common. Treatment records are frequently poorly maintained or non-existent and individual animal identification and traceability is often impossible. For resource-poor farmers, a scarcity of meat and the fear of economic loss may prompt the slaughter of casualty animals for food (Cannavan, 2005).

2.10.4 Drug Usage and Antibiotic Residues in Livestock Products in Nigeria

Livestock diseases are major limiting factors affecting productivity and profitability through increase mortality and morbidity rates, reduced rates of reproduction, weight gain and milk production. Bacterial diseases are global livestock challenges and are recognised as a major risk factor in livestock production systems (Aibinu *et al.,* 2007). Super-infections with bacterial agents also accompany other infections by viruses, protozoa and parasites. This engendered livestock farmers in the tropic spend more money on management and control of bacterial diseases. Disease constraints are estimated to cause losses of up to 30% of annual livestock output in developing countries, twice that estimated for developed countries (FAO, 1990a). According to Blench and Marriage (1999), several factors affecting the distribution of livestock in Nigeria include the following broad headings; ecology, feed availability, diseases, animal traction marketing systems and cultural preferences including religion. These factors make the sector to be very complex for structural organisation and monitoring.

In Nigeria like in many developing countries the livestock sector has not received appropriate commitments as it is not properly regulated and it is characterized by indiscriminate use of veterinary drugs including antibiotics which are easily obtainable over the counter without veterinary prescription and supervision (Dina and Arowolo, 1991; Dipeolu and Alonge,

2002). Veterinary drugs including antibiotics are usually available for purchase in open markets over the counter without veterinary prescription and they are usually sold through channels of multiple middlemen mostly traders without knowledge of animal health management (Dina and Arowolo, 1991; Dipeolu and Alonge, 2002). There are several brands of veterinary drugs imported and sold in veterinary shops, livestock markets and drug peddlers directly to the farmers who use the drugs routinely without veterinary diagnosis or prescriptions (Dina and Arowolo, 1991). These encouraged the proliferation and use of substandard or fake drugs thereby increasing the rate of drug misuse and widespread resistance (Dina and Arowolo, 1991, Olatoye, 2010). The practice is also of great economic concerns as wastages result from avoidable drug usage, if proper expertise is involved in the husbandry and veterinary care (Ogundipe, 1997).

The presence of antimicrobial residues in foods is of particular concern in developing countries, because legislation regarding maximum tolerance levels for marketed products is often lacking and violation of withholding time set to terminate drug therapy occurs regularly. In Nigeria various studies have been conducted confirming the misuse of drugs in food animals and residues deposition in meat and animal products (Oboegbulem and Fidelis, 1996; Dipeolu and Alonge, 2002; Kabir *et. al*., 2004). Oyekunle and Olubi, (1992) reported the occurrence of antibiotic residues in broiler meat while Dipeolu *et al.*, (2005) reported the presence of tetracycline residues in eggs marketed in Ogun State. Dipeolu and Ayinde, (2001) found tetracycline residue of 0.017-0.039µg/kg in pork produced around Abeokuta, Kabir *et al*., (2004) also reported the presence of antibiotics residues in slaughtered cattle and chicken in northern Nigeria. Most of these studies were employed on screening microbiological techniques that does not specifically classify and quantify the antibiotics, hence the degree of risks to the consumers could not been ascertained and there is the need to

quantify the levels of residues in meat, milk and eggs available for human consumption in Nigeria.

2.10.5 Nigerian Food and drug Safety Policies

Good food hygiene and safety with optimum nutritional health for all Nigerians though not explicitly expressed, is implicit in the objectives and strategies of the National Health Policy and the Abuja Health Declaration (FAO/WHO 2005). Nigeria as a member of the United Nations has been a signatory to all the Conventions and Declarations on Health issues including codex alimentarius commission and WTO. Various governments in Nigeria over the years have tried in several ways to make provision for the safety and wholesomeness of the nation's food supply. As far back as 1971 to date, several legislative provisions have been enacted in different statutes. These include:

a) Public Health Laws (1917) now known as Public Health Ordinance Cap 165 of 1958;

b) The Food and Drugs Decree, No. 35 of 1974;

c) The Standards Organization of Nigeria Decree No.56 of 1971;

d) The Animal Disease Control Decree No. 10 of 1988;

e) The Marketing of Breast Milk Substitute Decree No. 41 of 1990;

f) The National Agency for Food and Drugs Administration and Control (NAFDAC) Decree No. 15 of 1993.

Most State Governments in the country have promulgated meat edicts for assurance of safety of meat and meat products through proper inspection and hygiene practices. Efforts are been made to harmonise related laws and meat edicts through the proposed "veterinary public health (meat hygiene) act" at the National Assembly. The law when enacted will take care of

many gaps and lopsidedness in the food hygiene laws. The law is also to control transportation and slaughter of food animals and their inspection. It will also regulate interstate and international trade on food animal carcasses, meat and other animal by-products (Aliu, 2004).

Nigeria food and drug policies began with the food and Drug Act of 1974, followed by Drug and Related decree 19 of 1993, the national drug formulary and essential drug list established under Decree 43 of 1989 all of which has been encapsulated into National Agency for Food and Drug Administration (NAFDAC) decree 15 of 1993 and the amendment by decree 19 of 1999 for the control of drugs, processed foods and package, water. Animal disease control decree 19 of 1988 (repealed the Diseases of Animal Act 1917, 1962) empowered the Federal Livestock Division, of Federal Ministry of Agriculture to control exportation and importation of live animals, poultry egg, milk sense, other animal products and biological infections agents. It also provides for surveillance and control of trade animals within the country. Animal disease control decree 1988 (FGN, 1988) saddled the veterinary profession with responsibilities of prevention and control of animal diseases of socioeconomic and public health significance through the control and prevention of such diseases in livestock by ensuring good agricultural practices, good veterinary practices and proper inspection of animal products (meat, milk, egg etc) meant for human consumptions as part of food hygiene.

Meat inspection are being carried out by the Veterinary Department of the State Ministry of Agriculture in major abattoirs across the country, however, it is been efficient due to several factors including shortage of manpower, inadequate abattoir facilities and lack of coordinated livestock production which does not allow for traceability. Meat inspection is usually based on organoleptic visual inspection. In Nigerian abattoirs and slaughter slabs, butchering of meat are done on concrete floor with inadequate slaughtering basic facilities including lack of potable water resulting in unhygienic processing and handling of meat meant for public

consumption. Thus, high level of carcass contaminations with biological, chemical and physical hazards have been reported (Ojo *et al*., 2009;). Regulatory Control of livestock diseases in Nigeria especially those that are of economic and/or zoonotic importance are supposed to be jointly carried out by the Federal, State and to some extent, Local Governments Areas (LGAs). Each of these tiers has its own mandatory function or activity areas. The function of the Federal Government is mostly in the area of national policy formulation and implementation especially in the area of monitoring the activities of the States and LGAs to ensure compliance with laid down principles, standards and goals. The primary responsibility of disease control in their territories is that individual States who carry this out through the provision of Veterinary Clinics, diagnostic laboratories and other facilities such as a major abattoirs. Slaughter slabs are managed by Local Govt Councils.

Also, through the enactment NAFDAC decree No. 15, 1993 (FGN 1993) the National Agency for Food and Drug Administration and control (NAFDAC) was established to perform the following functions:

- Regulation and control the importation, exportation, manufacture, advertisement, distribution, sale, and use of food, drugs, cosmetics, medical devices, bottled water and chemicals;
    - Conducting appropriate tests and ensure compliance with standard specifications designated and approved by the council for the effective control of the quality of food, drugs, etc., as well as their raw materials and production, including processes in factories and other establishments;
    - Undertaking appropriate investigations into the production premises and raw materials for food, drugs, etc. and establish relevant quality assurance systems, including certification of the production sites and regulated products;

- Undertaking inspection of food, drugs etc.;
- Compiling standard specifications and regulations and guidelines for the production, importation, exportation, sale and distribution of food, drugs, etc.
- Undertaking registration of food, drugs, etc;
- Establishing and maintaining relevant laboratory or other institutions in strategic areas of Nigeria as may be necessary for the performance of its functions.

NAFDAC also participate in international food safety efforts of CAC. It has no paid attention to setting and maintaining essential veterinary drug list and veterinary drug residues control (Aliu, 2004). Unlike Food and Drug Act (1974) that has veterinary profession and scientists in their board, NAFDAC does not have representation of veterinary profession in the council and there are few veterinarians in its employment. However, section 8(g) of NAFDAC decree provides for the establishment of directorates, as may be required for proper performance of the functions. Veterinary Directorate have been advocated to reflect veterinary professions in the agency to adequately cater for veterinary drugs, biologics and pesticides chemicals. Also the National Drug Formulary and essential drug list Act 1989, does not include veterinary surgeon should be reviewed and define essential drug to satisfy and care for the needs of majority of animal and human population the list does not accommodate essential veterinary drugs, Biologics and pesticides chemicals. Counterfeit and fake drug (Miscellaneous Poisons) Act 1980 dealing with fake drug, does not have vet representation at both Federal and State task forces. The veterinary and toxicology training make veterinarians vast in the use and possible adverse effects of vet drugs in all species of terrestrial and aquatic animals. There are presumably many fake veterinary drugs that are not being monitored in Nigeria (Aliu, 2004). Therefore the structure and technical capability of

veterinary drug use control and monitoring are either lopsided or there is no coordinated effort on monitoring and control of antimicrobial use, residue and resistance in Nigeria. This study was designed to assess the practice of drug administration in poultry and cattle production and characterised the risks of antibiotics residues and resistance meat-borne *Escherichia coli* O157:H7 in the chicken and beef meat for human consumption in selected cities of south western Nigeria.

CHAPTER THREE

SURVEY OF THE USAGE OF ANTIBIOTICS USAGE BY CATTLE AND POULTRY PRODUCERS IN SOUTHWEST NIGERIA

3.1 Introduction

Veterinary drugs, including antibiotics are important inputs in livestock production. Approximately half of the antimicrobials produced today are used in animal production and they represent the largest proportion of pharmaceutical sales both in volume and money value of any drugs used in animal production (WHO, 2000). According to Mellon, *et al*., (2001), out of the overall annual 17.5 million kilograms production of antibiotics in the United States about 12.5 million kilograms are used for non-therapeutic purposes in livestock production while only 1.5 million kilograms are used for human medical therapy. However in Africa, reliable data on antibiotic consumption humans and animals are not readily available (Mitema *et al*., 2001). Whereas, developed nations are employing stringent control on the use of veterinary drugs to ensure consumer protection, the situation in developing countries is the opposite (Mitema *et al*., 2001; FAO/WHO/OIE, 2008).

In order to ensure food safety and prevent the side effects associated with the use of antibiotics, WHO and OIE recommended "Good Veterinary Practice in the use of Antimicrobial" (GVPA) products. GVPA is defined as a rational antibacterial therapy which is based on a combination of clinical judgement; laboratory diagnosis; epidemiological background and husbandry information about the flock to be treated (WHO, 2000). Drug manufacturers, regulatory agencies, veterinarians, livestock producers and their employees involved in administering the feed additive have collective responsibility for controlling unintended public health impacts of antibiotic use in food-producing animals (WHO, 2001). This work was therefore aimed at investigating the culture (i.e knowledge, atttitude and

practices - KAP) of cattle and poultry producers in south western Nigeria concerning the administration of antibiotics to their animals destined for human consumption.

3.3 Materials and methods

3.2.1 Sampling Procedures

Thirty poultry farmers were selected through multi-stage sampling of representative small, medium and large scale poultry farms in States under study. Willingness to participate in the survey and condition of confidentiality were among the criteria for the selection. Also twenty cattle producers at Ibadan and Lagos cattle markets and agropastoralist settlements at the peri-urban areas of the cities were also selected for the questionnaire interview. The respondents included flocks/herds owners or major operators such as farm managers or supervisors.

3.2.2 Questionnaire interview

The study was conducted using two sets of semi-structured questionnaire (Appendix IIa and IIb). The questionnaire was designed to assess the livestock production system, major health problems of their livestock, and the drug administration of veterinary drugs service in the study area. The first section was on the respondents' production experience, history and nature of the flocks or herds. The second section was on the disease status of the herd or flocks including their frequently occurrence, diagnosis and treatment sevices employed. The names of the diseases were translated from the local names and the description of symptoms by the respondents. The next section on antibiotics usage required the respondents to indicate the frequency of use antibiotics in feed or water and injection. They were also asked to specify, from a list of trade and generic names, the types of in-feed, in-water and injectable antibiotics used for their livestock. The final section of the questionnaire was on assessment of the respondents' knowledge and practice of withdrawal periods.

3.2.3 Data Analysis

The data were entered into Microsoft Office Excel Program after which simple descriptive statistic and chi square test were employed to analyze the data.

3.3 RESULTS

3.3.1 Livestock Farmers Characteristics

Out of the 30 poultry farmers interviewed, 33.3% were female while 66.7% were male. All the cattle producers were males. Their mean age and years of experience are shown on table 3.1. The majority of poultry producers (64%) had more than 10 years of poultry farming experience, with an average of 10.6 years, while the cattle producers had an average of 19.4 years cattle rearing experience. The education levels of the poultry farmers ranged from primary school certificate to university degree among them are three (6.7%) veterinary doctors while greater proportion (85.0%) of the cattle farmers did not have formal education (Figure 3.1). All the respondents reared the animals for sale and domestic consumption.

3.3.2 Flock/Herd Structure and Management Systems

The median cattle herd population reared by the respondents was 32 (range = 10-120), while the median poultry flock population was 4,500 (range=250 to 65,000) birds with 83.3% of farmers considered “large-scale” producers. Twenty (66.7%) of the poultry farmers were engaged in rearing of commercial layers while seven (23.3%) were broiler grow-out farmers, while three (10%) others engaged in the rearing of both layers and broilers in their farms and twenty farmers reared only commercial layers. Sixty percent of poultry farmers practiced a “deep litter” system while 23.3% (n = 7) practiced both deep litter and a “battery cage” system to house their flocks.

3.3.2 Feed and Feeding of the poultry and cattle

The results of the study showed that 83.3% of the poultry farmers were engaged in self feed milling for their chickens while 16.7% obtained feed from commercial feed millers. About 87.7% routinely added premixes and antimicrobials such as oxytetracycline and tylosine in feed as egg boosters or growth promoters and disease prevention. All (100.0%) cattle farmers fed the cattle mainly with grasses by pasture grazing with concentrates and grain offal as supplements. Salt lick mineral supplements were giving to the cattle by 60.0% of the farmers while 50.0% claimed they used herbal preparations in feeds and drinking water.

Table 3.1 Farmers characteristics and flock/herd structure

Variables		**Cattle farmers**	**Poultry farmers**
Gender	Male	20 (100.0%)	20 (66.7%)
	Female	0%	10 (33.3%)
Ownership	Owners	14 (70.0%)	12 (40.0%)
	Manager/Supervisor	6 (30.0%)	18 (60.0%)
Median age in years(range)		30 (14-60) years	36 (18-63) years
Mean years of experience in practice ±SD		19.4 ±10.7 years	10.6 ±4.5 years
Median Flock/herd Population (range)		32 (10-80) heads of cattle	4500 (250-62500) chickens

Source; Field survey 2006

3.3.3 Diseases Status of the Poultry and Cattle

The commonly encountered poultry diseases reported by the respondents include chronic respiratory diseases (CRD), helminthosis (worms), Newcastle disease, fowl typhoid (salmonellosis), coccidiosis, gumboro disease, fowl cholera, colibacillosis (yolk sac infection), infectious coryza, and lice infestation in that order (Figure 3). While the common diseases reported by the cattle farmers include helminthosis (worms), mastitis, ticks, cough/respiratory diseases, trypanosomosis abscesses, foot rots/lameness, skin infection/wounds in the frequencies shown in figures 3.2 and 3.3. These diseases were the major challenges which they claimed necessitated regular use of antibiotics for the animals.

Majority (86.7%) of the poultry farmers did not engage the services of veterinarians for disease diagnosis and drugs prescription. They claimed to recognise disease conditions based on their knowledge and experience of clinical signs and post mortem findings and only two of the poultry farmers 6.7% engaged laboratory confirmation of diseses diagnosis and antibiotic sensitivity tests for bacterial isolates. All (100%) the cattle farmers (interviewed) also claimed to recognise different diseases and did not engage the services of veterinarian for disease diagnosis drug prescription and use.

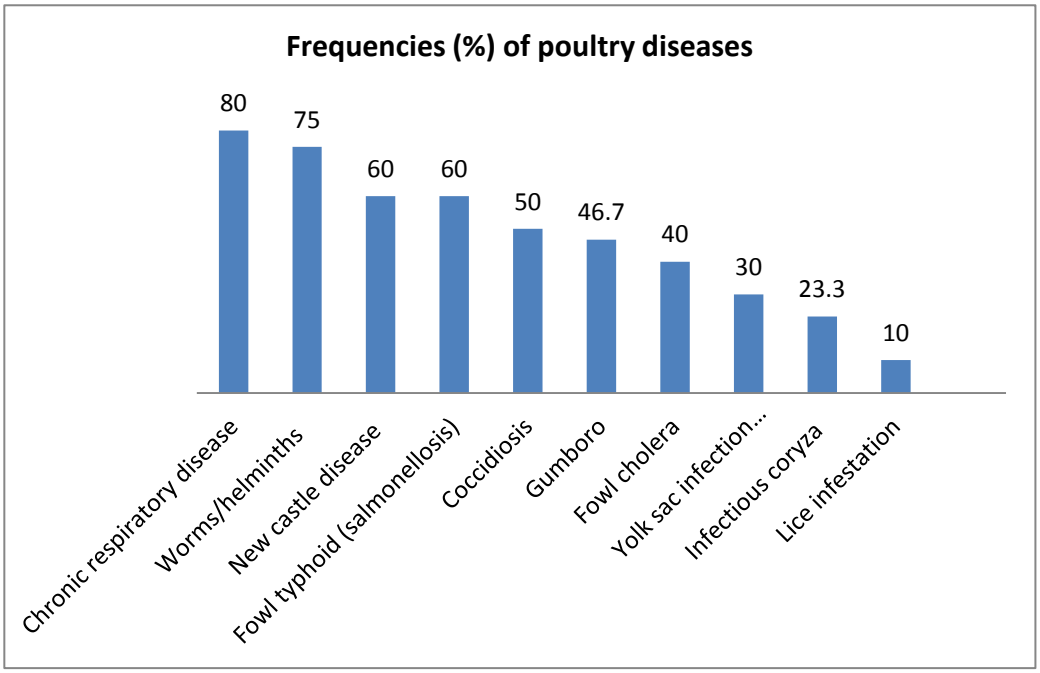


Figure 3.1: Commonly encountered poultry diseases reported by the respondents

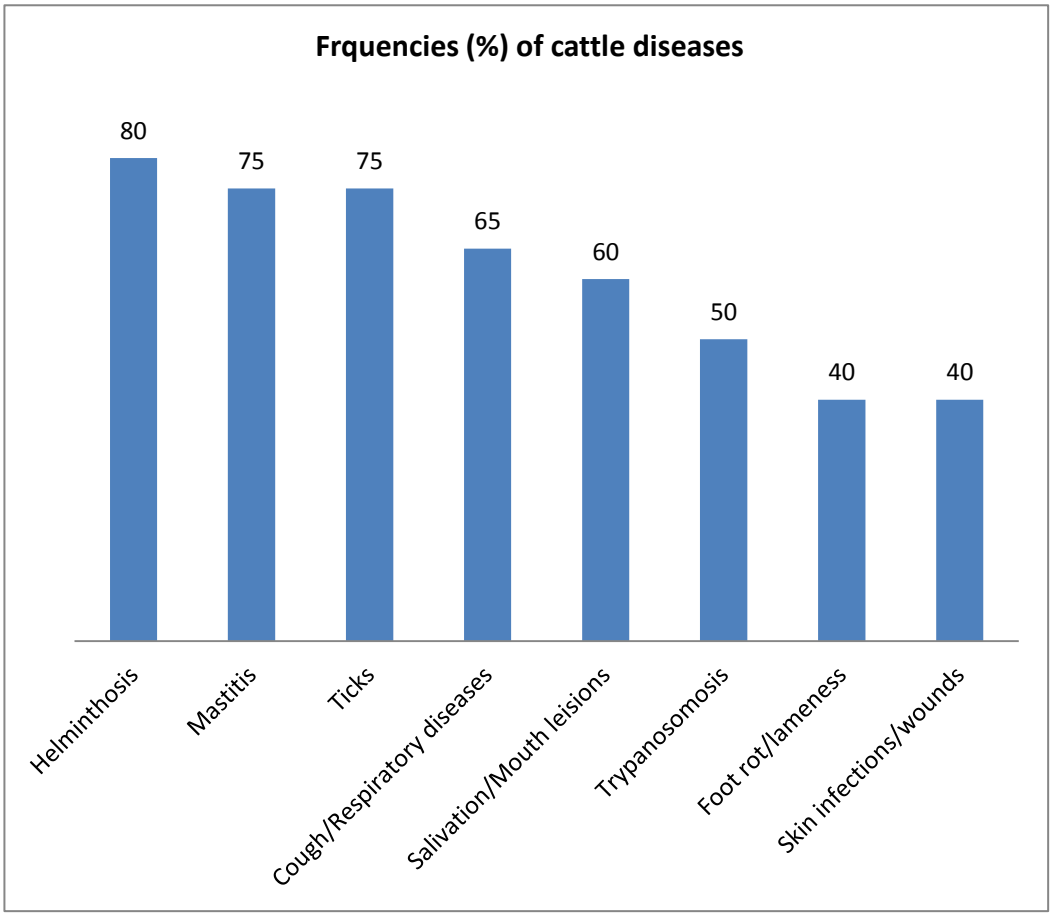


Figure 3.2: Commonly encountered cattle diseases reported by the respondents

3.3.4 The use of drugs in poultry and cattle

All the respondents (100%) used antibiotics for prevention and treatment of diseases. Table 3.2 show the frequencies of indications for the use of antibiotics in poultry and cattle. Majority 70.0% of the poultry farmers did not employ the services of veterinarian for prescription but were engaged in self administration of drugs to their animals. While 30.0% of the respondents reported that they treat their flocks based on professional judgement and prescription. However, all (100.0%) the cattle producers treated their animal by themselves through experience and they also claimed efficacy of herbal preparations in the treatment of cattle diseases. Records of diseases and treatments were not available in most of the surveyed farms. The majority of producers used at least 3 antibiotics (n=15) while seven other producers reported using between four and six different antibiotics. The majority of producers (87.7%) routinely added antibiotics to the feed for disease prevention and to improve production. Tetracyclines (oxytetracycline and chlortetracycline) were the most commonly used antibiotic (Figure 3.3). Approximately 50% of poultry farmers also used gentamicin while 20% of them employed fluoroquinolones (ciprofloxacin or (enrofloxacin) and chloramphenicol. The reported common routes of medication are oral in drinking water and by intramuscular injections. Also in cattle antibiotics were also frequently being used for the treatment of cattle infections and also prophylaxis or as anti-stress. The most frequently used antibiotics were oxytetracycline (figure 3.3) either as 5 to 10% short acting or 20% long acting (LA), procaine penicillin occasionally combined with dihydrostreptomycin. Intramuscular route was the most reported routes of administration of these drugs in cattle. These drugs were reported by 83.3% of the farmers to be sourced from veterinary retail shops, while 16.7% procured drugs directly from company's sales representatives and hawkers. Also, all the cattle farmers (100.0%) reported the therapeutic efficacy of herbal preparations.

