ASSESSMENT OF ANTIMICROBIAL RESIDUES, USE, PUBLIC HEALTH AWARENESS AND MANAGEMENT PRACTICES IN FISH FARMS IN KADUNA STATE, NIGERIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILMENT FOR THE AWARD OF MASTERS DEGREE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

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MARCH, 2014

DECLARATION

I declare that the work in this thesis titled ***"Assessment Of Antimicrobial Drug Use And The Occurrence Of Residues In Farmed Fish In Kaduna State, Nigeria"*** has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria under the supervision of Dr. E.C. Okolocha, Dr. J. Kabir and Professor P.I. Bolorunduro. The information derived from the literature has been duly acknowledged in the list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

This thesis entitled ***"ASSESSMENT OF ANTIMICROBIAL RESIDUES, USE, PUBLIC HEALTH AWARENESS AND MANAGEMENT PRACTICES IN FISH FARMS IN KADUNA STATE, NIGERIA"*** by Ladan Aliyu ALIYU meets the regulations governing the award of the degree of Master of Science in Veterinary Public Health and Preventive Medicine of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my brother, Malam Murtala Aliyu who had always encouraged and supported me when I am at crossroads and my aged mother, Hajiya Amina Umar who taught me the art of perseverance.

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ABSTRACT

This study was undertaken for the assessment of antimicrobial drug use and occurrence of residues in farmed fish in Kaduna State. A total of 300 fish samples from 30 farms in the three senatorial districts of Kaduna State were screened for the presence of antimicrobial drug residues using Premi$^{(R)}$Test. A cross sectional descriptive study was conducted using convenience sampling technique for fish collection. Ten fish each weighing 500g to 1kg were collected from earthen ponds, concrete ponds and plastic tanks in fish farms from the three senatorial districts. One hundred questionnaires were administered to fish farmers. Out of the 300 fish samples screened, an overall prevalence of 73.70% of antimicrobial drug residues was obtained, while prevalence's of 63%, 74% and 84% were obtained from Kaduna North, Central and South senatorial districts respectively. There was a significant association ($\chi^2 = 11.38$, df = 2, $p < 0.05$) between senatorial district and detection of antibiotic residue in the fish. From the questionnaire responses the use of antimicrobial drugs in fish ponds was common (91.86%); with tetracycline (63.41%) being the most frequently used antimicrobial agent. Self prescription and administration of drugs by farmers (67.86%) were more in number than those who consult veterinarians for prescription and drug administration. Level of awareness, dangers associated with consumption of antimicrobial drug residues in fish were very low. Furthermore, most fish farmers did not observe antimicrobial withdrawal periods. This study has established a high prevalence of antimicrobial drug residues in farmed fish in the study area. Awareness on the dangers and public health implications of antimicrobial drug residues in fish in Kaduna is low. Therefore, use of antimicrobial drug in aquaculture should be regulated, farmers should be encouraged to use immunostimulators or probiotics for disease control and prevention, in order to safeguard the general populace from the hazards associated with drug residues in fish.

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LIST OF ABBREVIATIONS

AHI	Animal Health Institute
ASM	American Society for Microbiology
BHAI	Brain Heart Infusion Agar…
CPE	Cytopathic Effect
CVM	Center for Veterinary Medicine
DNA	Deoxyribonucleic Acid
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FDLPC	Federal Department of Livestock and Pest Control
GMP	Good Management Practice
GVP	Good Veterinary Practice
HACCP	Hazard Analysis Critical Control Point
IOM	Institute of Medicine
LGAs	Local Government Areas
MOA	Ministry of Agriculture

MOE	Ministry of Environment
MRLs	Maximum Residue Limits
NAFDAC	National Agency for Food Drug Administration and Control
OIE	Office International des Epizootics
PCR	Polymerase Chain Reaction
RFTS	Rainbow Trout Fry Syndrome
SDA	Saboroud Dextrose Agar
SPSS	Statistical Package for Social Science
SWA	Sweet Wort Agar
TSA	Tryptic Soy Agar
USFDA	United States Food and Drug Administration
USGAO	United States General Accounting Officer
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Aquaculture is currently attracting much attention as an alternative to capture fisheries (Lovell, 1991). A growing amount of fish bought at the market place is coming from fish farms. As in many other intensive farming systems, the use of anti-microbial compounds in aquaculture to prevent or treat fish diseases is not unusual and sometimes necessary (Meyer, 1991; Schlotfeldt and Alderman, 1995). Following the discovery of the growth-promoting and disease-fighting capabilities of antibiotics, fish farmers, and livestock producers began to use drugs in animal feeds. Antibiotics have been used to treat humans as well as animals such as domestic animals, livestock, and aquatic animals such as fish for many years to prevent or cure infections among the stock population (Horinek, 2009).

The major route of transmission of resistant micro-organisms from animals to humans is through the food chain, and micro-organisms also have the potential to be resistant to many types of antibiotics (Akinbowale *et al.,* 2006). Pathogenic bacteria in some species of fish have been linked to bacteria in humans and it is believed that there should be a concern for safety of humans (Boinpally and Jiang, 2007).

As in other animal production sectors, antibiotics are used in aquaculture during both production and processing, mainly to prevent (prophylactic use) and to treat (therapeutic use) bacterial diseases contamination (FAO, 1997). Antibiotics have not always been used in a responsible manner in aquaculture, and in a number of reports, the control of the use of antibiotics has not provided a proper assurance of the prevention of risks to humans (FAO, 2000). The Food and Agriculture Organization (FAO), World Health Organization (WHO), the

International Office for Epizootics (OIE) and a number of national governments have already raised the issue of irresponsible use of antibiotics in all production sectors, with particular concern for the potential risks to public health. Many governments around the world have introduced, changed or tightened nation's regulations on the use of antibiotics in general and within the aquaculture sector particularly (Cahill *et al.,* 1966; Arthur *et al.,* 1996; FAO, 1997: USFDA, 1997).

The unintentional consumption of antibiotics in food chain is resulting in the development of antibiotic resistance in bacteria pathogenic to humans and this poses serious risk to human health globally (Cahill *et al.,* 1966; Angular and Griffin, 2000; Shears, 2001). Recognition of the risks associated with the direct and indirect effects on human health of both active and passive consumption of antibiotics has led to bans on the use of certain antibiotics in animal food production and the establishment of Maximum Residue Limits (MRLs) for those with known risks (Okerman *et al.,* 1989; 1998; Valset *et al.,* 1989; Arthur *et al.,* 1996; Calderon *et al.,* 1996; USFDA, 1997; FAO, 1997).

Growth promotion has been identified as one of the reasons why producers administer antibiotics to fish, therefore, antibiotic residues are expected to be found in fish, including the edible muscle tissue part (FDA, 1998a). Furthermore, food inspection services need fast, inexpensive and reliable screening systems for the testing of fish flesh in place of expensive, time-consuming chemical analytical methods for detection of the presence of antimicrobial residues.

The use of antibiotics in fish farming is of great public health concern; it is associated with a new hazard in fish production not encountered in wild captured species. The main hazards are

antibiotic residues and development of antimicrobial resistance in bacteria that may be transferred to consumers (Stevens *et al.,* 2012). Growing concerns over consumer health-related issues associated with the intake of antibiotic residues found in fish and meat products have led to an increased demand for reliable test methods (Nouws *et al.,* 1998; Reybroek *et al.,* 2000; Beverley and Sharman, 2001; Stead and Sharman, 2002).

Recently, the Premi®Test was released as a new broad-spectrum microbial screening test for the detection of large number of the most widely used antibacterial substances in fresh meat, meat products, kidney, fish and eggs (Geijp *et al.,* 1998). Premi®Test is based on the inhibition of the growth of *Bacillus stearothermophilus*, a bacterium very sensitive to many antibiotics and sulpha compounds (Korsrud *et al.,* 1998; Reybroek, 2000; Sporri and Stephen, 2000).

Premi®Test is used in order to prevent any edible animal product containing antibiotics above the legal limits entering the food chain (Arts and Witkemp, 1999). It is a fast, sensitive, reliable, easy-to-use and cost effective test covering a broad range of antibiotics (Geijp *et al.,* 1998; Arts and Witkemp, 1999; Beverly and Sharman, 2001; Koch and Dietrich, 2001; Stead and Sharman, 2002). Where conventional tests require overnight incubation (Shepherd and Poupard, 1975; Okerman *et al.,* 1998; Romero *et al.,* 2007), Premi®Test gives a reliable result in less than four hours. The use of this test allows one to take quick decisions on further processing of fish or meat and their products. Premi®Test kit can be used for screening single or large numbers of fish or meat samples (Geijp *et al.,* 1998; Arts and Witkemp, 1999; Beverly and Sharman, 2001; Koch and Dietrich, 2001; Stead and Sharman, 2002).