Table3.2: Indications for the use of Antimicrobials in Poultry and cattle

Indication for use	Poultry	Cattle
	Frequency (%)	Frequency (%)
Prophylaxis	26 (86.7)	20 (100.0)
Therapeautics	30 (100.0)	20 (100.0)
Egg booster	16 (53.3)	0 (0.0)
Growth Promotion	7 (23.3)	8 (40.0)

Source; Field survey 2006

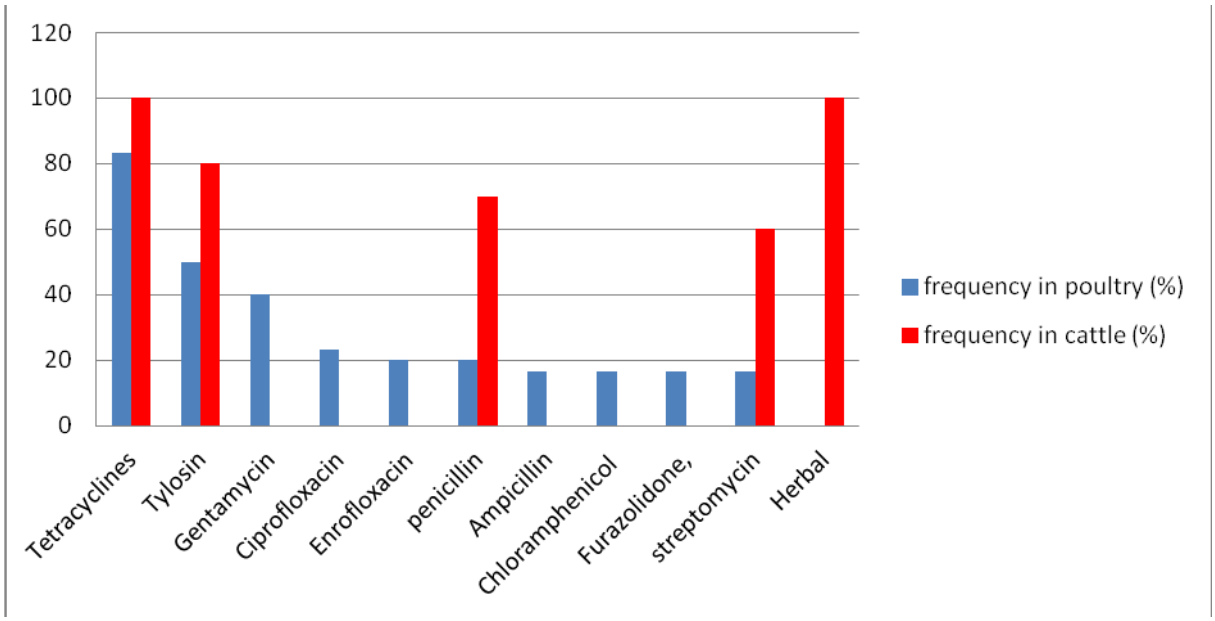


Figure 3.3: Commonly used antibiotics in poultry and cattle production

3.3.5 Farmers knowledge of withdrawal period and drug residues

An overall of 60% of the respondents claimed to have knowledge on antimicrobial withdrawal period but only 16.7% of the poultry farmers complied with withdrawal periods before culling, slaughter or sales of birds. Most of the respondents could not comply with withdrawal period as they could not understand the practicality of withholding the sales or consumption of their eggs or chickens. Culling and dressing of treated chickens were done by some farmers to avoid total loss. All the respondents who are egg producers admitted that they sold or ate their eggs immediately during and after the use of veterinary drugs in the flock for treatment or prophylaxis. On the cattle farms, milk and milk products from unaffected quarters of the udders of treated cows were reported being sold for consumption. Cattle that were given antibiotics prophylactically or as anti-stress were also sold for slaughter. Table 3.3 shows the levels of compliance of the farmers to withdrawal periods of the drugs. None of the cattle producers observed withdrawal periods while only 16.7% of the poultry farmers interviewed claimed that they observed the withdrawal periods. The results obtained on the farmers' knowledge of the effects of antimicrobial use and residues on human health vary from no effect to risks of cancers and drug resistance as shown in Figure 3.5.

Table 3.3: Compliance with Withdrawal Period among the Poultry and cattle farmers

Compliance	Poultry	Cattle
	Frequency (%)	Frequency (%)
Yes	5(16.7)	0 (0.0)
No	25 (83.3)	20 (100.0)

Source; Field survey 2006

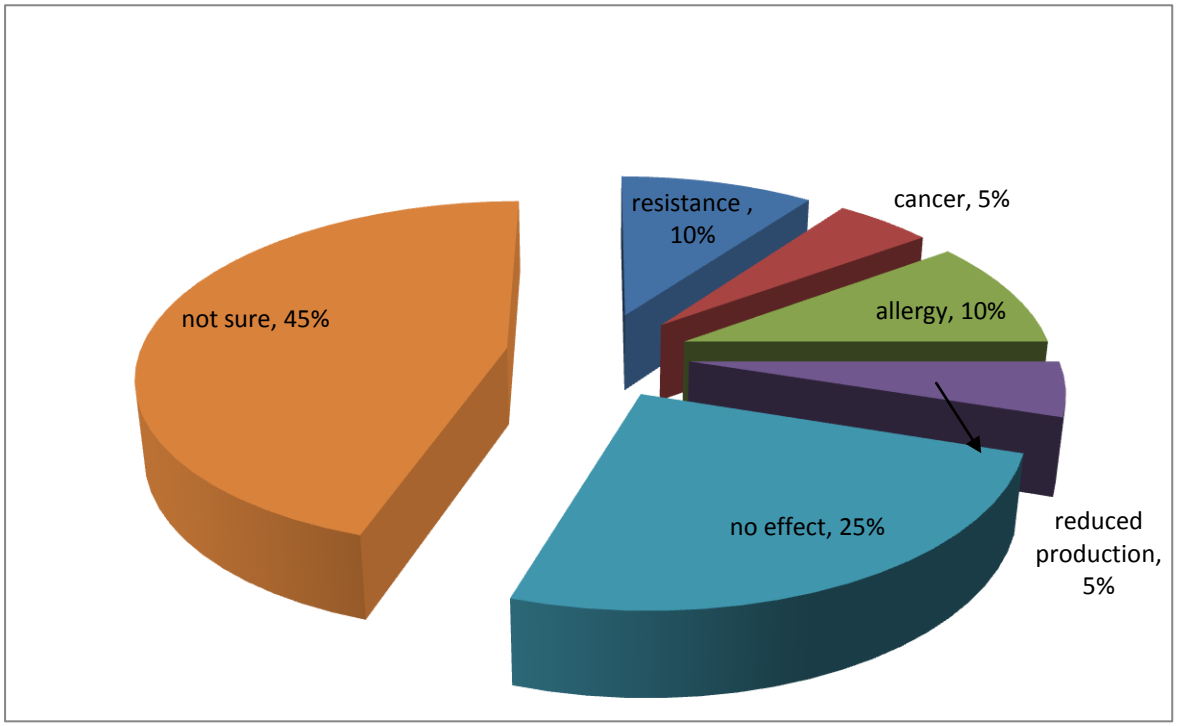


Figure 3.4: Farmers knowledge of the effects of drugs residues in egg/meat

3.4 Discussion

Agricultural use of antibiotics has become a very important public health concern (White *et al.*, 2001). This study showed that majority of cattle and poultry farmers interviewed depended on the use of different antimicrobial agents (including herbal preparations). The practice was indiscrimate, since majority of the farmers (86.7% poultry farmers and 100% cattle producers) did not engage the services of veterinarians or laboratories for disease diagnosis, antibiotic prescription and usage in these food animals. This practice is could be associated with the low education and lack of awareness of health risks of such practices. Also, since most of the farmers have more than 10 years of livestock rearing experience, there was a general assumption among them that they could recognise the disease status of their flocks or herds/ flocks. This attitude led to unprofessional disease diagnosis and drugs usage. Therefore, hypothesis (H_o1) is not rejected.

The prevalence of different diseases reported among the herds and flocks could have resulted from wrong diagnosis and improper dosing with drugs which could aid the development and spread of resistant strains of pathogens within the flocks and herds. The unrestricted access to veterinary drugs (including antibiotics) by the farmers due to over the counter availability of these drugs without veterinary prescription or supervision is an indication of lack of proper regulatory control and monitoring of the use of antibiotics in the study area. These agree with the findings of various authors in Nigeria and other developing countries (Okolo, 1986; Mitema *et al*., 2001; Dipeolu, 2002; Kabir *et al.*, 2004).

In this study most of the farmers (95%) were neither aware nor observed withdrawal periods before selling or consuming the poultry and cattle products, coupled with lack of proper records of treatment as reported in other studies in developing countries (Mitema *et al.*, 2001; Kabir *et al.*, 2004; Sasanya *et al.*, 2005) predisposed the meat to residues deposition.

therefore hypothesis (H_o2) is not accepted (rejected). Such practices without observance of the withdrawal period could result in the presence of residues in the meat, milk and eggs from the herds/flocks thereby predisposing the consumers to some levels of antibiotics residues and it attendant public health consequences (Riviere and Spoo, 1995). In the case of commercial laying birds the compliance with the withdrawal period during the course of antibiotics treatment was queried by the farmers on the ground of economic losses as they claimed that the proceed from the eggs were used to purchase for the chicken to boost egg production and to treat or prevent diseases which were the constant challenges. Hence withdrawal period was not practicable in eggs of treated laying birds. The indiscriminate use of several antibiotics by self prescription and medication by livestock producers could be due to: weak veterinary structure and inadequate control of veterinary drugs in developing countries (Dina and Arowolo, 1991; Fingleton, 2004; Noga, 2009).

Most of the antibiotics used in these animals are analogues of and fully cross resistant with important antibiotics that are used in human medicine (van den Bogaard, 2000). As a result of indiscriminate and regular exposure of food animals to antibiotics, the prevalence of resistant bacteria in the faecal flora of these animals is high. These resistant bacteria can be directly and indirectly, via foods of animal origin, transferred to humans and either colonize the human intestinal tract or exchange their resistance genes among the commensal and pathogenic bacteria (van den Bogaard, 1998). Witte, (1998) reemphasized the human health risks associated with the use of antibiotics in agriculture by citing specific examples of avoparcin-related, vancomycin-resistant enterococci disease transfer from animals to humans and the speculation about the relationship between *satA-gene*-mediated streptogramines-resistance development and the use of virginiamycin in food animals.

Other consequences of lack of accountability and inappropriate use of antibiotic in human, veterinary medicine and in agriculture include: shortened lifespan of an antibiotic's

usefulness, additional complications in surveillance, inability to predict resistance patterns and the consequences on human, animal and environmental health**.** Over-the-counter availability of antibiotics for domestic animals and the absence of professional oversight in many uses have been responsible for the frustration of regulatory officials on accountability and limit the ability to make a true estimate of the magnitude of resistance problems that threaten human and animal health (National Research Council, 1998).

In this study most of the farmers (95%) did not observe withdrawal periods with lack of proper records of treatment as reported in other studies in developing countries (Mitema *et al.*, 2001; Kabir *et al.*, 2004; Sasanya *et al.*, 2005) predisposed the meat to residues deposition. Dipeolu *et al*., (2005) reported that poultry farmers in Nigeria could not observe withdrawal periods following the use of antibiotics as feed additive and growth promoters, especially in laying birds as the poultry producers were selling eggs from both medicated and unmediated flocks for public consumption. Such practices enhance the presence of antibiotics residues at violative levels in food of animal origin against CAC and SPS standards and are inimical to food safety and public health. However, good animal husbandry and hygienic measures can prevent contamination and disease outbreak thereby reducing the need for antibiotics use. Appropriate use of antibiotics for food animals is crucial to the preservation of long-term efficacy of existing antibiotics, support animal health and welfare, and limit the risk of transfer of antibiotic residue and resistance pathogen among man, animals and the environment.

CHAPTER FOUR

DETECTION OF ANTIBIOTIC RESIDUES AND QUANTITATIVE ANALYSIS OF OXYTETRACYCLINE RESIDUES IN CATTLE AND CHICKEN MEAT IN SOUTHWEST NIGERIA

4.1 Introduction

Antimicrobials are among the inputs usually employed in commercial livestock production. Food animals treated with antibiotics and their edible products are required to be held for specific withdrawal periods so that their residues are depleted to safe level before such animal tissues can be used as food for human consumption (KuKanich *et al*., 2005). In Nigeria like most developing countries antibiotics are used in animals indiscriminately by livestock producers for the prevention and treatment of bacterial infection and the drugs are available over the counter (Dina and Arowolo 1991; Kabir *et al*., 2004). A greater proportion of cattle in Nigeria are reared by the nomadic herdsmen who administer chemotherapeutic agents without veterinary prescription (Dipeolu and Alonge, 2002; Kabir *et al*., 2004) while poultry farmers also routinely use antibiotics for their flocks. Correct dosage was unlikely being administered and the withdrawal periods are not usually observed.

Tetracyclines are broad-spectrum antibiotics that show activity against Gram-positive and Gram-negative bacteria, including mycoplasmas, chlamydiae, rickettsias, spirochetes, actinomycetes, and some protozoa (Sundin, 2003). This group of antibiotics have been used for more than 50 years for the treatment of bacterial infections in both humans and animals. They are among the most frequently used groups of antimicrobials animal-food production for prophylaxis, chemotherapy and growth promotion (Mitchell, *et al*., 1998; Karimuribo *et al*., 2005; Nonga *et al*., 2009). Despite early warnings about increasing resistances of microorganisms to tetracyclines and the banning of tetracyclines as growth promotors, more

than 65% of the antibiotics prescribed for veterinary therapeutic use within the European Community (2294 of 3494 tons) are tetracyclines (Sarmah *et al.*, 2006). In the United States, tetracyclines are the most commonly used antibiotics in a variety of animal species (disease treatment and prevention; growth promotion), plants, and humans (Mellon *et al*., 2001).

Consequently, residues of the commonly used tetracyclines (oxytetracycline, tetracycline, chlortetracycline and doxycycline) are found in meat, milk and eggs (Okerman *et al*., 1998). The adverse effects of tetracyclines and their residues on consumer's health include allergic reactions, discolouration of teeth, chronic nephrotoxicity, hepatotoxicity, skin hyperpigmentation, gastrointestinal disturbance due to the selective pressure of antibiotics on human gut micro flora and development of antibiotic-resistant bacteria (Robert, 1996; Goldfrank *et al*., 2002). The residual presence of tetracyclines in milk also causes technological difficulties in the milk processing industry (Heeschen and Bluthgen 1991).

Imprudent use of antibiotics could result in the occurrence of residues in meat, milk and eggs with consequent public health hazards (Heeschen and Bluthgen 1991). The widespread use of antimicrobials in livestock management and possible violations of MRLs require routine monitoring and surveillance of the use of veterinary drugs and drug residues. However, in Nigeria there are no residues inspections and monitoring programmes in place, coupled with the unregulated access to veterinary drugs by livestock producers the risks of residues in meat eggs or milk are very high. Therefore there is need for routine screening and surveillance of food of animal origin for residues of veterinary drugs.

Generally antibiotics residues screening of veterinary drugs is performed using microbiological inhibition methods, which allow their detection and/or semi-quantitative determination, and using specific rapid testing (Mitchell *et al*., 1998; Botsoglou and Fletouris 2001; Nonga, 2009). Traditionally, the methods require overnight (24h) incubation may be

very time-consuming and are considered as multi-residue screening tests for antibiotics. However, inhibition tests may be of high sensitivity but they lack specificity as they cannot distinguish among different forms of antibiotics, therefore every inhibition test can only be used as a tool for screening. In Nigeria various studies have been conducted on drug residues deposit in food animal products in Nigeria (Oboegbulem and Fidelis, 1996; Dipeolu and Alonge 2002; Kabir *et al.,* 2004) have demonstrated the presence of antibiotic residues in meat and animal products. Most of these studies were based on microbiological inhibition techniques that did not specifically classify and quantify the antibiotics and require 24 hours incubation. Also, there are no national MRLs or residues inspection and monitoring programmes in place, coupled with the unregulated access and use of veterinary drug (including antibiotics) by livestock producers in Nigeria. The risks of residues in meat, eggs or milk are therefore very high. To protect consumers from exposure to residues and the public health consequences, the FAO/ WHO Codex Alimentarius Commission and other national agencies have established and allocated MRLs for tetracyclines in food items. The maximum residual limit set by the EU legislation for tetracycline, oxytetracycline as well as chlortetracycline in raw cow milk is set to 0.1 mg/kg (100 ng/g) (Council Regulation 2377/90/EEC), while CAC (2009) recommended maximum residue limit (MRL) is 200µg/kg in muscle, 600µg/kg in liver and 1200µg/kg in kidney.

There is need for rapid screening of large quantity of meat, milk or eggs to protect the consumer against antibiotic residues; this requires rapid detection techniques for which several commercial kits have been developed. Premi®Test (DSM, the Netherlands), is a commercially available rapid microbiological screening kit developed for antibiotic residue detection. Premi®Test kit ampoule contain an agar medium, imbedded spores of *Bacillus stearothermophilus var. calidolacti*s which is sensitive to wide range of antibiotics. Premi®Test is an agar diffusion test with colour change indicator in the medium producing

result within 4 hours of incubation. Samples of chicken and beef sold for public consumption in Ibadan, Lagos and Akure were screened to determine the prevalence of antimicrobial residues in the tissues using Premi®Test kit and to analyse the levels of oxytetracycline residue in the samples.

4.2 Materials and Methods

4.2.1 Samples collection

Three municipal/metropolitan abattoirs namely, Oko-Oba, Bodija, Araromi abattoirs in Lagos, Ibadan and Akure respectively were visited where edible portions (muscle, kidney and liver) of beef were purchased in most cases or obtained through the effort of the meat inspectors and veterinary officers of the State Department of Veterinary Services from the abattoirs between June 2007 and May 2009. Broiler chicken liver and breast/thigh muscle from chicken slaughtering markets at Oko-Oba (Lagos), Mokola (Ibadan) and ten commercial broiler farms from Ibadan and Lagos were obtained. Chicken samples were collected from and Oko-Oba and Mokola chicken markets where live chickens from different farms in southwest poultry are sold live or dressed. Also ten commercial broilers grow-out farms were selected and prior visited to obtain appointment for the time of slaughtering of broiler chickens in Ibadan and Lagos.