1.2 Statement of Research Problem

The indiscriminate use of antibiotics for veterinary purposes has increasingly become a matter of public health concern. Presently, antibiotic resistance is a serious clinical and public health problem on a global basis. In the fish farming (aquaculture, mariculture) sector, the widespread use of antibiotics for treating bacterial diseases or as growth promoter has been associated with development of antibiotic resistance in pathogenic bacteria (Griffin, 2000; Shears, 2001).

The ingestion of antibiotic-contaminated fish has the potential of altering the human gut flora, thus favoring infections of micro-organisms such as *Salmonella* (Cabello, 2003; 2006). This may increase the risk of toxic and allergic phenomena that may be difficult to diagnose due to paucity of information on the ingestion of these agents (Husevag *et al.,* 1991; Cabello, 2003; 2006; Sorum, 2006).

The excessive use of antibiotics in aquaculture has had negative effects on human and animal health, and has also caused damage to the environment (Bjorlund *et al.,* 1990; Samuelsen *et al.,* 1992; Hektoen *et al.,* 1995; Grave *et al.,* 1999; Cabello, 2004; Wolf, 2004; Buschman *et al.,* 2006a; 2006b; Cabello, 2006). When consumed directly by humans as medicine, antibiotics may cause adverse side-effects, but these can generally be avoided through adhering to the recommended dose and duration of therapy. However, when antibiotics are unintentionally ingested as residues in food, the amount ingested may be above the permissible limit and may cause direct health concerns, such as aplastic anemia (USFDA, 1997; FAO, 1997). These direct effects pose significant risks to human health.

Furthermore, aquaculture is associated with novel routes for people to become exposed to resistant bacteria, or genes conferring resistance to a given antibiotic with roots in aquaculture.

For this reason, aquaculture may be, or may contribute to the pool of antibiotic resistant bacteria triggering infections in humans. Also, in the area of safety assurance, as in Hazard Analysis Critical Control Point (HACCP), and Good Management Practice (GMP), veterinarians, farmers and food producers require tools to screen and monitor wholesome and safe food from fish.

1.3 Justification of Research

Livestock and poultry production in Nigeria have not been able to satisfy the demand for quality animal proteins. Recent epidemics of bird flu that ravaged poultry industry discouraged many farmers from poultry keeping. Fish production is now becoming popular. This is associated with increased demand for fish and fish products in a country like Nigeria with a rapidly growing population (FAO, 2004). Besides, fish production is generally carried out on a small-scale, backyard and practiced by a wide variety of individuals both on part-time and full-time basis. Most part-time fish producers are untrained and do not adopt good management practices and bio-security measures (Amao *et al.,* 2006).

Poor fish farm practices predispose fish to infection. Most farmers realize that infection in fish ponds can be suppressed by regular antibiotic use and is done without veterinary supervision (FAO/WHO, 2003). Even when used by animal health personnel there is limited training of these personnel in chemotherapy and pharmaceutics in fish, particularly those kept under intensive management.

Due to the risks associated with indiscriminate use of antibiotic, it is necessary to carry out research to determine the presence of antibiotic residues in fish from commercial fish farms, and also the level of awareness of the populace on the deleterious effects of antibiotic residues (Olatoye and Basiru, 2013). Attention is urgently needed on aquaculture drug use because it is

probable that antibiotic selection pressure in aquaculture systems is intensifying (Neu, 1992). Furthermore, there is no government regulation on the use of drugs in fish in Nigeria. There also appears to be no record in the available literature on the extent of use of antibiotics and occurrence of residue in farmed fish in Kaduna State, Nigeria.

1.4 Aim of the Study

The aim of the study was to assess antimicrobial drug use and presence of residues in farmed fish in Kaduna, Nigeria.

1.5 Objectives of the Study

The specific objectives were to:

i. Assess the presence of antibiotic residues in tissues cultured fish in Kaduna State, Nigeria.
ii. Determine the pattern of use of antimicrobial drugs in fish farms in Kaduna State, Nigeria.
iii. Assess the management practices associated with antimicrobial drug residues in cultured fish in Kaduna State, Nigeria.
iv. Evaluate level of awareness of farm owners/attendants, Veterinarians and Para-veterinarians on the deleterious effects of antimicrobial drug residues in fish in Kaduna State, Nigeria.