The sampling was conducted between January 2007 and December 2009. Sample sizes of 200 each of cattle carasesses and 200 chicken were chosen using range 19 to 299 (at 1 to 15% non-compliance incidence at 95% CI) from CAC non-compliance prevalence table of random sampling for residue(Appendix v) according to CAC/GL, (2009) based on 15.6% and prevalence obtained by Dipeolu (2002) in beef and 33.1% by Kabir *et al*., (2004) in chicken from Nigeria.

Approximately 200 grams portions from retailed liver, kidney and muscle from 250 carcasses of cattle slaughtered for public consumption were obtained twice (during wet and dry seaons) from each municipal abattoir at Araromi (Akure), Bodija (Ibadan) and Oko-Oba (Lagos). Also, 200 breast muscle and liver samples of broiler chickens from Mokola and Oko-Oba live-birds slaughter markets and ten commercial broilers farms each from Ibadan and Lagos were aseptically collected into sterile plastic bags (Whirl-Pak, Nasco USA). The distributions of the samples are shown on table 4.1a and 4.1b. The samples were transported in cool boxes packed with ice packs to the laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, for analysis.

Table 4.1a: Distribution of beef samples

Location (Abattoir)	Wet season			Dry season			Total
	Kidney	Liver	Muscle	Kidney	Liver	Muscle	Total
Akure (Araromi)	250	250	250	250	250	250	1500
Ibadan (Bodija)	250	250	250	250	250	250	1500
Lagos (Oko-Oba)	250	250	250	250	250	250	1500
Total	750	750	750	750	750	750	4500

Table 4.1b Distribution of chicken samples

Location	Liver	Muscle	Total
Ibadan(Mokola market)	200	200	400
Ibadan (broiler farms)	200	200	400
Lagos (Oko-Oba market)	200	200	400
Lagos (broiler farms)	200	200	400
Total	800	800	1600

4.2.2 Screening of Beef and Chicken for Antimicrobial Residues

The prevalence of antimicrobial residues in the meat samples was determined by rapid test Premi®Test antibiotics screening kit following the manufacturer's (DSM, Netherlands) protocol. The test involved a simple process of meat juice extraction and incubation in agar imbedded with spores of *Bacillus stearothermophilus* var. *calidolactis* and containing acid-based indicator bromocresol purple. Premi®Test combines the principle of an agar diffusion test with colour change by the indicator. There active metabolism of the seeded micro-organism not inhibited by residue in negative samples makes the agar test changed colour from purple to yellow. But when the growth of the microorganism is inhibited (due to presence of an antibiotic at or above the limit of detection or LOD) the test remained purple.

Approximately $2cm^3$ each of kidney, liver and muscle was cut into the meat press to extract the meat juice from which 100µl was carefully pipetted onto each ampoule of the agar. The agar with the extract was allowed to stand for 20 minutes for pre-diffusion at room temperature and then flushed carefully twice with distilled water. Once the agar was drained of the extract and water the ampoules were closed with foil. These were incubated in the heating block for about 3hours at 64^0C after which the result were observed through colour changes. The colour of all ampoules was read at the moment the negative control changed colour from purple to yellow and the results were recorded as positive or negative for antimicrobial residues.

4.2.3 Chromatographic Analysis of Oxytetracycline Residue in beef and Chicken

4.2.3.1 HPLC Chromatographic conditions

HPLC Machine; Buck-Chrom® HPLC (Buck Scientific, USA) with UV/Visible detector was set at the following conditions: Wavelength set at 360nm, Stationary phase: reversed phase C18, 10µm Nucleosil 4.6 x 250mm ID column, Mobile phase: Methanol, Acetonitrile, 0.01M aqueous Oxalic acid (1:1.5:2.5) at pH 2, Flow rate: 1.5ml/minute, Injection volume: 20µl

4.2.3.2 Sample Preparation

Sixty samples of each of the meat types of cattle and chicken that were positive for antimicrobial residues by Premi®Test from each location were randomly selected for quantitative analysis of oxytetracycline residue. The samples were taken through three stages of preparation (*a*) homogenization and extraction of the sample residues by hydrochloric acid; (*b*) precipitation of proteins using acetonitrile and filtration; and, (*c*) clean-up by liquid-liquid partition with methylene chloride and hexane according to methods developed by Moats (1986) and used by Ibrahim and Moats (1994).

The extraction and clean-up procedures developed by Moats (1986) and used by Ibrahim and Moats (1994) was employed in this study. This involves Liquid - Liquid partitioning extraction procedures to obtain the analyte. The **extraction process** was done by cutting approximately 25g of each sample which was thoroughly homogenized thrice with 3 volume/weight of 1N hydrochloric acid. **Protein precipitation** was done by adding 8ml of the homogenate which was thoroughly swirled with 32mls acetonitrile in a conical flask and allowed to stand for 5minutes after which the supernatant was filtered through a plug of glass wool on the stem of a glass funnel. The **sample clean-up** was achieved by adding 20 mls of the filtrate to 20mls hexane and 20mls methylene chloride in a separatory funnel and

vigorously shaken resulting in separation into two layers. About 4mls of the water layer containing the analyte were collected to scintillation vials for HPLC analysis.

4.2.2.3 Preparation of Standard Curve

One hundred milligrams of oxytetracycline hydrochloride analytical standard was accurately weighed and put in a 100 ml volumetric flask, the powder was dissolved in 100 ml of methanol to produce a stock solution of 1,000 ppm. The absorbance was determined using UV-Spectrophotometer to be 360 nm. Serial dilutions of the stock solution were carried out to give the following dilutions: 1: 100 (10 ppm), 1: 200 (5 ppm), 1: 400 (2.5 ppm), 1: 500 (1.25 ppm), 1: 1000 (0.1 ppm), 1: 10000 (0.01 ppm), 20µl of the final concentrations were used to prepare the standard curves obtained from the chromatograph of the standard solution (figure 4.3). The corresponding concentrations of these dilutions (ppm were: 10, 5, 2.5, 0.1, and 0.01) were injected to the HPLC machine to obtain the calibration curve, the peak areas were plotted against the corresponding concentrations, the best line of fit was plotted using Microsoft Excel programmes.

4.2.2.4 Procedure for HPLC analysis of oxytetracycline

The analysis and quantification of the oxytetracycline residues in the analytes were performed at the Chemical Analysis Laboratory of the Nigeria Institute of Science Laboratory Technology (NISLT), Ibadan using a high-performance liquid chromatography machine (Buck chrome) equipped with a constant flow pump and a variable wavelength UV detector set at 360nm and flow rate of 1.5mls/min. Elution of oxytetracycline from the analyte was done on a nucleosil C- 18 (10µm, 250 x 4.0mm 1D) column with mobile phase; Methanol-Acetonitrile-0.01M aqueous Oxalic acid solution, pH 2.0 (1: 1.5: 2.5) as described by Muriuki *et al*., (2001). The mobile phase was filtered through 0.22µm membrane filter using vacuum pump and regularly degassed by sonication. The HPLC machine was also flushed at

interval with blank methanol and mobile phase was allowed to run through the machine for equilibration and conditioning during which stable baseline was obtained on the recorder monitor. The column and tubing were regularly checked to ensure leak proof, 20µl of analyte from each sample (in duplicate) was injected to the column when the machine gave instruction "waiting for pulse injection". Oxytetracycline was eluted on the C-18 column and resolution occurred in the detector resulting in peaks (chromatographs) shown on the monitor with the peak areas and retention times recorded by the computer recorder (figure 4.4). The mean peak areas of the samples corresponding to the retention time between 2.8 to 3.5 minutes obtained from the reference standard were recorded as positive for oxytetracycline. Quantification of oxytetracycline residues in the samples were obtained from calculation by substituting the mean peak areas in the linear equation obtained from the calibration curve of the standard.

4.2.2.5 Recovery Experiment

This was to determine the sensitivity of the procedure and the rate of residue recovery from the extraction and detection procedures. About 25g each of liver kidney and muscle from antibiotic free broiler chicken were accurately weighed and spiked in triplicate with 4 ppm and 8ppm of the analytical standard oxytetracycline solutions. The tissues were allowed to equilibrate for about 30mins before proceeding with extraction and further HPLC analysis as above to determine the recovery rate.

4.2.2.6 Statistical Analysis

The data were analyzed using descriptive (percentage, range, mean ± SD) and inferential statistics. Prevalences of antibiotic residues were compared by chi-square ($\chi 2$) test while the mean oxytetracycline residues levels in different tissues were analysed by student's t-test,

ANOVA followed by Tukey's multiple comparison test at a confidence level of 95% ($p < 0.05$) using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA).

4.4 Results

4.3.1 Prevalence of antimicrobial residues in beef

Out of a total of 4500 tissues of cattle samples screened from cattle the three abattoirs in both dry and wet seasons 2042 (45.4%) were positive for antimicrobial residues. The results of screening of the different edible portions of bovine carcasses from the three abattoirs are shown on table 4.2. The highest prevalence (62.8%) occurred during the wet season and in the liver samples from Bodija abattoir in Ibadan.

Results from Bodija abattoir indicated an overall prevalence of 53.3% during the wet season compared with 43.2% during the dry season. Also meat from Oko-Oba abattoir yielded an overall prevalence of 47.2% and 41.8% during the dry and wet seasons respectively. While beef samples from Araromi abattoir had total prevalence of 44.9% and 43.4% prevalence of antimicrobial residues during the wet and dry seasons respectively. Liver and kidney samples yielded more residue prevalence than the muscle.

Table 4.2 Premi^{R}Test Screening of beef for antimicrobial residues

Location City (abattoir)	**Type of samples**	**DRY SEASON**		**WET SEASON**	
		No of samples	No +ve with Premi®Test (%)	No of samples	No +ve with Premi®Test (%)
Akure (Araromi)	Kidney	250	112 (44.8)	250	123 (49.3)
	Liver	250	124 (49.6)	250	128 (51.8)
	Muscle	250	74 (29.6)**	250	86 (34.4)**
	Total	**750**	**310 (41.3)**	**750**	**337 (44.9)**
Ibadan (Bodija)	Kidney	250	99 (39.6)	250	110 (44.0)
	Liver	250	126 (50.4)	250	157 (**62.8**)*
	Muscle	250	102 (40.8)**	250	133 (53.2)*(**)
	Total	**750**	**327 (43.2)**	**750**	**400 (53.3)**
Lagos (Oko-Oba)	Kidney	250	109 (43.6)	250	125 (50.0)
	Liver	250	117 (46.8)	250	123 (49.2)
	Muscle	250	88(35.2)**	250	106 (42.4)**
	Total	**750**	**314 (41.8)**	**750**	**354 (47.2)**

*, ** Significantly difference $p<0.05$

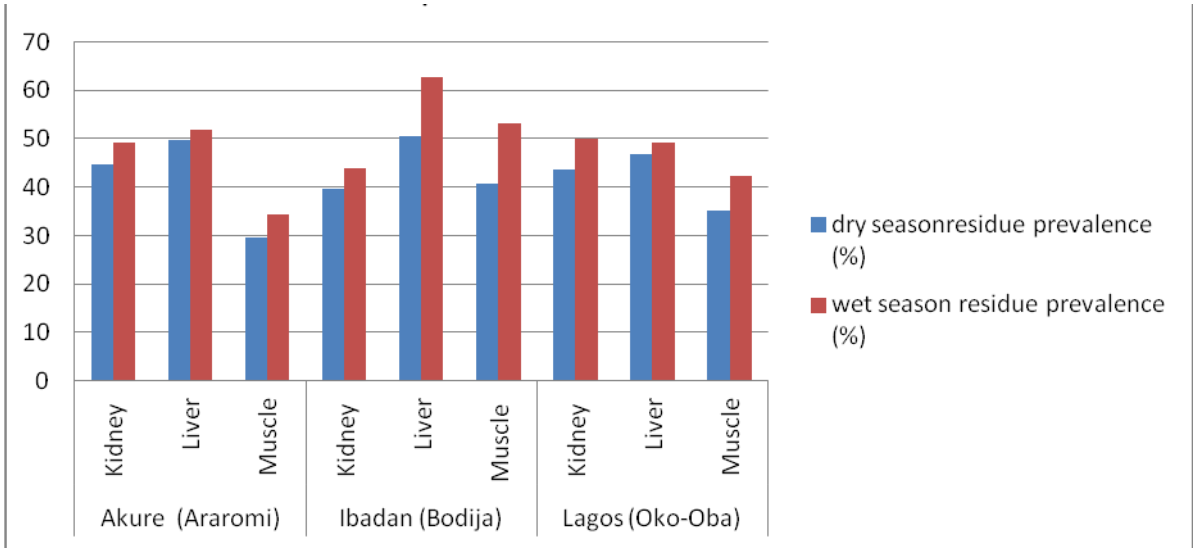


Figure 4.1: Seasonal prevalence of antimicrobial residues in beef from abattoirs in the cities

4.3.2 Prevalence of antimicrobial residues in chicken

The overall prevalence of antimicrobial residues obtained from all the chicken samples was 69.1%. The prevalence in the different chicken tissues across the different locations is shown on table 4.3 and figure 4.2. The liver of chicken from market yielded higher prevalence than the liver and muscle from farms. Table 4.3, figure 4.2 and appendix II showed the patterns of antimicrobial residues prevalence in the chicken samples from Ibadan and Lagos. The data indicated that more chicken samples from Ibadan contained the residues than from Lagos but the difference was no statistical significant. However, the prevalence of antimicrobial residue in the chicken liver was significantly higher ($p<0.05$) than in the chicken muscle. Also the results showed that the prevalence of antimicrobial residues in chicken is higher than in cattle.

Table 4.3: Prevalence of antimicrobial residues in chicken from Ibadan and Lagos

Location	Portion of chicken	No of samples (n)	No +ve with Premi®Test (%)
Ibadan (market)	Liver	200	160 (80.0)
	Muscle	200	144 (72.0)
Ibadan (farms)	Liver	200	144 (72.0)
	Muscle	200	134 (67.0)
	Total	**800**	**582 (72.8)**
Lagos (market)	Liver	200	160 (80.0)
	Muscle	200	120 (60.0)
	Liver	200	124 (62.0)
Lagos (farms)	Muscle	200	120 (60.0)
	Total	**800**	**524 (65.5)**

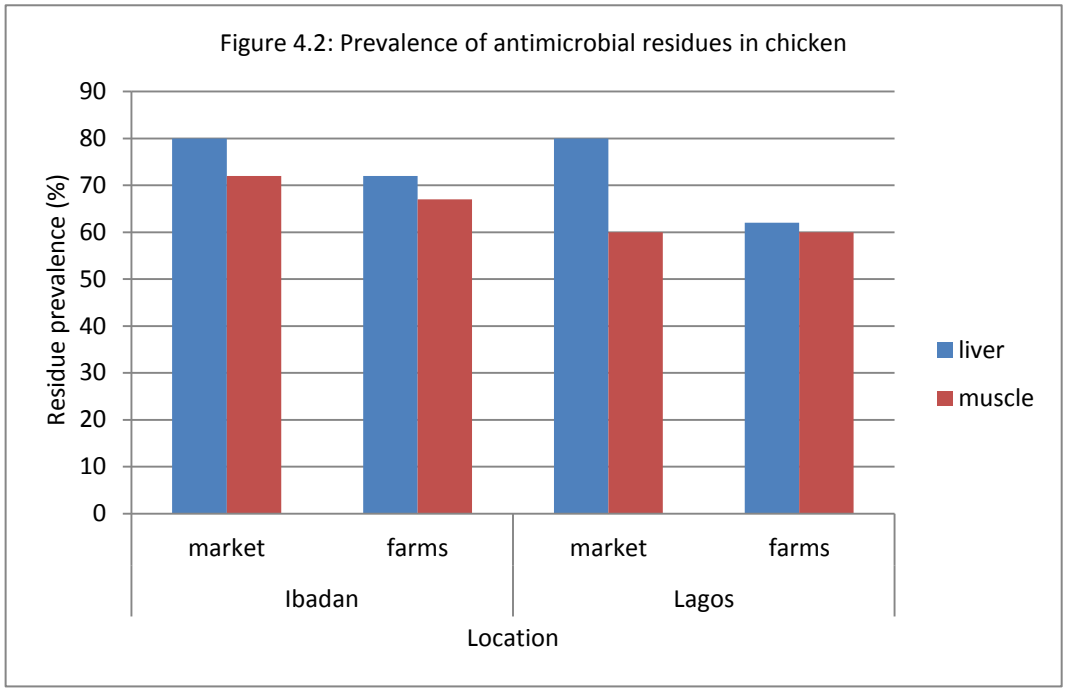


Figure 4.2 Prevalence antimicrobial residues in chicken meat by Premi®Test Screening.

4.3.3 Results of oxytetracycline residue

4.3.3.1 Calibration Curve and Recovery Experiment

The results of the standard concentrations and the peak areas were ploted as the standard curve (figure 4.3) with using linear regression equation The linear equation Y = a + b X was obtained where Y = peak area (cm^2), a = Y-intercept, b = the slope, X = concentration of the oxytetracycline (ppm), y = 20.7x + 8.611 and R^2 value of 0.993, where y is the peak area and x is the concentration in ppm. The R^2 value > 0.9 showed the linearity. The detection limit for oxytetracycline was 0.01ppm while the retention time of the oxytetracycline ranged between 2.8 to 3.5 minutes. The mean retention time for the oxytetracycline is 3.02 minutes while the detection limit was 0.01ppm. The mean recovery of oxytetracycline from the spiked tissues was between 80.0%-92.5% as shown in table 4.4 with recovery was highest in the muscle followed by kidney. The chromatographs of oxytetracycyline standard and tissue residues are shown in figures 4.4a and 4.4b.

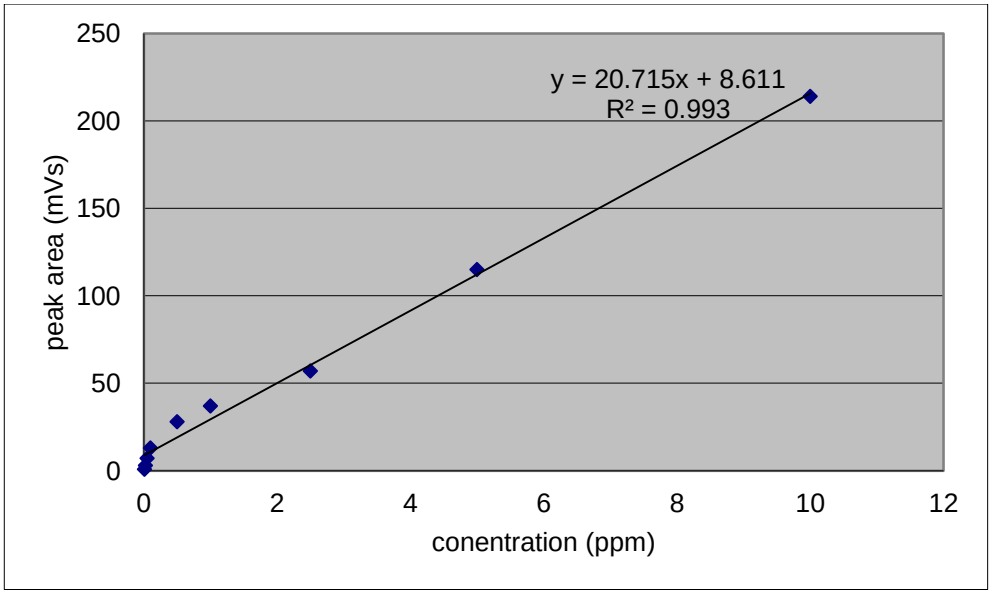
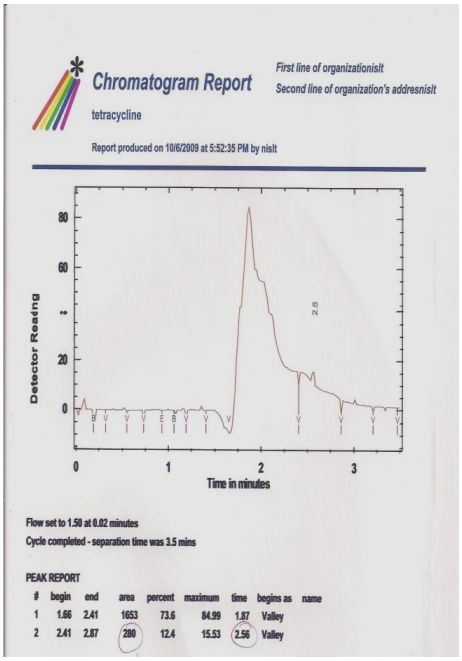


Figure 4.3 Calibration curve of oxytetracycline analytical standard.



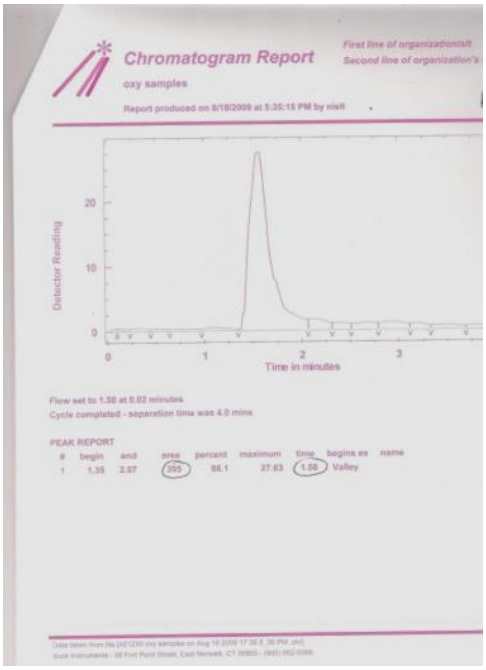
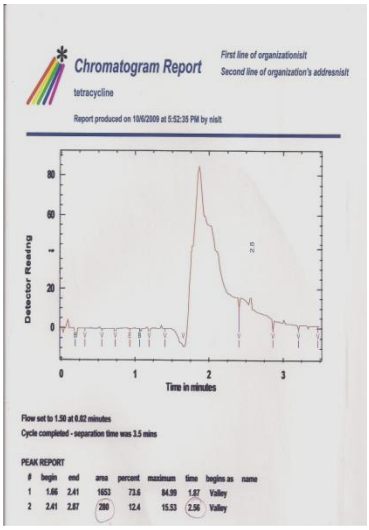
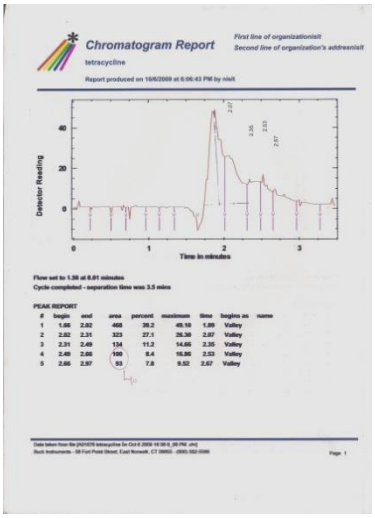
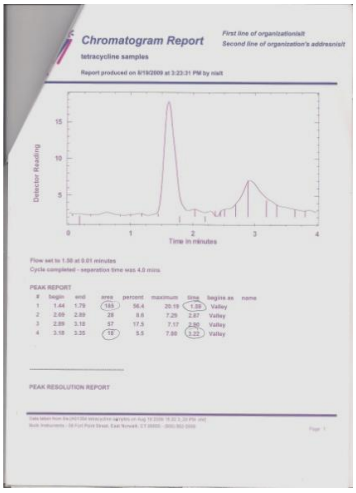
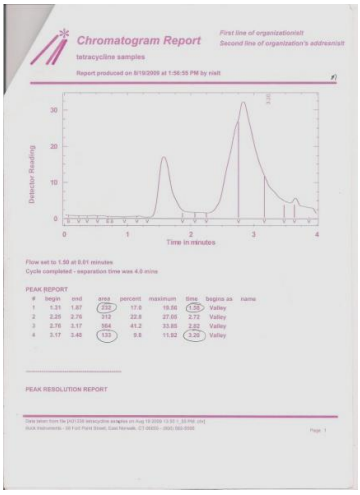


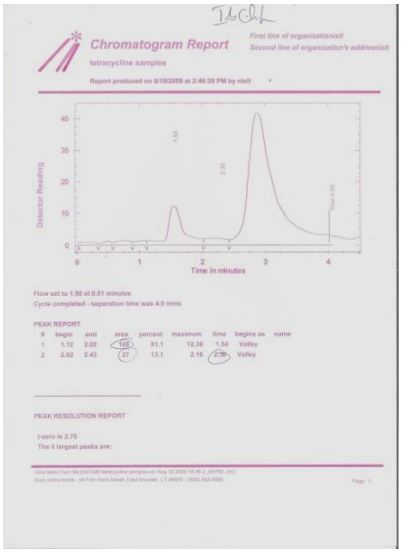
Figure 4.4a: Chromatographs of oxytetracycline standard











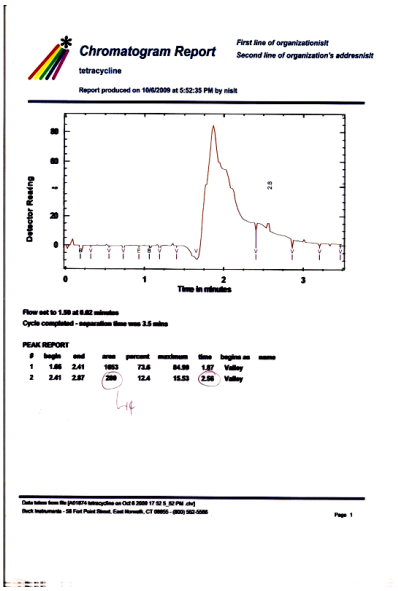


Figure 4.4b: Chromatographs of oxytetracycline in meat samples

Table 4.4: Recovery of oxytetracycline from spiked tissues.

Sample	Concentration of Oxtetracycline (ppm)	Mean recovery (ppm)	Percentage recovery %(SD)
Muscle	8	7.2	90.0 (0.35)
Kidney	8	7.0	87.5 (0.45)
Liver	8	6.8	85.0 (0.46)
Muscle	4	3.7	92.5 (0.21)
Kidney	4	3.6	90.0 (0.32)
Liver	4	3.2	80.0 (0.30)

4.3.3.2 Prevalence of oxytetracycline residue in beef

The overall prevalence of 56.2% oxytetracycline residue was obtained in beef across the study area with their distributions in the different abattoirs during the different seasons shown in table 4.5. The prevalence of 41.9% of the samples contained oxytetracycline above MRL (table 4.6). Also in the chicken, an overall prevalence of oxytetracycline residue obtained was 68.1% with 54.8% of the chicken containing the residue above the MRL.

Table 4.5 Seasonal prevalence of oxytetracycline residue in beef

Abattoir	Wet season			Dry season		
	Kidney (%) n=60	Liver (%) n=60	Muscle(%) n=60	Kidney(%) n=60	Liver (%) n=60	Muscle(%) n=60
Akure (Araromi)	38 (63.3)	36 (60.0)	33 (55.0)	31 (51.7)	34 (56.7)	29 (48.3)
Ibadan (Bodija)	44 (73.3)	37 (61.7)	34 (56.7)	35 (58.3)	32 (53.3)	28 (46.7)
Lagos (Oko-Oba)	34(56.7)	30(50.0)	29(48.3)	36(60.0)	36(60.0)	32 (50.7)
Overall oxytetracycline prevalence in beef= 607 (56.2%), n=1080						

Table 4.6: Prevalence of oxytetracycline in beef above the MRLs

Abattoir	Wet season			Dry season		
	Kidney (%) n=60	Liver (%) n=60	Muscle (%) n=60	Kidney (%) n=60	Liver (%) n=60	Muscle (%) n=60
Akure (Araromi)	16 (26.7)	26 (43.3)	31 (51.7)	27 (45.0)	18 (30.0)	24 (40.0)
Ibadan (Bodija)	32 (53.3)	28 (46.7)	34 (56.7)	18 (30.0)	24 (40.0)	27 (45.0)
Lagos (Oko-Oba)	19(31.7)	24 (40.0)	28 (46.7)	24 (40.0)	25 (41.7)	27 (45.0)
Overall oxytetracycline >MRLs in beef= 452 (41.9%), n=1080						

4.3.3.3 Prevalence of xytetracycline residue in beef from Ibadan (Bodija) abattoir

Table 4.7 shows the results of HPLC analysis of the samples from Bodija abattoir (Ibadan) with 73.3, 61.7 and 56.7% of the kidney, liver and muscle respectively having detectable oxytetracycline residue during the wet season, while 58.3, 53.3 and 46.7% of the meat respectively also contained oxytetracycline residue during the dry season. The mean oxytetracycline concentrations in the different meat types ranged between 766.2 and 1544.0 µg/kg during the wet season and between 555.8 and 1354.0 µg/kg in dry season. These results also showed that variable proportions of the meat contained the residues above MRLs (violative levels).

Table 4.7: Oxytetracycline residue in beef from Ibadan (Bodija) abattoir

Location (season)	**Ibadan (wet season)**			**Ibadan (dry Season)**		
Portion of carcasses	Kidney	Liver	Muscle	Kidney	Liver	Muscle
Range concentration(µg/kg)	598.1-5059.0	163.6-2544.0	211.9-2790.0	163.6-3736.0	110-2191.0	67.1-1245.0
Mean residue level ±SD (µg/kg)	1544±870.8[a]	917.5 ±465.2 [a]	766.2 ±513.9 [a]	1354 ±696.5 [b]	949.5 ±505.6 [b]	555.8 ±277.8 [b]
Samples positive for oxytetracycline (%) n=60	73.3	61.7	56.7	58.3	53.3	46.7
Samples with oxytetracycline levels above MRLs (%)	53.3	46.7	56.7	30.0	40.0	45.0

Superscripted values [a, b] in columns are significantly different ($p < 0.05$).

4.3.3.4 Prevalence of oxytetracycline residue in beef from Akure (Araromi) abattoir

The results of the quantitative (HPLC) analysis of meats from Araromi abattoir in Akure as shown in table 4.8 indicated that oxytetracycline was detectable in 63.3, 60.0 and 55.0% of kidney, liver and muscle samples respectively during the wet season and 51.7, 56.7 and 48.3% of the meat respectively during the dry season. The mean concentrations of oxytetracycline residue obtained from the different meat types in this abattoir ranged between 587.7 and 1185.0 µg/kg with variable proportions containing the residue above codex (CAC) recommended MRLs (table 5.5) There mean residue concentrations in muscle liver and kidney were significantly different ($p< 0.05$) with kidney having the highest followed by liver. However, there was no significant difference ($p>0.05$) in the tissues residue concentrations of wet season compared with the dry season.

Table 4.8: Oxytetracycline residue in beef samples from Akure (Araromi) abattoir

Location (season)	**Akure (wet season)**			**Akure(dry Season)**		
Portion of carcasses	**Kidney**	**Liver**	**Muscle**	**Kidney**	**Liver**	**Muscle**
Range mean concentration(µg/kg)	482.2-2915.0	163.6-2544.0	163.6-1660.0	308.4-2770.0	86.4-1737.0	67.1-1187.0
Mean residue level ±SD (µg/kg)	1185 ±581.1	945.2* ±541.2	692.6** ±376.2	1162 ±685.1	661.7* ±387	587.7** ±321.4
Proportion of samples with detectable oxytetracycline (%) n=60	63.3	61.7	55.0	51.7	56.7	48.3
Samples with oxytetracycline levels above MRLs (%)	6.4	10.4	12.8	4.0	7.2	9.6

Superscripted values*, ** in columns are significantly different ($p < 0.05$).

4.3.3.5 Prevalence of oxytetracyline residue in beef from Lagos (Oko-Oba) abattoir

The HPLC results of bovine meat samples from Oko-Oba abattoir in Lagos as shown in table 4.9 indicate that oxytetracycline residue was detectable in 56.7, 50.0 and 48.3% of kidney, liver and muscle respectively during the wet season and in 60.0, 60.0 and 50.78% of the different meat types respectively during the dry season from the same abattoir. The mean concentrations of oxytetracycline were 1267 ±476.4 SD, 904.9 ±421.4SD and 729.6 ±374.3 SD µg/kg in kidney, liver and muscle respectively. The results also indicated that 31.7, 40.0 and 46.7% of kidney, liver and muscle respectively from Oko-Oba abattoir respectively contained oxytetracycline at concentrations above the CAC recommended MRL during the wet season and in 40.0, 41.3and 45.0% of the different meat types respectively during the dry season. There mean residue concentrations in muscle liver and kidney were significantly different ($p< 0.05$), but there was no significant difference ($p<0.05$) in the tissues residue concentrations of wet season compared with the dry season.

Table 4.9: Oxytetracycline residue in beef from Lagos (Oko-Oba) abattoir

Location (season)	**Lagos (wet season)**			**Lagos (dry Season)**		
Portion of the carcasses	Kidney	Liver	Muscle	Kidney	Liver	Muscle
Range of oxytetracycline residue	76.7-2409.0	163.6-1708.0	178.1-1926.0	308.4-2819.0	240.8-1824.0	67.1-1709.0
Mean concentration ±SD(µg/kg)	1267.0 ±476.4 ^{e}	904.9±421.4^{f}	729.6±374.3^{g}	1436.0±554.1^{e}	761.7±375.6^{f}	546.2 ±373.8^{g}
Samples positive for oxytetracycline (%) n=60	56.7	50.0	48.3	60.0	60.0	50.7
Samples with oxytetracycline levels above MRLs (%)	31.7	40.0	46.7	40.0	41.7	45.0

Superscripted values $^{e, f, g}$ in columns are significantly different ($p < 0.05$).

4.3.3.6 Prevalence of oxytetracycline residue in chicken samples from farms and markets in Lagos and Ibadan

HPLC quantitative analysis of the chicken samples indicated oxytetracycline residue prevalence of 71.7 and 75.0% in liver and muscle respectively from Ibadan poultry farms, and also prevalence of 63.3 and 60.0% were obtained in liver and muscle respectively from Ibadan chicken market. Oxytetracycline residue prevalence from Lagos poultry farms were 53.3 and 60.0% in liver and muscle respectively and also 41.7 and 56.7% prevalence in chicken liver and muscle respectively from Lagos market. The overall mean concentration of oxytetracycline residue obtained from chicken muscle and liver samples in this study were 1042.0±122.8 and 615.0±91.8µg/kg respectively. The distribution of the mean residue levels in the chicken across the market and farm locations are shown in table 5.8 with a range of 41.7 to 75.0% of the samples having oxytetracycline above the CAC MRLs (table 5.9). Chicken samples from Ibadan farms had the highest prevalence of the residue.

Table 4.10: Oxytetracycline residue in chicken from Ibadan.

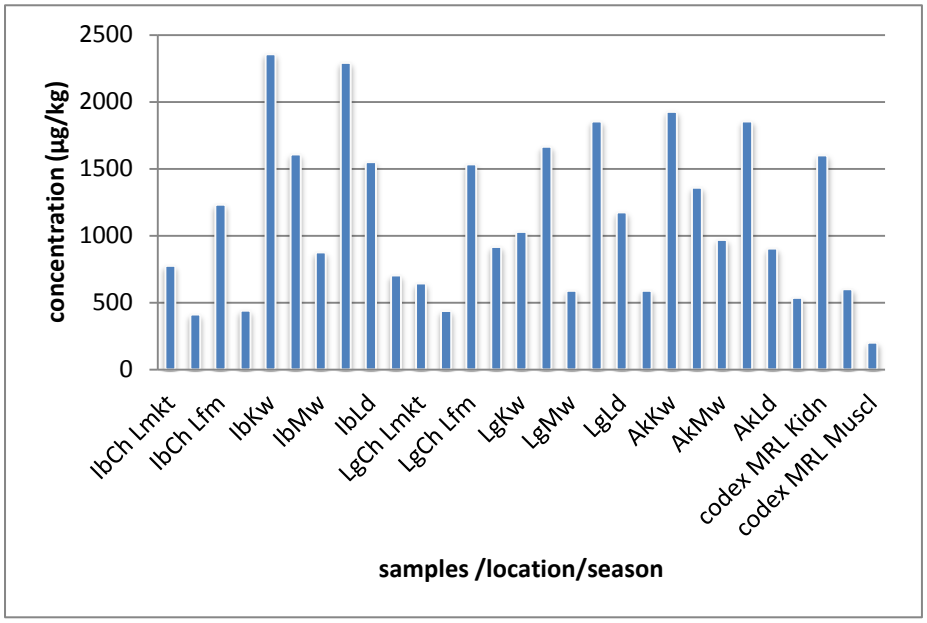
Location	**Ibadan (farm)**		**Ibadan (market)**	
Portion of carcasses	**Liver**	**Muscle**	**Liver**	**Muscle**
Range of oxytet residue (µg/kg)	299-2481	164-1129	270-1911	183-1525
Mean concentration ±SD(µg/kg)	1156 ±528 [i]	566.8 ±270.7 [i]	986.1 ±449.1 [j]	559.4 ±349 [j]
Proportion of samples with detectable oxytetracycline residue (%) n=60	43 (71.7)	45 (75.0)	40 (63.3)	36 (60.0)
Proportion with oxytetracycline above MRL	32 (53.3)	36 (60.0)	25 (41.7)	34 (56.7)

Superscripted values [i, j] in columns are significantly different ($p < 0.05$).

Table 4.11: Oxytetracycline residue in chicken from Lagos

Location	**Lagos (farm)**		**Lagos (market)**	
Portion of carcasses	**Liver**	**Muscle**	**Liver**	**Musce**
Range concentration(µg/kg)	164-2674	231-1853	19-1901	48-1660
Mean concentration ±SD(µg/kg)	1130 ±614.4 [l]	752.4 ±393.7 [l]	895.7 ±473.8 [k]	583.8 ±396 [k]
Proportion of samples with detectable oxytetracycline residue (%) n=60	42 (70.0)	35 (58.3)	43 (71.7)	45 (75.0)
Proportion with oxytetracycline above MRL (%)	34 (56.7)	35 (58.3)	31 (51.7)	36 (60.0)

Superscripted values [l, k] in columns are significantly different ($p < 0.05$).



***Keys**:

IbK_W = Kidney wet/s (Ibadan), IbL_W = Liver wet/s (Ibadan), IbM_W = Muscle wet/s, (Ibadan) IbK_D = Kidney dry/s (Ibadan), IbL_D = Liver dry/s (Ibadan), IbM_D = Muscle dry/s (Ibadan), IbCh L_{mkt} = chicken liver (Ibadan market), IbCh M_{mkt}= chicken muscle (Ibadan market), IbCh L_{fm} = chicken liver (Ibadan farm) IbCh M_{fm} = chicken muscle (Ibadan farm), LgCh L_{mkt} = chicken liver (Lagos market), LgCh M_{mkt} chicken muscle (Lagos market), LgCh L_{fm} = chicken liver (Lagos farm), LgCh M_{fm} = chicken muscle (Lagos farm), LgK_W= kidney wet/s (Lagos), LgL_W = Liver wet/s (Lagos), LgM_W= Muscle wet/s (Lagos), LgK_D= kidney dry/s (Lagos), LgL_D= liver dry/s (Lagos), LgM_D=muscle dry/s (Lagos), AkK_W= Kidney wet/s (Akure), AkL_W= Kidney wet/s (Akure), AkM_W= Muscle wet/s, (Akure) AkK_D = Kidney dry/s (Akure), AkL_D = Liver dry/s (Akure), AkM_D = Muscle dry/s

Figure 4.5: Mean oxytetracycline residue levels in beef and chicken from southwest Nigeria.

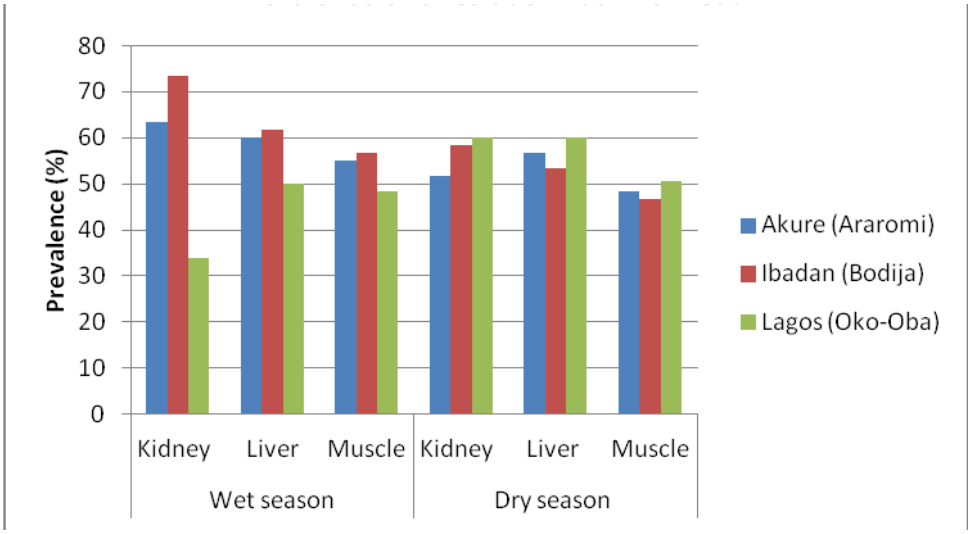


Figure 4.6: Seasonal prevalence of oxytetracycline residue in cattle

4.3.4 Hypotheses testing

Statistical analyses of antimicrobial residues prevalence in the meats types obtained by Premi®Test across the different abattoirs and during the different seasons of the year were obtained by chi-square test. There was no significant difference ($p<0.05$) in the overall prevalence of antimicrobial residues across the three abattoirs but the overall residue prevalence of antimicrobial residues in chicken (69.1%) was significantly higher ($p<0.05$) than in beef (45.4%), therefore the null hypothesis (H_O3) is rejected. Also, the prevalence of antibiotic residues in beef during the wet season was significantly higher ($p<0.05$) than the prevalence during the dry season, hence the null hypothesis (H_O4) is rejected.