1.5 Research Questions

i. Do cultured fish raised in fish farms in Kaduna State, Nigeria contain antimicrobial drug residues?
ii. What is the pattern of use of antimicrobial drugs in fish farms in Kaduna, Nigeria?

iii. Are the management practices in fish farms in Kaduna State, Nigeria associated with occurrence of antimicrobial drug residues in cultured fish?

iv. What is the level of public awareness on the deleterious effects in humans following consumption of antimicrobial drug residues in fish and fish by-products?

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture

Aquaculture is the rational rearing of fish and other aquatic organisms in man-made ponds, reservoirs, cages or other enclosures in lakes and coastal waters (Lovell, 1991). It is one of the most ancient occupations of man, having been practiced in China and Egypt more than 400 years ago (Abbot, 1924; Shang, 1972; Oladosu and Ajasin, 2012). Aquaculture in the tropics remains a small-scale project, run as community farms, homestead ponds or becomes integrated into rural development schemes. The major objective of fish farming is for the provision of high quality protein for urban and rural communities. Often pond fish is the major protein supply to such communities; sometimes it merely supplements supplies from other sources (Reay, 1979).

According to the UN Food and Agriculture Organization (2010), aquaculture is growing more rapidly than all other animal food-production sectors. Its contribution to global supplies of several species of fish, crustaceans and molluscs increased from 3.9% of total production by weight in 1970 to 33% in 2005. It has been estimated that fisheries and aquaculture supplied the world with about 110 million metric tons of food fish per year, providing a per capita supply of 16.7 kg (live weight equivalent) (FAO, 2010). Of this supply, 47% is derived from aquaculture production. However, this production is hampered by unpredictable mortalities that may be due to negative interactions between fish and pathogenic bacteria. Farmers solve this problem frequently by the use of antibiotics to treat the bacterial diseases (Cabello, 2006).

2.1.1 Development and status of aquaculture

Aquaculture has developed from the initially subsistence level (small-scale farming operation) into the multi-million dollar enterprise for catfish, trout, milkfish and oyster farming industries consisting of many relatively small units (Ajana *et al.,* 2006). Virtually integrated industrial-level aqua-farming is relatively rare, although there are clear trends toward the establishment of such particularly in developed countries. Naturally, there are many intermediate organizational levels found (Holden and Reed, 1972).

Farm size may vary from less than a hectare to a thousand or more hectares owned and operated by individuals, co-operatives, government corporations or private companies (Oladosu and Ajasin, 2012). The enterprise may be solely devoted to the production and sale of seed or baitfish, or for the production of aqua-foods (including sport fish), industrial products like pearls, or raw materials for other industries like agar and marine colloid manufacture (Sorum, 2000). However, most of present day aquaculture is oriented to the production of human food.

2.1.2 Importance of aquaculture

The importance of fish as an agricultural commodity cannot be over-emphasized, considering its food value, the relative affordability compared to other animal protein sources and the contribution to the economy of nations in terms of job and wealth creation (Webber, 1972; Eng, 1973; Pillay, 1973; Delgado *et al.,* 2003). Global demand for fish is ever increasing which creates more opportunities for enhanced foreign exchange earnings (Dawson and Steinberg, 1973; Viveen *et al.,* 1985; Ajana *et al.,* 2006). Furthermore, fish being one of the cheapest sources of animal protein has the potential for the eradication of hunger and reduction of

malnutrition and the infant mortality associated with it especially in the developing world (Delgado *et al.,* 2003).

2.1.3 Pre-requisites for aquaculture

Aquaculture is becoming a more concentrated industry, with fewer, but much larger, farms. Infectious diseases are always a hazard and may cause significant stock losses and problems with animal welfare. Intensive aquaculture (shrimp and fish farming) has led to growing problems with bacterial diseases, the treatment of which now requires the intensive use of antimicrobials. Various authors have emphasized the negative effects of using antimicrobial agents in fish farms (Alderman and Hastings, 1998; Miranda and Zemelman 2002; Cabello, 2006; Fernández -Alarcón, 2010). For a successful aquaculture, there are basic conditions that must be met. The pre-requisites include: good water quality, suitable site for the farm, reduce contamination by pathogens and good nutrition and management practices.