The mean oxyteracycline residue concentrations were analysed by ANOVA and Tukey's Multiple Comparison Test. There was no significant difference in the mean oxytetracycline residue concentration ($p<0.05$) in the different meat types across the different abattoirs, but the levels of oxytetracycline residue in beef during the wet season were significantly higher tha residue levels during the dry season ($p<0.05$) therefore, H_O5 is rejected.

The mean residue concentrations from in the chicken liver were significantly higher ($p<0.05$) than in the chicken muscle but there was no significant difference ($p< 0.05$) between the mean residue in chicken from farms and the markets within and across the cities. However, the mean concentration of oxytetracycline residue in chicken was significant higher ($p< 0.05$) than mean concentration in beef (H_O6 is therefore rejected).

The overall mean concentration of oxytetracycline residue levels obtained in bovine kidney, liver and beef were 1324.7±148.0, 856.6±118.0 and 651.7±101.3µg/kg were respectively were significantly higher ($p<0.5$) than the CAC recommended MRLs of 1200, 600 and 200 µg/kg oxytetracycline in kidney, liver and muscle respectively (H_O7 is therefore rejected).

4.4 Discussion

The extensive use of veterinary drugs in animal production resulting from gigantic growth and intensification of animal production in order to meet the increasing population demand for animal protein have become a major global public health concern (Botsoglou and Fletouris, 2001; Jafari *et al.,* 2007). There are several national and international regulatory control and monitoring efforts to ensure safety of livestock products meant for human consumption. This study determined the prevalence of antimicrobial residues edible portions of cattle and chicken slaughtered for public consumption in three selected cities of southwest Nigeria. The results of this study showed higher prevalence in the samples of antimicrobial residue in both cattle and chicken than those obtained in previous studies by other workers in different parts of Nigeria. The total prevalence of 45.6% obtained in cattle and chicken samples respectively from this study were higher than 8.0, 7.4, 16.7 and 44.0% prevalence obtained by Oboegbulem and Fedelis, 1996; Kabir *et al.*, 1999 and Dipeolu and Alonge, (2002) in slaughtered cattle respectively across the country. Also the 69.1% residue prevalence in chicken samples from the present study is higher than 33.1%, prevalence obtained by Kabir *et al*., (2004). While a recent study by Ezenduka *et al*., (2011) also reported 36.0 and 30% of commercial eggs from farms and retail outlets of Enugu, Nigeria positive for antibiotic residues respectively.

The higher prevalence of antimicrobial residues obtained in the present study compared with the previous studies by other authors in Nigeria could be due to a better sensitivity of the test kit than the conventional overnight culture methods. Also the test organism (*Bacillus stearothermophilus var. calidolactis*) in Premi®Test has been shown to be sensitive to wide range of antibiotics (Stead *et al*., 2004). This could account for the sensitivity as a broader screening method that was able to detect many of the antibiotics routinely used by livestock producers in the country. Stead *et al*., (2004) and Popelka *et al*., (2005) confirmed the broad

spectrum and sensitivity of Premi®Test with the ability to detect many antibiotic residues below MRLs. These authors also concluded that the assay will relieve the chemical analytical resources of food inspection laboratories and contribute to less positive animals, safer products and better consumer protection thereby assuring the quality of animal food products, which is beneficial for the consumers and the producers. More so, another great advantage of the Premi®Test is the short time of the analysis (<4 hours) thereby ensuring quicker release of meat negative for antibiotics for public consumption unlike the conventional overnight microbial inhibition tests that were used by the previous author in Nigeria. Coupled with the ease of operation of the Premi®Test, its sensitivity and the rapid results obtainable within 3 to 4 hours the method is more practicable at all the levels of slaughter plants and slabs for antibiotics screening of large number of meat samples.

Also, this study also showed that residue prevalence obtained in chicken was higher than in cattle. This may be as a result of more frequent administration of different antibiotic combinations simultaneously in feed and water in poultry than in cattle, since poultry are reared under intensive management system with more challenges of infections compared to cattle which are reared on semi-intensive and extensive management system. Dipeolu *et al*., (2005) also reported routine antibiotics dosing of chicken in the markets against stress and diseases until they are sold for the fear of economic loss due to mortalities. Dipeolu *et al*., (2005) also reported that eggs from medicated chickens are sold usually sold for public consumption because poultry farmers could not adhere to withdrawal periods of antibiotics for fear of economic losses.

Nonga *et al*., (2009) also obtained a prevalence of 70.0% antibiotic residues in commercial broilers in Tanzania using microbial inhibition test while Muriuki *et al*., (2001); Shitandi and Sternejo (2001) detected 45.6% and 21.0% prevalence in Kenyan beef and milk respectively. These results are comparable to high prevalence obtained in this study thereby indicating

widespread of antibiotic residues in meat across developing countries as a result of indiscriminate use of antibiotics in these nations. This implied that the consumers were ingesting some level of antibiotics in food animal products which has significance public health consequences.

Antimicrobial residue prevalence in the liver samples was the highest followed by kidney and lowest in the muscle. These results are consistent with the findings of several authors since the liver and kidney are the major organs of drug metabolism and excretion. Higher prevalence of residues were also obtained in chicken than beef this could be due to increase frequency of use of more antibiotics in poultry for prophylaxis and as feed additives which were reared by intensive system than in the cattle by extensive management system. The higher prevalence of antibiotic residues obtained during the wet season than during the dry season. This could correlates to higher prevalence of bacterial diseases in the wet season requiring more antibiotics usage in wet season than the dry seasons.

According to CAC and other national residue regulatory monitoring and surveillance programs, confirmation of the identity and concentration of tissue residues by chemical analysis are required to compared with the MRLs in order to quantitatively assess the residue risk.

This study detected oxytetracycline from 56.2% and 64.2% of the meat samples from cattle and chicken that positive by Premi®Test respectively in the study area. The results showed a higher prevalence than 45.6% obtained by Muriuki *et al.,* (2001) in a survey of beef samples from Kenya slaughter. There has not been HPLC quantification of oxytetracycline residue in Nigerian meat. However, Dipeolu and Alonge, (2002) obtained tetracycline residue prevalence of 16.63, 15.0 and 13.34% in liver, kidney and muscles from Ogun and Lagos States (Nigeria) markets and 17.22, 16.11 and 6.67% of goat, cattle and pig meat containing

streptomycin residue ranging from 0.06 to1.99µg/g from southwest (Dipeolu and Alonge (2002). While Ibrahim *et al.,* (2010) obtained 44% total residue in beef of which 26% were oxytetracycline from Sokoto abattoir, also, Fagbamila *et al*., (2010) in a recent survey of commercial eggs in some part of northern Nigeria obtained 3.6% positive for antimicrobial residues with only 0.1% tested positive for tetracycline residue. These authors employed microbial inhibition method could not have detected protein bounded antibiotics in these tissues that required physicochemical extraction. More so, the size of zone of inhibition is determined by several factors such as the sensitivity of the test organism, inoculum size, pH of the medium. MIT is prone to false positive and negative but it is good for screening large number of samples as the first line of action in residue monitoring and control. Therefore the higher prevalence obtained in this study could be due to the specificity and sensitivity of HPLC method employed in this work as validation.

The residue concentrations vary with the meat types, the mean concentrations of 1324.7±148.0, 856.6±118.0 and 651.7±101.3µg/kg were obtained in bovine kidney, liver and muscle were respectively and 1042.0±122.8 and 615.0±91.8µg/kg in chicken liver and muscle respectively. The mean residue levels obtained were higher than codex MRLs for each meat type (200, 600 and 1200 µg/kg for muscle, liver, and kidney). In a similar study Muriuki *et al*., (2001) reported mean oxytetracycline residues of 1380 µg/kg in kidneys, 1090 µg/kg in liver and 790 µg/kg muscle of cattle in Kenya. Also in a survey conducted by Al-Ghamdi *et al*., (2000) in Saudi Arabia showed that antibiotic residue were present in 69.7% of broiler and 60%) layer chicken samples. While Salehzadeh *et al*., (2006) also obtained oxytetracycline residues 95.55% of farms survey in Iran with 27.77%, 95.55% and 18.88% of muscle, liver and kidney samples containing residues of oxytetracycline above MRLs respectively using HPLC.

These results indicate that a large proportion of cattle and chicken meat being slaughtered for human consumption in Nigeria and other developing countries contain varying detectable levels of oxytetracycline residue above MRLs which could result in public health hazards. Also the prospect of international trade in meat products envisage by most developing countries through WTO due to increase livestock production in this region cannot be achieved with such products that cannot meet Codex SPS standards. This implies a huge loss of potential foreign exchange.

There is stringent regulation of drug use in food animals and residue control in developed countries with resultant low level of residue violation. Okerman *et al*., (1998b) reported that 1.2% of chicken meat samples and 2.7% of pork meat samples, purchased from retail outlets contained residues belonging to the tetracycline family. De Wasch *et al.,* (1998) reported that two out of 523 pork and none of the 1768 chicken samples containing oxytetracycline residue at levels higher the maximum residue limit, while Oka *et al.,* (2001) obtained the overall incidence of 30.9% tetracycline residues in beef and pork from Japan, while Nhiem *et al*., (2006) detected residue prevalence of 5.5% in pork from Vietnam.

The results of this study also showed that the mean oxytetracycline levels were higher in liver and kidney than muscle, but the level in cattle muscle was higher than in chicken muscle. This could be due to the facts that cattle were administered with intramuscular injectable brands of the drugs while chicken were dosed through feed and water. However, there was no significance difference in the mean concentrations of oxytetracycline across the different locations and during the different climatic seasons. This could imply that there was no difference in livestock husbandry practices across the region and that oxytetracycline was commonly used throughout the year.

CHAPTER FIVE

ISOLATION AND ANTIBIOTICS SUSCEPTIBILITY OF *ESCHERICHIA COLI* O157:H7 FROM BEEF AND CHICKEN IN IBADAN AND LAGOS

5.1 Introduction

There are growing concern of bacterial adaptation and evolution resulting in the emergence of a number of zoonotic microorganisms in the food and water. Food-borne disease is a global public health concern. Mead *et al*., (1999) reported an estimated food-borne of 76 million illnesses, 325,000 hospitalizations and 5,000 deaths annually in United States and in the United Kingdom, an estimated 2.37 million cases of food-borne gastroenteritis occurred in 1995 (Adak *et al.,* 2002). Available data from United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) indicated that 13 million kg of ground beef were contaminated with *E. coli* O157:H7 on August 12, 1997 and 9.5 million kg of beef trimmings and ground beef potentially contaminated with *E. coli* O157:H7 on July 19, 2002 (Sofos, 2008). *Escherichia coli* is a widespread intestinal commensal organism found in human and animal resulting from faecal contamination or contamination during food animal slaughter it is often found in soil, water, feaces and foods.

Shiga toxin-producing *E. coli* (STEC) O157:H7 has emerged as a major foodborne pathogen and a threat to public health following its initial identification in a 1982 outbreak of illness associated with the consumption of undercooked ground beef (Riley *et al*., 1983). There are many pathogenic strains causing a variety of illness in man and animals with associated clinical features and virulence factors depending on the serogroups from a food safety perspective, the EHEC groups are most important. Specifically, *E. coli* O157:H7 and O157: NM (non-motile) are recognized as major etiologic agents in hemorrhagic colitis (HC) and haemolytic-uremic syndrome (HUS) in humans (Thielman and Guerrant, 1999). As with

other food-borne bacterial pathogens, the potential health hazard presented by foodborne *E. coli* is influenced by numerous factors, ranging from variations in farm-rearing practices and processing, storage, handling and cooking in the home (WHO, 2000). Cattle and other ruminants were identified as the main reservoir for human infections (Nataro and Kaper, 1998; Gyles, 2007). Several outbreaks have been associated with other food commodities such as milk, lettuce and chicken (Rangel, *et al*., 2005). The bacteria is highly infectious for human being at a very low infectious dose of 10 to 100 organisms (Willshaw, *et al*., 1993)

Antibiotics resistant *E. coli* has been reported over the past 50 years since the chemotherapeutic and growth promotion uses of antibiotics (Orden *et al*., 1999; Lambie *et al*., 2000). Studies in the UK found that, in the late 1950s, tetracycline resistance was already detectable in *E. coli* isolates from chickens and pigs fed rations containing less than 100 g tetracycline/ton (Smith, 1967). Resistance to other antibiotics was detected as new agents were introduced for therapeutic and growth promotion purposes (Smith, 1967; Anderson, 1968). Antimicrobial resistant food-borne pathogens are acquired primarily through consumption of contaminated food of animal origin or water (Mead *et al.*, 1999; National Research Council, 1998). Information on the magnitude of the public health burden due to resistant food-borne pathogens indicates that the situation is complex and differs by country. It is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter, storage and distribution systems, the availability of clean water, and proper cooking and home hygiene, among others (WHO, 2000). The major concern on the public health threat of foodborne illness is infection by antimicrobial-resistant strains that lead to more intractable and severe diseases (Helms *et al*., 2002; Martin *et al*., 2004). This situation is further complicated by the potential of resistant bacteria to transfer their resistance determinants to resident comensals of the human microflora and other pathogenic bacteria.

Several data have been published on resistance in *E. coli* originating from retail raw meat products (Meng and Doyles, 1997; Zhao *et al*., 2001), resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries including Nigeria (Hart and Kariuki, 1998; Aibinu *et al.*, 2007; Okeke *et al.*, 2005; Ojo *et al*., 2009). Unhygienic floor dressing of carcasses is a common practice in Nigeria resulting in carcass contamination and isolation of pathogenic microorganisms from meat and slaughtering facilities in Nigeria (Umolu *et al*., 2006, Ojo *et al*., 2009).

Several countries of the developed nations have established national surveillance programmes whereby they continuously assess bacterial susceptibility to antimicrobials among zoonotic and commensal bacteria isolated from humans and animals. Such programs as Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP), Denmark; The National *Antimicrobial* Resistance Monitoring System (NARMS), USA; Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM), Japan; Monitoring Antimicrobial Resistance And Antibiotic Usage Animals, the Netherlands (MARAN); Indian Initiative for Management of Antibiotic Resistance (IIMAR); Canada (CIPARS); Sweden (SVARM) and Norway (NORM-VET).

However, there are no sufficient data on the susceptibility of zoonotic and commensal bacteria isolated from food animals and no national surveillance programmes on the antimicrobial susceptibility in Nigeria. The importance of resistance determinants derived from animals is still largely unknown and the the role of meat-borne bacteria as vectors in the transmission, spread and development of cross-resistance to several antibiotics requires critical scrutiny. White *et al*., (2004) suggested the need for continuous research on the ecology and epidemiology of major foodborne pathogens, and surveillance of retail food (including meat) products in order to characterize and mitigate food-borne bacterial resistance. Additionally, several authors emphasised the need for continuous assessment of

the public health consequences of the use of antimicrobials in the animal husbandry (Smith *et al*., 2003; Hald *et al*., 2004; Phillips *et al*., 2004). Antimicrobial drug susceptibility profiles and genetic strain typing methods are useful epidemiologic tools to determine the sources of infections, including potential links between food animals and persons (Okeke *et al*., 2007). Considering the nature of veterinary drugs use in livestock and the prevalence of resistance pathogens in animal production and processing environment, this work was aimed at isolation and assessment the resistance patterns of meat-borne *E. coli* O15:H7 in beef and chickens processed for human consumption in Lagos and Ibadan, southwest Nigeria.

5.2 Materials and Methods

5.2.1 Sterilization of Glass Wares and Preparation of Culture Media

All glass wares including petri dishes, Bijuo bottles, universal bottles, media bottles measuring cylinders, conical flask and others were thoroughly washed and rinsed with clean water and sterilised in the hot air oven at 160^{O}C for 1hour before use.

5.2.2 Preparation of microbiological media for bacterial isolation and identification:

The culture media were sterilized by moist heat under pressure in the autoclave at 121^{O}C for 15 minutes and prepared according to manufacturers' recommendation. After sterilization by autoclaving, the solid media were allowed to cool to about 45^{O}C in media bottles on the bench to cool and then about 20ml was poured into sterile covered petri dishes and were allowed to gel. Air bubbles were removed from the surfaces of some of the agar plates before solidification and were kept in the refrigerator until they were to be inoculated.

5.2.3 Samples and Sampling Procedures:

One hundred samples (about 25grams) of beef were randomly obtained from Bodija and Oko-Oba abattoirs during the wet season and repeated in the dry season and also 100 samples each of broiler chicken meat were randomly and aseptically collected from Mokola slaughter market, Oko-Oba chicken slaughter market and poultry farms in Ibadan and Lagos (20 samples from ten farms) shortly after meat dressing. The samples were obtained by carcass surface scrapings (excision method) with sterilised meat inspection knife and superficial cutting about $5cm^2$ piece of tissue of about 3mm thick from four different portions of each carcass, the knife was rinsed with about 100ml peptone water into sterile sample bags (Whirl-Pak Nasco, USA). The sample bags were placed into icebox which was subsequently transported to the Food and Meat Hygiene Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for the bacteriology within 24hours. The samples were collected from April 2008 to June 2009.

5.2.4 Bacterial isolation

Each meat sample was thoroughly homogenised with 25ml peptone water and incubated overnight at 37^oC. A wire loop full of the broth culture was separately inoculated each onto 7% sheep blood agar (Oxoid®) and MacConkey agar (Oxoid®). The plates were incubated aerobically at 37°C for 24hrs. The bacterial isolates were identified by their cultural characteristics, morphology, gram and biochemical reactions according to standard methods described by Barrow and Felthman (1993). Red to pink (lactose fermenters) colonies, Gram negative rod, motile, indole positive, catalase positive, oxidase negative, and showed the standard biochemical characteristics of *E. coli* described by Barrow and Felthman (1993) were further subcultured onto CT-Sorbitol-MacConkey (CT-SMAC) agar plates, and incubated at 37 °C at 24 hours. Colonies that were colourless to pale, flat and smooth,

circular or serrated at the edge were selected as presumptive non-sorbitol fermenting *E. coli* for serological test. The bacterial isolates were identified by their cultural characteristics, morphology, Gram staining and biochemical reactions according to standard methods described by Barrow and Felthman (1993).

5.2.5 Identification and Biochemical Tests

a. Gram staining

Sterile wire loop was used to pick a discrete colony of suspected *E. coli* from the agar plate onto a clean glass slide. The colony was emulsified in a small drop of normal saline and allowed to dry in the air. The smear was heat-fixed by passing over flame. The glass slides was labelled and arranged on a rack and were stained as follows: smears were flooded with methyl violent and allowed the stain to act for about 15 seconds. Excess stain was rinsed off with a little water. After which Gram's iodine was poured and allowed to act for about 15 seconds which was washed off with water. This was followed by flooding with acetone which was almost immediately rinsed off under running water. Subsequently safranin was poured and allowed to act for 30 seconds, washed off and dried by blotting paper. The stained smears were then examined under microscope with oil immersion using X100 objective lense (Barrow and Feltham, 1993).

b. Haemolysis test

Haemolytic activity of the isolates was tested by culturing the isolates in 7% sheep blood agar (Oxoid Columbia blood agar) and incubating at 37°C for 24 h. The isolates that produced haemolysis are presumptive EHEC and were further characterised.

c. Catalase test

Catalase test demonstrates production of catalase enzyme by the organism. Catalase releases oxygen from hydrogen peroxide. Members of the family *Enterobactereacea* including *E. coli* are catalase positive. Smear of suspected colony was put on a clean glass slide. And then a drop of 3% hydrogen peroxide was added and observed for effervescence after gently rocking the slide (Barrow and Feltham, 1993).

d. Oxidase Test

A redox dye (tetramethyl-P-phenydiamine) was added to a strip of filter paper and the culture of the colour of the filter paper from white to deep purple. *E. coli* and other members of the family *Enterobactereacea* are oxidase negative (Barrow and Feltham, 1993). This test demonstrates the presence of oxidase enzyme that catalyses the transport of electron between the bacterium and the dye.

e. Motility Test

Motility was demonstrated by the hanging drop method according to Barrow and Feltham, (1993). Plasticine was made into a circle and placed on a cover slip; a drop of overnight culture of the organism in buffer peptone water was dropped at the centre of the circle. A microscope slide was placed on the plasticine and then inverted. This was viewed at X40 objective under microscope.

f. Indole Test

Few drops of Kovac reagent (5 g *p*-dimethylaminobenzaldehyde dissolved in mixture of 75 ml amyl alcohol and 25 ml concentrated sulphuric acid) was added to a pure culture of the organism in peptone water. The mixture was shaken. The appearance of a pink or red ring layer at the upper surface of the mixture indicated positive result while the absence of the red ring indicated a negative result **(**Barrow and Feltham, 1993).

g. Citrate utilization test

Pure colony was inoculated into Simmon citrate agar according to Barrow and Feltham, (1993) in bijou bottles. These were incubated at 37^{o}C for 18-24 hours citrate positive isolates changed the colour of the agar from green to blue while negative ones did not produce colour change. *E. coli* is citrate negative.

h. Sugar fermentation test

The appropriate sugar solution (1%) was prepared by dissolving 1g of the sugar in peptone water to which Andrade's indicator has been added (10ml of indicator/litre of peptone water). The sugar solution was dispensed into bijou bottle and sterilized in the autoclave at 121^{o}C for 5minutes. Bacterial isolates were inoculated into a set of sugar solution and incubated for 12-18 hours at 37^{o}C. Positive organisms that have fermented sugar changed the colour of the solution from colourless to pink while those that did not ferment sugar did not produce colour change.

i. *E. coli* O157 latex agglutination test:

This test was carried out using *E. coli* O157 latex agglutination test kit (Oxoid® DRO 120M, UK) according to the manufacturer's recommendation and protocol. The refrigerated reagent was allowed to thaw at room temperature before use. A drop of the test latex was dispensed at the edge of the circle on the reaction card provided. 2-3 loopfuls of saline was added to the center of the circle in both tests and control on reaction card. A portion of suspected colony was picked with a sterile stick and carefully emulsified in a saline drop. The mixing sticks provided was used to spread the mixture over the entire area of the ring the same was done with the control latex and control organism. The card was rocked in a circular motion while observing for visible agglutination within one minute by the test latex and identified as *E. coli*

O157 while those with no agglutination were considered negative. Sorbitol-positive *E. coli* ATCC25922 was used as negative control.

5.2.6 Determination of Antibiotic Susceptibility and resistance pattern of *E. coli* O157 isolates

The E.coli O157 isolates were further tested for their susceptibility to antimicrobial agents by disc-diffusion method according to Bauer *et al*., (1966) and Cheesbrough, (2000). The isolates were screened for their susceptibility to some commonly used antibiotics using commercially available multo-disks purchased from Abtek Biological Ltd, England containing nitrofurantoin (200µg), cefuroxime (25µg), norfloxacin (30µg), cotrimoxazole (25µg), gentamycin (10µg), tetracycline (30 µg), ciprofloxacin (25µg), nalidixic acid (30µg), chloramphenicol (30µg) and ampicillin (25 µg) were used. *E. coli* NCTC 10418 was used as positive control. About 3ml of overnight nutrient broth culture of the pure colonies was flooded on the surface of Nutrient agar plates while the excess is carefully discarded into disinfectant. The inoculated plates were allowed to dry for about 20 minutes. Subsequently, the antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37^{O}C for 16-24 hours. Inhibitory zones of growth were observed, measured and recorded. The result was interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) now known as Clinical Laboratory Standard Institute (CLSI) guideline, (2005). The ssusceptibility of the isolates was characterized according to the breakpoints recommended by the NCCLS and designated as susceptible, intermediate or resistant. Intermediate strains were grouped with the sensitive isolates. Zones of inhibition above 2mm were recorded as sensitive, while the discs with no inhibition or below 2mm were recorded as resistant.

5.2.7 Statistical Analysis

The positive results were express in percentages as prevalence rates for both beef and chicken meat. Statistical comparison of the prevalence rates and the frequencies of resistance among isolates obtained from different meat types and sources were obtained by chi-square ($\chi 2$) test.

5.3 Results

5.3.1 Prevalence of *E. coli* O157:H7 in Beef and Chicken

Based on colonial morphology, microscopy and the biochemical tests *E. coli* were *isolated* from which *E. coli* O157:H7 serotype was confirmed with the agglutination test.

Out of a total of 400 each of beef and chicken samples examined in this study 186 and 129 isolates of *E. coli* were isolated from beef and of chicken respectively. Seventy nine (19.8%) and 58 (14.5%) of isolates were confirmed positive for *E. coli* O157:H7 serotype from beef and chicken respectively. The prevalence of this pathogen in the beef from Bodija metropolitan abattoir were 26% and 31% during dry and wet season respectively while beef from Oko-Oba yielded the prevalence of 10% and 12% during the dry and wet season respectively (table 5.1).

Results of the chicken samples showed prevalence of 13% in Ibadan chicken slaughter markets, 18% in broiler from Ibadan farms, 14% in Lagos chicken slaughter market and 13% in chicken from Lagos farms (table 5.1). Beef from Bodija abattoir (Ibadan) had the highest contamination with this organism. Contamination was also highest in the meat obtained during the wet season than the dry season.

The prevalence of *E. coli* O157 was significantly higher in beef ($p<0.05$) compared to chicken (19.8% vs. 14.5%). The prevalence of beef at the Bodija abattoir is also significantly higher than that of Oko-Oba abattoir, Lagos ($p<0.05$).

Table 5.1 Distribution of *E. coli* O157 isolated from beef and chicken

Carcass type	Sample location (season)	No of chicken samples (n)	No of NSF* *(E. coli)* isolated	No of *E. coli* O157 isolated
Cattle (beef)	Bodija (dry s.)	100	45	26
	Bodija (wet s.)	100	63	31
	Oko-Oba (dry s.)	100	36	10
	Oko-Oba (wet s)	100	42	12
Subtotal		400	186 (46.5%)	79 (19.8%)
Chicken	Ibadan (market)	100	38	13
	Ibadan (farms)	100	33	18
	Lagos (market)	100	36	14
	Lagos (farms)	100	22	13
Subtotal		400	129 (32.3%)	58 (14.5%)
Total		800	215 (26.9%)	137 (17.1%)

5.3.2 Antibiotics susceptibility and resistance pattern of *E. coli* O157 isolates

Antibiotic susceptibility profile showed that all the *E. coli* O157 isolates from beef and chicken were resistant to one or multiple antibiotics. The frequencies of resistance by the isolates from beef and chicken to the individual antibiotic are shown in table 5.2 and 5.3 respectively. The isolates exhibited resistance to all the antibiotics with tetracycline resistance (91.1 and 89.7% in beef and chicken respectively) being the highest. Nine different resistant patterns were identified with the isolates from chicken and beef as shown on tables 5.4 and 5.5

Table 5.2: Frequencies of Antibiotics Resistant *E. coli* O157:H7 isolates from Beef

Antibiotics	No of resistant isolates (prevalence %)	Distribution of resistant isolates			
		Bodija (d. season)	Bodija (w. season)	Oko-Oba (d. season)	Oko-Oba (w. saeson)
Ampicillin	18 (22.8)	8	4	3	3
Cefuroxime	40 (50.6)	12	18	4	6
Ciprofloxacin	6 (7.6)	1	1	2	1
Chloramphenicol	20 (25.3)	7	9	2	2
Cotrimoxazole	15 (19.0)	7	5	2	2
Gentamycin	12 (15.2)	5	4	1	2
Nalidixic	8 (7.6)	3	2	1	2
Nitrofurantoin	60 (80.0)	18	23	8	11
Norfloxacin	15 (19.0)	18	25	9	8
Tetracycline	72 (91.1)	24	29	9	10

Table 5.3: Frequencies of antibiotics resistant *E. coli* O157:H7 isolates from chicken

Antibiotics	No of resistant isolates (%)	Source of isolates			
		Ibadan market	Ibadan farm	Lagos market	Lagos farm
Ampicillin	16 (27.6)	5	3	3	5
Cefuroxime	35 (60.3)	8	8	9	10
Ciprofloxacin	10(17.2)	2	4	2	2
Chloramphenicol	30 (51.7)	7	11	6	6
Cotrimoxazole	16 (27.6)	5	4	3	4
Gentamycin	15 (25.9)	2	6	3	4
Nalidixic	8 (13.8)	2	1	2	3
Nitrofurantoin	45 (80.8)	8	14	10	13
Norfloxacin	17 (29.3)	3	6	3	5
Tetracycline	52 (89.7)	12	17	12	11

Table 5.4: Antibiotic resistance patterns of *E. coli* O157:H7 isolated from beef from Lagos and Ibadan abattoirs

Pattern of resistance	Isolates from beef			
	Bodija (dry s.)	Bodija (wet s.)	Oko-Oba (dry s.)	Oko-Oba (wet s.)
Am,C,Cf,Co,Gn Na,Nf,Te	4	6	2	0
Am,C,Cf,Co,Gn,N,Te	3	5	0	3
Am,C,Cf,N,Na,Te	5	3	0	3
Am,C,Cf,Cp,N,Te	3	2	2	0
Am,C,Co,N,Te	0	5	0	2
Am,C,N,Te	2	3	1	1
Am,C,Cf,N	1	0	3	0
N,Cp,Nf,Te	2	4	0	2
Te	6	3	2	1
Total	26	31	10	12

Table 5.5: Antibiotic resistance patterns of *E. coli* O157:H7 isolated from chicken from Lagos and Ibadan markets and farms

Pattern of resistance	**Isolates from chicken**			
	Ibadan chicken (market)	**Ibadan chicken (farms)**	**Lagos chicken (market)**	**Lagos chicken (farms)**
Am,C,Cf,Co,Gn Na,Nf,Te	4		1	3
Am,C,Cf,Co,Gn,N,Te	0	1	1	2
Am,C,Cf,N,Na,Te	2	0	2	0
C,Cf,Cp,N,Te	0	2	0	2
Am,C,Co, Gn,N,Te	3	3	3	4
Am,C,N,Te	0	2	6	2
Am,C,Cf,N	2	3	2	0
N,Cp,Nf,Te	1	0	0	1
Te	1	4	2	1
Total	13	18	14	15

5.4 Discussion

E. coli is one of the common microflora of gastrointestinal tract of human being and animals including poultry but may become pathogenic to both (Jawetz *et al*., 1984; Fairbrother and Nadeau, 2006). Although most isolates of *E. coli* are usually non-pathogenic but they are considered as indicators of faecal contamination in food. It is use as an indicator bacterium because it acquires antimicrobial resistance faster than other conventional bacteria and transferred to pathogenic bacteria (Von Baum and Marre, 2005; Miranda *et al.*, 2008). *E. coli* O157:H7 or Shiga toxin producing *E. coli* (STEC) is a major cause of food borne disease globally (Armstrong *et al*., 1996).

This study demonstrated a high prevalence of *E. coli* contamination of beef and chicken from which the pathogenic *E. coli* O157:H7. The high prevalence of contamination of meat from the different abattoirs and slaughter houses shown in this study is an indication organism for the presence of unacceptable contamination with bacteria. Seasonal prevalence of the contamination also showed significantly higher prevalence of the organism contamination during the wet season than in the dry season at the different abattoirs. This could be as a result from higher proliferation of the organisms and poor hygiene during the wet season. Also the prevalence of the isolates was higher in cattle than in chicken could have resulted from better hygiene practices at poultry slaughter houses than those practiced at the cattle abbatoirs. The high level of carcass contamination obtained in this study could be due to unhygienic slaughtering and meat processing engaged in these abattoir and slabs, where butchering of meat were done on concrete floor with inadequate basic slaughtering facilities including lack of potable water (Abiola, 1995). Also lack of the practice of hazard analysis critical control programme during the slaughtering process could have predisposed the carcasses to much contamination and subsequently the meats with multi resistant *E. coli* and other pathogens (Turtura *et al*., 1990; Umolu *et al.*, 2006).

The study also confirmed that food animals are the major reservoirs of *E. coli* O157:H7 which could possess antimicrobial resistant genes that contaminate meat and milk meant for public consumption. The bacterium have also been isolated from live cattle, meat and milk from other parts of the country by different researchers (Ojo *et al.,* 2009; Aibinu *et al.*, 2007; Luga *et al.,* 2007). Umolu *et al*., (2006) isolated multiple resistant strains of E.coli in meat from slaughtered cattle in Edo State Nigeria while Aibinu *et al.,* (2007) also isolated *E. coli* O157 from cattle, pig, chicken sheep and humans in Lagos and Ogun States. Daini and Adesemowo, (2008) also found the resistance of *E. coli* from Nigeria in 54% and 88% strains against gentamicin and tetracycline respectively. Also high resistance of enterotoxigenic *E. coli* has been reported by other authors across the globe. For example in England and Wales up to third isolated *E. coli* from pigs were multi-resistant (Mazel and Davis, 1998). In Canada almost all (93%) of tested isolates were resistant to tetracycline, and a similar number (91%) were resistant to sulphonamides.

Contamination of meat and other animal products with entero-pathogenic bacteria and their contribution in the epidemiology of antibiotic resistant in man and animal are of global food safety concern (White *et al*., 1998). Most of the isolates obtained in this study were resistant to multiple antibiotics with variable resistance patterns. The multiple antimicrobial resistances and the highest resistance to tetracycline obtained in this study were similar to the patterns obtained by Aibinu *et al.,* (2007) who obtained 94.4% resistant tetracycline strains of E. coli O157:H7isolated from animals and man. This could be due to the high frequencies of use of tetracycline among livestock in Nigeria as growth promoter for routine chemoprophylaxis. Tetracycline are readily available in different dosage forms and in combination with other antibiotics and vitamins and accessible to livestock producers. This study also confirms the widespread resistance to most commonly used antimicrobial agents in both human and animal health practice in Nigeria. The public health significance of

these findings is that antimicrobial resistant bacteria from food animals may colonize the human population via the food chain, contact through occupational exposure, or waste runoff from meat production facilities to the neighbourhood.

The multiple antibiotic resistance partterns exhibited by all the isolates obtained in this study is similar to the findings from Nigeria and other parts of the world (Rahman *et al*., 2001; Khan *et al*., 2002 Zhao *et al*., 2005; Umolu *et al*., 2006; Aibinu *et al*., 2007; Ojo *et al*., 2009;). Antimicrobial use and/ or especially abuse have been considered to be the most vital selecting force to antimicrobial resistance of bacteria (Okeke *et al*., 1999). There are well established evidence that antibiotics can lead to the emergence and dissemination of resistant *E. coli* which can then be passed into people via food or direct contact with infected animals. These resistant microbes also function as a potential source in the transportation of antimicrobial resistance to human pathogens (Van de Bogaard *et al.*, 2001; Schroeder *et al.*, 2002). The high prevalence of antibiotic resistant bacteria in this study as obtained from other developing countries has been associated with several factors including indiscriminate and uncontrolled use due to unregulated access of non-professional to different classes of antimicrobial over-the-counter (Dina and Arowolo, 1991; Hart and Kariuki, 1998; Okeke *et al.*, 1999). Selective pressures exerted by unregulated use of antibiotics as a growth promoter in food animals have created large reservoirs of transferable antibiotic resistance in the ecosystem and to bacterial pathogens of humans (Witte, 1998). These antibiotic resistant strains can ultimately replace the drug sensitive microorganisms from antibiotic saturated environment (Van de Boogard and Stobberingh, 2000).

This study revealed that retail raw meats are often contaminated with food-borne pathogens in variable prevalence and antibiotic resistance patterns; thereby stressing the need for prudent and regulated use of antimicrobial in food animals, increased implementation of hazard analysis of critical control point (HACCP) and consumer food safety education efforts.

CHAPTER SIX

CONLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The findings in this study elucidated the importance of antibiotics usage in cattle and poultry and provided the quantitative analysis of the prevalence and levels of antimicrobial residues and *E. coli* O157:H7 contamination in beef and chicken meat being consumed in cities of south western Nigeria. The pathogens could have been carried over along production and meat processing line water troughs have been shown to support *E. coli* O157, and be a source of colonisation.

The widespread and unrestricted usage of different antibiotics in food animals without adequate diagnosis, prescription and supervision by veterinarians contribute greatly to the selective pressure on microorganisms and resulting in multiple resistant strains of bacteria and the deposit of residues of these drugs in the meat. This was evident by the high prevalence of resistance *E. coli* O157:H7 and antibiotic residues in the meat. Also the common practices of unhygienic meat processing and floor dressing of carcasses predisposed the meat to contamination by both the flora and pathogenic bacteria such as the *E. coli* O157:H7.

The multiple drug resistance patterns exhibited in this study is of public health importance as *E. coli* generally is a common bacterial flora of humans and animals that carries transmissible plasmids that enhance and maintain the dissemination of antibiotic resistance genes among other pathogenic and commensal bacteria. In addition the risks of intestinal and extra-intestinal infections of consumers with the resistant *E. coli* O157:H7 from the contaminated could account for the incidence of renal failure among hospitalised patient in Nigeria.

Tetracycline resistance was the most common resistance among the pathogen in this study could have resulted from the drug being one of the most commonly used antibiotics in animal production. Different brands of tetracycline were reported being administered orally in feeds and water or by injection to both cattle and poultry.

This study also provided quantitative data on the prevalence of oxytetracycline residue in cattle and chicken meat that were being consumed in cities of southwest Nigeria. The results of this study revealed that greater proportion of the meat being consumed in southwest Nigerian cities contained oxytetracycline residues above international food safety standards. The meat could also contain residues of several other antibiotics that are available for use in livestock. The high proportion of cattle and chicken meat samples containing residues of antimicrobials could be due to the misuse and lack of strict regulation and control of antimicrobial use Nigeria livestock production. This therefore requires urgent public health attention by the consumers and appropriate regulatory agencies in the country. More so, Nigeria being a member of WTO has the potential of participating in international meat and other livestock trade that could increase foreign earning if appropriate SPS measures are observed to regulate the industry.

6.2 Contributions to Knowledge

a. This work demonstrated the inadequate structure, practice and lack of awareness of food safety objective in food animal production among livestock producers in southwest Nigeria and the unregulated practices of animal heath management.

b. Another major contribution of this work is that it is the first quantitative prevalence analysis of oxytetracycline residue in meat in Nigeria using HPLC analytical method comparable with international food safety regulation (CAC) protocols for confirmation of violative levels of residue. This study showed that high proportion

(37.8 to58.8%) of beef and chicken being consumed in southwest Nigerian cities contained antimicrobial residues especially oxytetracycline residues above the codex maimum residue limits (MRLs).

c. *E. coli* that is generally considered a conmensal has been assuming pathogenic status also of major role in the dissemination of antibiotic resistance genes, this work elucidate the prevalence and antibiotic resistance status of the highly pathogenic *E.coli* O157:H7 contamination of beef and chicken sold for human consumption in the southwest Nigeria. This work also attempted to associate the high prevalence of resistant STEC of tetracycline and residue of oxytetracycline in chicken and beef with the indiscriminate usage of antibiotics among the livestock farmers.

6.3 Recommendations

Food animals and foods of animal origin are traded worldwide and the occurrence of antimicrobial resistance is a global problem. Programmes monitoring the occurrence and development of resistance and consumption of antimicrobial agents are strongly desirable. It is recommended that large scale epidemiological survey covering all the regions in Nigeria be carried out to fully evaluate the consumer safety of food animal products (meat, milk and egg) concerning resistant food-borne pathogens and antibiotic residues in the country.

According to WHO, global initiatives and the establishment of common guidelines and systems controlling resistance in all countries must pay closer attention to the national monitoring of distribution, prescription and usage in the developing countries (including Nigeria). There is need for establishment of national pharmaco-epidemiological surveillance program on antibiotic usage, residues and resistant food-borne pathogens in man and animal, especially along the food chain to protect meat consumers Nigeria.

Application of HACCP from farm to fork in food animals and meat production and processing is also recommended to monitor and control chemical and microbial hazards along the food chain. This should involve proper structuring of livestock production in the country, regular extension education for livestock producers, marketers and processors on good animal husbandry practice to ensure safe animal protein supply.

Veterinary supervision of livestock production for proper diseases diagnosis, prescription of antibiotics and observance of withdrawal period in food animal production in Nigeria are equally recommended. Good management practices and vaccinations of livestock as alternatives to antibiotic prophylaxis are also recommended. Vaccination of poultry has been very effective in reducing the incidence of food-borne pathogens among livestock. The

feeding of probiotics ("beneficial bacteria") to livestock to competitively exclude the pathogens is also recommended as a good alternative to antibiotic chemotherapy.

Hygienic meat production and processing practices should be promoted among the butchers and other meat handlers. The improvement of abattoir facilities to include laboratory infrastructure for routine testing or monitoring of food-borne microbial and chemical hazards is also recommended. This involve establishment of modern abattoirs meat storage and supply chain with potable water. Adequate water treatment such as chlorination will help to reduce the incidence *E. coli* in livestock farms and abattoir water.

Rapid residue screening kits such as Premi®Test should be made available at the meat inspection laboratories. Establishment of reference laboratories within the Local Government Areas, States and Regions of the countries is also recommended for confirmation of chemical and microbial safety of food of animal origin. These will greatly enhance Nigeria's participation in international meat trade and boost our foreign investment.

There is also the need to review and amend the enabling laws on animal disease control, handling of veterinary drugs and meat inspection in Nigeria to ensure safety of meat consumers. There is need to create Veterinary Directorate at National Directorate for Food and Drug Administration and Control to function like the Centre for Veterinary Medicine of FDA and EUCVM. There must also be stricter regulations and enforcement on the use of antibiotics accompanied by strategies to educate the public, physcians, and veterinarians on the appropriate use of antibiotics.

Pharmaco-epidemiological studies are required on safety of food of animal origin through molecular tracking of food-borne pathogens, the mobility and transfer of antibiotic resistance genes (markers) along the food chain. There is also the need for national quantitative risks surveillance of meat, milk, egg and honey for residues of antibiotics and

other growth promoters which are inimical to public health are highly desired. The true impact of antibiotic use as growth promoters need to be accessed since antibiotic resistance and residues are important factors in international livestock trade between different countries of the world and human migrations.

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APPENDIXES

Appendix i: Codex Definitions on antibiotics and residue

Acceptable Daily Intake (ADI): An estimate by JECFA of the amount of a veterinary drug, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk (standard man = 60 kg).

Bioavailable Residues: Those residues that can be shown, by means of an appropriate method (e.g. Gallo-Torres method) to be absorbed into systemic circulation when fed to laboratory animals.

Bound Residue: This is a residue derived from the covalent binding of the parent drug or a metabolite of the drug and a cellular biological soluble or insoluble macromolecule. These residues are not extractable from the macromolecule by exhaustive extraction, denaturation or solubilization techniques. They do not result from the incorporation of metabolized, radiolabelled fragments of the drug into endogenous compounds, or the same macromolecule by normal biosynthetic pathways.

Egg: The fresh edible portion of the spheroid body produced by female birds, especially domestic fowl. The edible portions of the egg include the yolk and egg white after removal of the shell.

Extractable Residue: Those residues extracted from tissues or biological fluids by means of aqueous acidic or basic media, organic solvents and/or hydrolysis with enzymes (e.g. sulfatase or glucuronidase) to hydrolyze conjugates. The extraction conditions must be such that the compounds of interest are not destroyed

Good Practice in the Use of Veterinary Drugs (GPVD): Is the official recommended or authorized usage including withdrawal periods, approved by national authorities, of

veterinary drugs under practical conditions. The maximum residue limit for veterinary drugs (MRLVD) can be assured with good practice in the use of veterinary drugs. Good Veterinary Practice of antimicrobial products is the rational antibacterial therapy which is based on a combination of clinical judgement, laboratory diagnosis, clinical knowledge, epidemiological background and husbandry information about the flock to be treated. The usage of antimicrobials should not replace fundamental shortcomings in husbandry, biosecurity measures and prophylactic hygiene. The administration of antimicrobial products in disease situations is supposed to be complimented with good farm management and properly-designed immunization programs.

Marker Residue: A residue whose concentration decreases in a known relationship to the level of total residues in tissues, eggs, milk or other animal tissues. A specific quantitative analytical method for measuring the concentration of the residue with the required sensitivity must be available.

Maximum Residue Limit for Veterinary Drugs (MRLVD): Is the maximum concentration of residue resulting from the use of a veterinary drug (expressed in mg/kg or µg/kg on a fresh weight basis) that is recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in or on a food. It is based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the Acceptable Daily Intake (ADI), or on the basis of a temporary ADI that utilizes an additional safety factor. It also takes into account other relevant public health risks as well as food technological aspects. When establishing an MRL, consideration is also given to residues that occur in food of plant origin and/or the environment. Furthermore, the MRL may be reduced to be consistent with good practices in the use of veterinary drugs and to the extent that practical analytical methods are available.

Meat: The edible part of any mammal.

Milk : Milk is the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing.

Muscle: Muscle is the skeletal tissue of an animal carcass or cuts of these tissues from an animal carcass that contains interstitial and intramuscular fat. The muscular tissue may also include bone, connective tissue, tendons as well as nerves and lymph nodes in natural portions. It does not include edible offal or trimmable fat.

Non-Extractable Residues: These residues are obtained by subtracting the extractable residues from the total residues and comprise:

i) Residues of the drug incorporated through normal metabolic pathways into endogenous compounds (e.g. amino acids, proteins, nucleic acid). These residues are of no toxicological concern.

ii) Chemically-bound residues derived by interaction of residues of parent drug or its metabolites with macromolecules. These residues may be of toxicological concern

Poultry: Means any domesticated bird including chickens, turkeys, ducks, geese, guinea-fowls or pigeons.

Regulatory Method of Analysis: A method that has been legally enacted and/or validated in a multi-laboratory study and can be applied by trained analysts using commercial laboratory equipment and instrumentation to detect and determine the concentration of a residue of a veterinary drug in edible animal products for the purpose of determining compliance with the MRL.

Residues of Veterinary Drugs: Include the parent compounds and/or their metabolites in any edible portion of the animal product, and include residues of associated impurities of the veterinary drug concerned.

Screening Method: This is a rapid, relatively inexpensive, and rugged field method used for testing for a specific substance or closely related group of substances which are sufficiently selective and sensitive to allow at least semi-quantitative detection of residues in contents in accordance with the established maximum limit.

Temporary Acceptable Daily Intake (TADI): Used by JECFA when data are sufficient to conclude that use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but are insufficient to conclude that use of the substance is safe over a lifetime. A higher-than-normal safety factor is used when establishing a temporary ADI and an expiration date is established by which time appropriate data to resolve the safety issue should be submitted to JECFA.

Tissue: All edible animal tissue, including muscle and by-products.

Tissue, Control: Tissue from animals not treated with veterinary drugs of the same species, sex, age and physiological status as the target species.

Tissue, Dosed: Tissue from animals of the test species that have been treated with the drug according to its intended use.

Tissue, Spiked or Fortified: Tissue containing known concentrations of the analyte added to the sample of control tissue.

Total Residue: The total residue of a drug in animal derived food consists of the parent drug together with all the metabolites and drug based products that remain in the food after administration of the drug to food producing animals. The amount of total residues is

generally determined by means of a study using the radiolabelled drug, and is expressed as the parent drug equivalent in mg/kg of the food.

Validated Method: This is an analytical method which has been subjected to a multi-laboratory study for accuracy, precision, reproducibility performance and ruggedness. Concise written procedures for sample selection, preparation and quantitative analysis are provided for inter-laboratory quality assurance and consistency of results, on which an appropriate regulatory method of analysis can be established.

Veterinarian Client-Patient Relationship: The relationship is recognized when the livestock enterprise, premises and husbandry practices are known to the veterinarian as a result of a recent professional visit to the site and the veterinarian is available for emergency on site consultation and is responsible for preventative medicine programmes.

Veterinary Drug: Any substance applied or administered to any food-producing animal, such as meat or milk producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behaviour.

Withdrawal Time and Withholding Time: This is the period of time between the last administration of a drug and the collection of edible tissue or products from a treated animal that ensures the contents of residues in food comply with the maximum residue limit for this veterinary drug (MRLVD). Meat and eggs must be withheld from human consumption until residues are depleted below the tolerance limits set by the authorities. Withdrawal periods, ranging from a few days to a few weeks vary according to the drug used, dosage, route of administration, and animal species and are defined as the time required for 99% of the animals in a population (treated according to label instructions) to be free of drug residues above the tolerance level.

Appendix IIa: QUESTIONNAIREs ON ANTIBIOTICS USAGE IN FOOD ANIMALS IN NIGERIA (cattle)

UNIVERSITY OF IBADAN

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

QUESTIONNAIRE ON ANTIBIOTICS USAGE IN CATTLE IN NIGERIA

Dear Respondents,

This questionnaire survey is an academic exercise on the use of antibiotics in food animal (especially poultry and cattle) production in Nigeria. All information supplied will be treated with utmost confidentiality and used for research only.

Thank you for your time and cooperation.

(A) CATTLE OWNERS BACKGROUND INFORMATION

1. Age: 10-20 ( ) 21-30 ( ) above 30 ( )

2. Educational level: primary ( ) secondary ( ) tertiary ( ) others specify….

3. Location of the farm (Local Government Area)……

(B) PRODUCTION DATA

1. Types of farming system: Intensive ( ) Extensive ( ) Semi intensive ( )

2. Population of the herd 1-5 ( ) 5-10 ( ) 11-20 ( ) above 20 ( )

3. Nature of the herd: Calves (# =), Heifers (# =), Bull (# =), Cow (# =)

4. Purpose of production; Milk ( ) Meat ( ) Draft ( )

5. Breeds of cattle: White Fulani/Bunaji ( ) Ndama ( ) Sokoto Gudali ( ) Kuri ( ) Others ……

6. Years of experience in cattle production: 1-5 years ( ) 5-10 years ( ) above 10 years ( )

(C) FEEDS AND FEEDING

1. What do you feed the animals on? Commercial feed and concentrate ( ) Pasture ( )both ( )

2. Source of feed: Self milled ( ) Commercial milled ( )

3. Do you use drug as feed additives? Yes ( ) No ( )

4. If yes state the particular drugs …….

5. Why do use it?

(D) CATTLE DISEASES AND HEALTH

1. Tick the common symptoms of cattle disease you observed?

Diarrhea ( ) Wound/Abscess ( ) Nasal discharge ( ) Coughing ( ) Weight loss ( ) lameness/foot Rot ( ) Mouth lesion/salivation ( )

2. What is the average mortality? This month ( ) this week ( ) Yesterday ( )

3. How do you diagnose disease on the farm? Through clinical signs ( ) mortality ( ) post mortem findings ( ) laboratory findings ( )

4. Who does the diagnoses and treatment of disease on the farm? Vet doctors ( ) animal health officer ( ) self by experience ( ) others ( )

5. Do you do laboratory test before treatment? Yes ( ) No ( )

6. Do you always follow the manufacturer's instruction on drug use? Most often ( ) often ( ) never ( )

7. State your reasons? .....

(E) DISEASES TREATMENT AND PREVENTION

1. Do you use antibiotics for preventive purpose? Yes ( ) No ( )

2. If yes to what extent? Very often ( ) often ( ) rarely ( ) very rare ( )

3. Commonly used antibiotics: Oxtetracycline ( ) Penicillin ( ) Gentamycin ( ) Enrofloxacin ( ) Neomycin ( ), Streptomycin ( ) others ( )

4. Source of the antibiotics: Manufacturers ( ) Manufacturer's representatives ( ) Vet/Agrochemical stores/shops ( ) Hawkers ( ) Feed mill ( )

5. Do you rely on Veterinary Doctor for disease treatment? Yes ( ) No ( )

6. Do you observe the recommended withdrawal periods when using drugs? Yes ( ) no ( )

7. If yes, how……

8. Do you know of any effect of drugs remains in cattle meat and milk? Yes ( ) no ( )

9. If yes, which one…….

(F) POLICY

1. Are you aware of any law on drug use in your animal? Yes ( ) No ( )
2. If yes which ones? ……
3. Are these laws actively enforced by the necessary authorities? Not enforced ( ) Enforced regularly ( ) Enforced irregularly

COMMENT

Please comment freely on drug distribution and use in Nigeria. ……

……

……...

Appendix IIb: QUESTIONNAIRE ON ANTIBIOTICS USAGE IN POULTRY IN NIGERIA

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, FACULTY OF VETERINARY MEDICINE UNIVERSITY OF IBADAN

Dear Respondent,

This questionnaire is designed to assess the pattern of antibiotics usage in poultry industry. Please respond to the questions appropriately, your confidentiality is guaranteed. All information shall be used for academic purpose.

Thank you for your time and cooperation.

(A) FARM BACKGROUND INFORMATION

1. Respondent: Owner { } worker { }
2. Age: 21-25{ } 26-30{ } Above 30{ }
3. Education level: Primary { } Secondary { } OND { } HND Graduate { } others specify……..
4. Location of the farm [Local Government Area] …….

(B) PRODUCTION DATA

1. Type of Farming system Small Scale{ } Commercial { }

2. Management System: Deep litters { } Battery cage

3. Population of birds …….

4. Purpose of production: Chicken { } Egg { } Day old chicks { }

5. Types of birds: pullet { } Cockerel { } Broilers { }

(C) FEEDS AND FEEDING

1. What is the source of your feeds? Self milled { } Commercial feeds { }

2. Do you add drug[s] to your feed? Yes{ } No{ }

3. If yes state the particular drug

4. When do you use drug? When birds are sick { } When mortality is recorded { }To boost production { }

(D) POULTRY DISEASES AND HEALTH

1. What are the common poultry diseases you do encounter?

i……. ii……

iii……. iv……..

v..……..

2. What is the average mortality?

   This month { } this week { } Yesterday { }

3. How do you diagnose diseases on the farm? Through clinical signs{ }
   Mortality { } post mortem findings { } laboratory finding { }
4. Who does the diagnosis and treatment of disease on the farm? Vet Doctors { }
   animal health officer { } self/ by experience { } others…….

5. Do you perform laboratory text before treatment? Yes { } No{ }
6. Do you always follow the manufacturer's instruction on the use of drug's? Most often { }Very often { } often { } never{ }
7. State your reason(s)

(E) TREATMENT

1. Do you use antibiotics for preventive purpose? Yes { } No { }
2. If yes to what extent? Very often { } often { } Rarely { } very rare { }
3. Tick the common antibiotics you normally use oxytetracyline { } Penicillin { } Enroflaxacin { } Neomycin { } Gentamicin { } others…….
4. Source of antibiotics; Vet. Shop { } Feed mill { } by farm vet. { }
5. Do you rely on vet to complete the dosage ?Yes { } No { }
6. Do you observe any withdrawal period recommended by manufacturer when using antibiotics? Yes { } No { }
7. If yes, How

   ……

   ……..
8. Do you know of any effect of drug remains in chicken meat? Yes { } No { }
9. If yes, which one.....

Appendix IIIa: List of Respondent Poultry Farmers

S/N	Name and Location	Average Flock Size	Flock Type (L= Layers, B= Broilers)
1	Abrahamsum Farms, Akure	10,000	L
2	Adegoke Farms, Akure	15,000	L
3	Ben-K Farms, Ikotun, Lagos	10,000	B
4	Busyvice Farm, Agege, Lagos	8000	L & B
5	Covenant Farm, Olodo, Ibadan	2,500	L
6	December Farm, Igbesa, Lagos	40,000	L & B
7	Dutel Farm, Ijaye, Lagos	25,000	L
8	Fabak Farm, Agege, Lagos.	9,000	L
9	Farm Support Services, Olodo, Ibadan	42,500	L
10	Fomalko Farm, Agbowo, Ibadan	8,000	L
11	Goodhealth Farm, Igbesa, Lagos	60,000	L & B
12	Iloti Farm, Ikorodu, Lagos	20,000	B
13	Jofa Farms, Akure	40,000	L& B
14	Lazana Farms, Ojodu, Lagos	24,000	L
15	Mirth Agric Farm, Asejire, Ibadan	35,000	L

16	Mrs Ojo, (Principal) Farms, Akure	5,000	L
17	Mustard Seed, Ojodu, Lagos	2,500	L
18	New Earth Farm, Badagry, Lagos	20,000	L
19	Ola Farms, Oko-Oba, Lagos	20,000	L & B
20	Olabosco Farm, Igbesa, Lagos	62,500	L
21	Ologun Farm, Ogudu, Lagos	5000	B
22	Omotosho Farms, Lagos	1,500	L
23	Providence Farms, Lagos	3,000	L
24	Ritlab Farm, Ogba, Lagos	650	B
25	S&S Farm, Epe, Lagos	10,000	L
26	Sachel Farm, Badagry, Lagos	2,500	B
27	Zartech Farms Ibadan	63,500	L & B
28	Bronco Farms Oluyole, Ibadan	50,000	B
29	Bamfot Farm, Ife-Rd, Ibadan	30,000	L
30	Ola-Omolola Farms Ibadan	5,500	L

Appendix IIIb: List of Respondent Cattle Producers

S/N	Name and Location	Average Herd Size (heads of cattle)
1	Ahaji Dauda Eleran, Oba Ile Akure.	82
2	Alfa Mahamud, Akinsawe,yana Offa, Ibadan	34
3	Alfa Mumin, Akinsawe,Iyana Offa, Ibadan	80
4	Alfa Saheed, Akinsawe,Iyana Offa, Ibadan	28
5	Alhaji Bala, Ojodu, Lagos	73
6	Alhaji Bello, Akinsawe,Iyana Offa, Ibadan	120
7	Deacon Bamidele, Oda-Road, Akure	35
8	Alhaji Mohamed, Ayede, Egbeda, Ibadan	84
9	Alhaji Tijani, Ayede, Egbeda, Ibadan	115
10	Alhaji, Yekeeni Ojodu, Lagos	77
11	Dr Adejumo, Apata, Ibadan	24
12	Mr Dauda Sheu, Bodija, Ibadan	26
13	Mr Jamiu Lawal, Ojodu, Lagos	38
14	Mr Raufu Junaidu, Bodija, Ibadan	45
15	Mr Sule Lawal, Akufo farm Ibadan	34

16	Mr Sule, Ayede, Egbeda, Ibadan	86
17	Mr Sunday Adedokun, Bodija, Ibadan	22
18	Mr Sunday Adeniyi, Iddo road, Ologuneru, Ibadan.	40
19	Mr Yusuf Taiwo Oko-Oba Lagos.	73
20	Pastor Olusayo, Akinyele, Ibadan	48

Appendix IV: Codex Alimentarius Commission Maximum Residue Limits for Tetracylines (Chlortetracycline/Oxytetracycline/Tetracycline) in Foods

Acceptable Daily Intake (group ADI for chlortetracycline, oxytetracycline and tetracycline): 0-30 µg/kg body weight (50th JECFA, 1998).

Species	**Tissue**	**MRL (µg/kg)**	**CAC**	
Cattle	Muscle	200	26th (2003)	
Cattle	Liver	600	26th (2003)	
Cattle	Kidney	1200	26th (2003)	
Cattle	Milk (µg/l)	100	26th (2003)	
Fish	Muscle	200	26th (2003)	Applies only to oxytetracycline.
Giant prawn (Paeneus monodon)	Muscle	200	26th (2003)	Applies only to oxytetracycline.
Pig	Muscle	200	26th (2003)	
Pig	Liver	600	26th (2003)	
Pig	Kidney	1200	26th (2003)	
Poultry	Muscle	200	26th (2003)	

Poultry	Liver	600	26th (2003)
Poultry	Kidney	1200	26th (2003)
Poultry	Eggs	400	26th (2003)
Sheep	Muscle	200	26th (2003)
Sheep	Liver	600	26th (2003)
Sheep	Kidney	1200	26th (2003)
Sheep	Milk (µg/l)	100	26th (2003)

CAC/MRL 02-2009; JECFA Evaluation: 45 (1995); 47 (1996); 50 (1998); 58 (2002)

Appendix v: Table of Number of samples required to detect at least one non-compliant result with pre-defined probabilities in a population having known non-compliance residue prevalence.

Non-compliant prevalence (% in a population)	Minimum number of samples required to detect a non-compliant result with a confidence level of:		
	90%	95%	99%
35	6	7	11
30	7	9	13
25	9	11	17
50	11	14	21
15	15	19	29
10	22	29	44
5	45	59	90
1	230	299	459
0.5	460	598	919
0.1	2302	2995	4603

Appendix vi: Preparation of reagents and buffers

a. 1N Hydrochloric Acid

Molecular Formula = HCl; % Purity = 36%; Molar mass = 36.5

Specific gravity 1180g/L

Molarity = % purity X Sp. gravity/100 X molar mass

= 36 X 1180/100 X36.5

= 11.64M

M1V1 = M2V2

V2 = M1V1/M2

= 1X1000/11.64

= 85.91ml

85.91ml of concentrated HCl was added to distilled water and made up to 1000ml (1L) to obtain 1N HCl.

b. 0.01M Oxalic acid

Molar mass = 126.07

Molarity = 0.01

g/dm^3 = 126.07 X 0.01

= 1.126g

1.126g oxalic acid salt was dissolve in 1L of deionised water to make 0.01M solution.

c. Mobile Phase

The mobile phase comprised of Methanol: Acetonitrile: 0.01M Oxalic acid (1:1.5:2.5). Using volumetric flask 200ml of methanol, 300ml of acetonitrile and 500ml of 0.01oxalic acid were measured and mixed together to give 1000ml of the mobile phase in corked Duran's bottle. This was subsequently degassed in the ultrasonic water bath (sonicator) to release the dissolved gas.

Methanol (HPLC grade) used as blank to flush the HPLC column was also degassed regularly in the sonicator.

Appendix vii: Statistiscal Tables oxytetracycline residue in beef and chicken

ANOVA table of Oxytetracycline residue in Akure beef

	AkKD	AkLD	AkMD	AkKW	AkLW	AkMW
Number of values	31	34	29	38	36	33
%	51.7	56.7	48.3	63.3	60.0	55.0
Minimum	308.4	86.36	67.07	482.2	163.6	163.6
25% Percentile	665.7	344.6	265	730.8	554.6	453.2
Median	887.7	634.3	607.9	1139	940.8	549.8
75% Percentile	1322	931.2	825.2	1530	1192	960.2
Maximum	2770	1757	1187	2915	2544	1660
Mean	**1162**	**661.7**	**587.7**	**1185**	**945.2**	**692.6**
Std. Deviation	685.1	387	321.4	581.1	541.2	376.2
Std. Error	123.1	66.36	59.69	94.27	90.19	65.49
Lower 95% CI of mean	910.7	526.7	465.5	994	762.1	559.2
Upper 95% CI of mean	1413	796.7	710	1376	1128	826
Sum	36021	22497	17044	45029	34028	22855

ANOVA table of Oxytetracycline residue in Lagos beef

	LgKW	LgLW	LgMW	LgKD	LgLD	LgMD
Number of values	34	30	29	36	36	31
%	56.7	50.0	48.3	60.0	60.0	51.7
Minimum	76.7	163.6	178.1	308.4	240.8	67.1
25% Percentile	948.3	646.3	453.2	1144	453.2	308.5
Median	1243	815.3	694.6	1356	742.9	453.4
75% Percentile	1429	1284	885.3	1733	863.6	646.5
Maximum	2409	1708	1926	2819	1824	1709
Mean	1267	904.9	729.6	1436	761.7	546.2
Std. Deviation	476.4	421.4	374.3	554.1	375.6	373.8
Std. Error	81.7	76.93	69.5	92.35	62.6	67.14
Lower 95% CI of mean	1100	747.6	587.2	1248	634.6	409.1
Upper 95% CI of mean	1433	1062	871.9	1623	888.7	683.3
Sum	43067	27148	21157	51687	27420	16932

ANOVA table of Oxytetracycline residue in Ibadan beef

	IbKW	IbLW	IbMW	IbKD	IbLD	IbMD
Number of values	44	37	34	35	32	28

%	73.3	61.7	56.7	58.3	53.3	46.7
Minimum	598.1	163.6	211.9	163.6	110	67.1
25% Percentile	1182	583.6	453.4	916.7	668	260.1
Median	1419	815.3	680.3	1322	888	549.8
75% Percentile	1689	1202	863.8	1805	1180	728.4
Maximum	5059	2210	2790	3736	2191	1245
Mean	1544	917.5	766.2	1354	949.5	555.8
Std. Deviation	870.8	465.2	513.9	696.5	505.6	277.8
Std. Error	131.3	76.48	88.13	117.7	89.38	52.5
Lower 95% CI of mean	1280	762.4	586.9	1115	767.2	448.1
Upper 95% CI of mean	1809	1073	945.5	1594	1132	663.5
Sum	67956	33946	26051	47401	30384	15563

ANOVA table of Oxytetracycline residue in Lagos and Ibadan chicken

	IbCh Lmkt	IbCh Mmkt	IbCh Lfm	IbChMfm	LgCh Lmkt	LgCh Mmkt	LgCh Lfm	LgCh Mfm
Number of values	60	60	60	60	60	60	60	60
Minimum	0	0	0	0	0	0	0	0
25% Percentile	0	0	0	0	0	24	0	0
Median	762	308	776.5	260	728.5	255.5	757.5	525.5
75% Percentile	1202	660.5	1660	695	1129	743	1547	936

Maximum	2626	1525	10880	2206	1901	1660	12811	5474
Mean	775.4	410.8	1232	438.6	642	437.9	1533	916.5
Std. Deviation	687.9	428.9	1855	469.2	570.5	426.6	2590	1455
Std. Error	88.8	55.38	239.4	60.58	73.66	55.07	334.4	187.8
Lower 95% CI of mean	597.7	300	753.4	317.4	494.6	327.7	863.8	540.7
Upper 95% CI of mean	953.1	521.6	1712	559.8	789.3	548.1	2202	1292
Sum	46524	24646	73949	26315	38517	26273	91978	54988

Tukey's Multiple Comparison Test (chicken)				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
IbCh Lmkt vs LgCh Lfm	-757.6	4.492	P < 0.05	-1492 to -23.42
IbCh Mmkt vs IbCh Lfm	-821.7	4.873	P < 0.05	-1556 to -87.57
IbCh Mmkt vs LgCh Lfm	-1122	6.655	P < 0.001	-1856 to -388.1
IbCh Lfm vs IbChMfm	793.9	4.708	P < 0.05	59.75 to 1528
IbCh Lfm vs LgCh Mmkt	794.6	4.712	P < 0.05	60.45 to 1529
IbChMfm vs LgCh Lfm	-1094	6.49	P < 0.001	-1829 to -360.2
LgCh Lmkt vs LgCh Lfm	-891	5.284	P < 0.01	-1625 to -156.9
LgCh Mmkt vs LgCh Lfm	-1095	6.494	P < 0.001	-1829 to -360.9

Tukey's Multiple Comparison of Mean Oxytetracycline residue Test (beef)

Parameter Comparison	Mean Diff.	Q	P value	95% CI of diff
* AkKD vs AkMD	574.2	6.018	P < 0.01	80.37 to 1068
*AkKD vs AkLD	500.3	5.454	P < 0.05	25.55 to 975.0
* AkKD vs AkMD	574.2	6.018	P < 0.01	80.37 to 1068
AkLD vs IbLD	-287.8	3.164	P > 0.05	-758.7 to 183.0
AkMD vs AkMW	-104.8	1.115	P > 0.05	-591.4 to 381.7
AkMD vs LgMD	41.54	0.4354	P > 0.05	-452.3 to 535.4
AkMD vs IbMD	31.9	0.326	P > 0.05	-474.6 to 538.4
AkKW vs AkKD	23.03	0.2576	P > 0.05	-439.6 to 485.7
* AkKW vs AkMW	492.4	5.602	P < 0.05	37.54 to 947.3
AkKW vs LgKW	-81.7	0.937	P > 0.05	-533.0 to 369.6
AkKW vs IbKW	-35950%	440%	P > 0.05	-782.8 to 63.87
*AkKD vs AkLD	500.3	5.454	P < 0.05	25.55 to 975.0
*AkKD vs AkMD	574.2	6.018	P < 0.01	80.37 to 1068
AkKD vs AkKW	-23.03	0.2576	P > 0.05	-485.7 to 439.6
AkKD vs LgKD	-273.8	3.025	P > 0.05	-742.2 to 194.6
AkKD vs IbKD	-192.4	2.112	P > 0.05	-663.9 to 279.1
AkLD vs AkMD	73.95	0.792	P > 0.05	-409.3 to 557.2
AkLD vs AkLW	-283.6	3.21	P > 0.05	-740.7 to 173.6
AkLD vs LgLD	-99.98	1.132	P > 0.05	-557.1 to 357.2
AkLD vs IbLD	-287.8	3.164	P > 0.05	-758.7 to 183.0
AkMD vs AkMW	-104.8	1.115	P > 0.05	-591.4 to 381.7
AkMD vs LgMD	41.54	0.4354	P > 0.05	-452.3 to 535.4

AkMD vs IbMD	31.9	0.326	P > 0.05	-474.6 to 538.4
AkKW vs AkLW	239.7	2.791	P > 0.05	-204.9 to 684.4
*AkKW vs AkMW	492.4	5.602	P < 0.05	37.54 to 947.3
AkKW vs LgKW	-81.7	0.937	P > 0.05	-533.0 to 369.6
AkKW vs IbKW	-359.5	4.395	P > 0.05	-782.8 to 63.87
AkLW vs AkMW	252.7	2.838	P > 0.05	-208.0 to 713.4
AkLW vs LgLW	40.3	0.4413	P > 0.05	-432.3 to 512.9
AkLW vs IbLW	27.77	0.3212	P > 0.05	-419.8 to 475.3
AkMW vs LgMW	-37	0.3935	P > 0.05	-523.6 to 449.6
AkMW vs IbMW	-73.65	0.8159	P > 0.05	-540.8 to 393.5
LgKW vs LgLW	361.8	3.91	P > 0.05	-117.1 to 840.6
*LgKW vs LgMW	537.1	5.753	P < 0.05	53.91 to 1020
LgKW vs LgKD	-169.1	1.914	P > 0.05	-626.2 to 288.1
LgKW vs IbKW	-277.8	3.293	P > 0.05	-714.3 to 158.7
LgLW vs LgMW	175.4	1.823	P > 0.05	-322.5 to 673.2
LgLW vs LgLD	143.3	1.569	P > 0.05	-329.3 to 615.9
*LgLW vs IbKW	-639.5	7.312	P < 0.001	-1092 to -186.9
LgLW vs IbLW	-12.53	0.1381	P > 0.05	-482.2 to 457.1
LgMW vs LgMD	183.4	1.922	P > 0.05	-310.5 to 677.2
LgMW vs IbMW	-36.65	0.3926	P > 0.05	-519.9 to 446.6
* LgKD vs LgLD	674.1	7.743	P < 0.001	223.5 to 1125
* LgKD vs LgMD	889.6	9.829	P < 0.001	421.2 to 1358
LgKD vs IbKD	81.44	0.9288	P > 0.05	-372.4 to 535.2
LgLD vs IbLD	-187.8	2.093	P > 0.05	-652.3 to 276.6

LgMD vs IbMD	-9.644	0.1001	P > 0.05	-508.0 to 488.8
*IbKW vs IbMW	778.2	9.227	P < 0.001	341.7 to 1215
IbKW vs IbKD	190.1	2.273	P > 0.05	-242.8 to 623.1
IbLW vs IbMW	151.2	1.724	P > 0.05	-302.9 to 605.4
IbLW vs IbLD	-32.04	0.3593	P > 0.05	-493.5 to 429.4
IbMW vs IbMD	210.4	2.232	P > 0.05	-277.5 to 698.2
IbKD vs IbLD	404.8	4.481	P > 0.05	-62.73 to 872.4
*IbKD vs IbMD	798.5	8.526	P < 0.001	313.8 to 1283
IbLD vs IbMD	393.7	4.119	P > 0.05	-101.0 to 888.4

Key: * significant difference

IbKd = Ibadan Kidney (dry season); IbMd = Ibadan Muscle (dry season); IbLd = Ibadan Liver (dry season), IbKw = Ibadan Kidney (wet season) ; IbMw = Ibadan Muscle (wet season); IbLw = Ibadan Liver (wet season)

AkKd = Akure Kidney (dry season); AkMd = Akure Muscle (dry season); AkLd = Akure Liver (dry season), AkKw = Akure Kidney (wet season); AkMw = Akure Muscle (wet season); AkLw = Akure Liver (wet season)

LgKd = Lagos Kidney (dry season); LgMd = Lagos Muscle (dry season); LgLd = Lagos Liver (dry season), LgKw = Lagos Kidney (wet season); LgMw = Lagos Muscle (wet season); LgLw = Lagos Liver (wet season)

Tukey's Multiple Comparison Test of oxytetracycline residue in chicken (Ibadan and Lagos)

Tukey's Multiple Comparison Test	Mean Diff.	Q	P value	95% CI of diff
*IbCh Lmkt vs IbCh Mmkt	426.6	5.528	P < 0.01	90.29 to 763.0
IbCh Lmkt vs IbCh Lfm	-169.7	2.227	P > 0.05	-501.9 to 162.5
*IbCh Lmkt vs IbCh Mfm	419.2	5.467	P < 0.01	85.02 to 753.4
IbCh Lmkt vs LgCh Lmkt	90.32	1.218	P > 0.05	-232.9 to 413.5

*IbCh Lmkt vs LgCh Mmkt	402.2	5.476	P < 0.01	82.08 to 722.4
IbCh Lmkt vs LgCh Lfm	-143.8	1.929	P > 0.05	-468.7 to 181.0
IbCh Lmkt vs LgCh Mfm	233.7	3.007	P > 0.05	-104.9 to 572.3
*IbCh Mmkt vs IbCh Lfm	-596.3	8.072	P < 0.001	-918.3 to -274.4
IbCh Mmkt vs IbCh Mfm	-7.421	0.09979	P > 0.05	-331.5 to 316.7
*IbCh Mmkt vs LgCh Lmkt	-336.3	4.687	P < 0.05	-649.1 to -23.58
IbCh Mmkt vs LgCh Mmkt	-24.43	0.3439	P > 0.05	-334.0 to 285.1
*IbCh Mmkt vs LgCh Lfm	-570.5	7.907	P < 0.001	-884.9 to -256.0
IbCh Mmkt vs LgCh Mfm	-193	2.559	P > 0.05	-521.6 to 135.6
*IbCh Lfm vs IbCh Mfm	588.9	8.027	P < 0.001	269.2 to 908.7
IbCh Lfm vs LgCh Lmkt	260	3.676	P > 0.05	-48.22 to 568.3
*IbCh Lfm vs LgCh Mmkt	571.9	8.172	P < 0.001	266.9 to 876.9
IbCh Lfm vs LgCh Lfm	25.88	0.3639	P > 0.05	-284.1 to 335.8
*IbCh Lfm vs LgCh Mfm	403.4	5.42	P < 0.01	79.02 to 727.7
IbCh Mfm vs LgCh Lmkt	-328.9	4.617	P < 0.05	-639.3 to -18.47
IbCh Mfm vs LgCh Mmkt	-17.01	0.2412	P > 0.05	-324.2 to 290.2
*IbCh Mfm vs LgCh Lfm	-563	7.861	P < 0.001	-875.2 to -250.9
IbCh Mfm vs LgCh Mfm	-185.6	2.477	P > 0.05	-512.0 to

				140.9
*LgCh Lmkt vs LgCh Mmkt	311.9	4.604	P < 0.05	16.66 to 607.1
LgCh Lmkt vs LgCh Lfm	-234.1	3.397	P > 0.05	-534.5 to 66.21
LgCh Lmkt vs LgCh Mfm	143.3	1.982	P > 0.05	-171.8 to 458.5
*LgCh Mmkt vs LgCh Lfm	-546	8.012	P < 0.001	-843.1 to -249.0
LgCh Mmkt vs LgCh Mfm	-168.6	2.354	P > 0.05	-480.6 to 143.5
*LgCh Lfm vs LgCh Mfm	377.5	5.192	P < 0.01	60.63 to 694.3

Key: * significant diff.

IbChLmkt = Ibadan chicken Liver (market); IbChMmkt = Ibadan chicken muscle (market); IbChLfm = Ibadan chicken Liver (farm); IbChMfm = Ibadan chicken muscle (farm); LgChLmkt = Lagos chicken Liver (market); LgChMmkt = Lagos chicken muscle (market); LgChLfm = Lagos chicken Liver (farm); LgChMfm = Lagos chicken muscle (farm)

One-way analysis of variance (ANOVA) of oxytetracycline in chicken & beef

P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	16
F	3.474
R squared	0.05236

Tukey's Multiple Comparison Test of oxytetracycline residue in chicken-beef (Ibadan and Lagos)

Tukey's Multiple Comparison Test	Mean Diff.	Q	P value	95% CI of diff
IbCh Lmkt vs IbLD	-775	3.21	P > 0.05	-1963 to 412.9
IbCh Lmkt vs IbMD	72.47	0.3001	P > 0.05	-1115 to 1260
IbCh Lmkt vs LgLD	-397.9	1.641	P > 0.05	-1591 to 795.1
IbCh Lmkt vs LgMD	186.8	0.7737	P > 0.05	-1001 to 1375
IbCh Lmkt vs IbLW	-831.5	3.444	P > 0.05	-2019 to 356.4
IbCh Lmkt vs IbMW	-100.5	0.4163	P > 0.05	-1288 to 1087
IbCh Lmkt vs LgLW	-887.7	3.676	P > 0.05	-2076 to 300.3
IbCh Lmkt vs LgMW	186.8	0.7737	P > 0.05	-1001 to 1375
IbCh Mmkt vs IbLD	-1140	4.72	P > 0.05	-2328 to 48.27

IbCh Mmkt vs IbMD	-292.2	1.21	P > 0.05	-1480 to 895.8
IbCh Mmkt vs LgLD	-762.5	3.145	P > 0.05	-1955 to 430.5
IbCh Mmkt vs LgMD	-177.8	0.7365	P > 0.05	-1366 to 1010
IbCh Mmkt vs IbLW	-1196	4.954	P < 0.05	-2384 to -8.223
IbCh Mmkt vs IbMW	-465.1	1.926	P > 0.05	-1653 to 722.8
IbCh Mmkt vs LgLW	-1252	5.187	P < 0.05	-2440 to -64.36
IbCh Mmkt vs LgMW	-177.8	0.7365	P > 0.05	-1366 to 1010
IbCh Mmkt vs LgLD	-762.5	3.145	P > 0.05	-1955 to 430.5
IbCh Mmkt vs LgMD	-177.8	0.7365	P > 0.05	-1366 to 1010
IbCh Mmkt vs IbLW	-1196	4.954	P < 0.05	-2384 to -8.223
IbCh Mmkt vs IbMW	-465.1	1.926	P > 0.05	-1653 to 722.8
IbCh Mmkt vs LgLW	-1252	5.187	P < 0.05	-2440 to -64.36
IbCh Mmkt vs LgMW	-177.8	0.7365	P > 0.05	-1366 to 1010
IbCh Lfm vs IbLD	-318	1.317	P > 0.05	-1506 to 870.0
IbCh Lfm vs IbMD	529.6	2.193	P > 0.05	-658.4 to 1718
IbCh Lfm vs LgLD	59.2	0.2441	P > 0.05	-1134 to 1252
IbCh Lfm vs LgMD	643.9	2.667	P > 0.05	-544.1 to 1832
IbCh Lfm vs IbLW	-374.5	1.551	P > 0.05	-1562 to 813.5
IbCh Lfm vs IbMW	356.6	1.477	P > 0.05	-831.4 to 1545
IbCh Lfm vs LgLW	-430.6	1.783	P > 0.05	-1619 to 757.4
IbCh Lfm vs LgMW	643.9	2.667	P > 0.05	-544.1 to 1832
IbChMfm vs IbLD	-1112	4.605	P > 0.05	-2300 to 76.09
IbChMfm vs IbMD	-264.3	1.095	P > 0.05	-1452 to 923.6
IbChMfm vs LgLD	-734.7	3.03	P > 0.05	-1928 to 458.3
IbChMfm vs LgMD	-150	0.6213	P > 0.05	-1338 to 1038
IbChMfm vs IbLW	-1168	4.839	P > 0.05	-2356 to 19.59
IbChMfm vs IbMW	-437.3	1.811	P > 0.05	-1625 to 750.6
IbChMfm vs LgLW	-1225	5.071	P < 0.05	-2412 to -36.55
IbChMfm vs LgMW	-150	0.6213	P > 0.05	-1338 to 1038
LgCh Lmkt vs IbLD	-908.5	3.763	P > 0.05	-2096 to 279.5
LgCh Lmkt vs IbMD	-60.98	0.2525	P > 0.05	-1249 to 1127
LgCh Lmkt vs LgLD	-531.3	2.191	P > 0.05	-1724 to 661.6

LgCh Lmkt vs LgMD	53.36	0.221	P > 0.05	-1135 to 1241
LgCh Lmkt vs IbLW	-965	3.997	P > 0.05	-2153 to 223.0
LgCh Lmkt vs IbMW	-234	0.969	P > 0.05	-1422 to 954.0
LgCh Lmkt vs LgLW	-1021	4.229	P > 0.05	-2209 to 166.8
LgCh Lmkt vs LgMW	53.36	0.221	P > 0.05	-1135 to 1241
LgCh Mmkt vs LgCh Lfm	-1095	4.535	P > 0.05	-2283 to 92.87
LgCh Mmkt vs LgCh Mfm	-478.6	1.982	P > 0.05	-1667 to 709.4
LgCh Mmkt vs IbLD	-1113	4.608	P > 0.05	-2301 to 75.39
LgCh Mmkt vs IbMD	-265	1.098	P > 0.05	-1453 to 922.9
LgCh Mmkt vs LgLD	-735.4	3.033	P > 0.05	-1928 to 457.6
LgCh Mmkt vs LgMD	-150.7	0.6242	P > 0.05	-1339 to 1037
LgCh Mmkt vs IbLW	-1169	4.842	P > 0.05	-2357 to 18.89
LgCh Mmkt vs IbMW	-438	1.814	P > 0.05	-1626 to 749.9
LgCh Mmkt vs LgLW	-1225	5.074	P < 0.05	-2413 to -37.25
LgCh Mmkt vs LgMW	-150.7	0.6242	P > 0.05	-1339 to 1037
LgCh Lfm vs LgCh Mfm	616.5	2.553	P > 0.05	-571.5 to 1804
LgCh Lfm vs IbLD	-17.48	0.07238	P > 0.05	-1205 to 1170
LgCh Lfm vs IbMD	830	3.438	P > 0.05	-357.9 to 2018
LgCh Lfm vs LgLD	359.7	1.483	P > 0.05	-833.3 to 1553
LgCh Lfm vs LgMD	944.4	3.911	P > 0.05	-243.6 to 2132
LgCh Lfm vs IbLW	-73.97	0.3064	P > 0.05	-1262 to 1114
LgCh Lfm vs IbMW	657.1	2.721	P > 0.05	-530.9 to 1845
LgCh Lfm vs LgLW	-130.1	0.5389	P > 0.05	-1318 to 1058
LgCh Lfm vs LgMW	944.4	3.911	P > 0.05	-243.6 to 2132
LgCh Mfm vs IbLD	-634	2.626	P > 0.05	-1822 to 554.0
LgCh Mfm vs IbMD	213.5	0.8844	P > 0.05	-974.4 to 1401
LgCh Mfm vs LgLD	-256.8	1.059	P > 0.05	-1450 to 936.2
LgCh Mfm vs LgMD	327.9	1.358	P > 0.05	-860.1 to 1516
LgCh Mfm vs IbLW	-690.5	2.86	P > 0.05	-1878 to 497.5
LgCh Mfm vs IbMW	40.55	0.168	P > 0.05	-1147 to 1229
LgCh Mfm vs LgLW	-746.6	3.092	P > 0.05	-1935 to 441.3
LgCh Mfm vs LgMW	327.9	1.358	P > 0.05	-860.1 to 1516