2.1.3.1 Good water quality

There must be constant supply of water of good (or suitable) quality and quantity. Provision of potable water is a pre-requisite for good productivity and good health management. Water for fish culture must be provided from sources free of pathogens and toxicants (Goldburg and Triplett, 1997). Underground waters are the most suitable in this regard. If surface water must be used, it must be subjected to treatments that ensure that pathogens, toxicants and sediments are effectively removed (Dietz *et al.,* 2005). This is done mostly through sedimentation, filtration and ultraviolet irradiation. Regular monitoring of water in culture facilities is also critical to good fish health management (Oladosu and Ajasin, 2012).

2.1.3.2 Suitable Site

According to Pillay (1977) for a conventional drainable fish ponds, the site chosen must have a suitable topography, suitable soil, must contain less vegetation and suitable for organisms to cultures.

2.1.3.3 Reduce contamination by pathogens

Biosecurity measures must be put in place to reduce contamination by pathogens. These include procurement of fingerlings and brood-stock from reputable disease free farms, and quarantine of new entrants which must be disinfected where necessary (Dorson, 1972; Klontz, 1972). Routine disinfection of production facilities (in between production cycles), equipment, utensils and other materials used in the farm (before and after use). Production facilities must not be shared between farms to avoid cross contamination. Furthermore, ponds and rearing tanks should be screened from predators such as vectors of disease agents (Abd El-Ghany and El-Ashram, 2008).

2.1.3.4 Good nutrition and management practices

Growth and survival of fish is greatly dependent on nutrition, husbandry and management practices. Good husbandry practices mean better health condition and low mortality, and fish fed good quality diet in the right quantities will exhibit vigor and vitality, grow faster and develop better resistance to pathogens (Ajana *et al.,* 2006). Fish eat to satisfy energy requirements; hence ensuring that efficient energy/protein ratio is pertinent. In addition, stress and trauma will decline with reduced handling, and the chances of secondary pathogenic infections will decrease marginally (Oladosu and Ajasin, 2012).

Moreover, when there is disease incidence, prompt, specific and definitive diagnosis of the disease becomes imperative for effective control (Abd El-Ghany and El-Ashram, 2008). In

addition, diagnosis must take cognizance of the possibility of the environment of multiple pathogens in a disease incidence (Roberts and Shepherd, 1974; Sano *et al.,* 1997). The probability of multiple involvements of pathogens is higher where there is damage to skin or gill surfaces via trauma or parasitic infestation, which can be secondarily infected by bacterial and fungal pathogens (Snieszko, 1972; Shepherd and Poupard, 1975; Inglis *et al.,* 1993).

2.2 Aquaculture Practices and Systems

Fish production by culture can be classified into two broad groups based on the intensity of production and number of fish species cultured (Greenfield, 1970; Oladosu and Ajasin, 2012). Aquaculture whether extensive, semi-intensive, or intensive may be carried out in fresh water (fresh water aquaculture) or in brackish water (brackish water aquaculture) or in 100 % sea water (mariculture).

2.2.1 Classification based on production intensity

2.2.1.1 Extensive aquaculture

Fish raised in this system depend solely on natural food production in the pond, thus, yield per unit area is usually low. Extensive cultivation normally involves large areas, low levels of capital investment per unit area and low general management. The system is characterized by low inputs, low stocking density and availability of planktons and the other live food materials in the pond and no supplemental (artificial) feeding, or pond fertilization. In this system the fish once stocked is left to the natural carrying capacity of the pond water, the yield per unit area is low. This type of culture is done in reservoirs and undrainable ponds, and they tend to be labor intensive (Smith *et al.,* 1994).

2.2.1.3 Semi-intensive aquaculture

In the semi-intensive aquaculture, the ponds are fertilized with organic or inorganic fertilizers. Beside, pelleted feed, other supplemental feeding, if applied, are given in small quantities occasionally and generally consists of locally available materials in the form of kitchen wastes, rice bran, groundnut cake and poultry offal. The yield is intermediate between those obtained from intensive and extensive cultures. Most aquaculture in the tropics is of this type. Under this system, ponds are fertilized in order to enhance phytoplankton growth and supplemental (artificial) feed is also required. Yield per unit area is moderately higher than extensive system but lower than in the intensive aquaculture system (Ajana *et al.,* 2006).

2.2.1.2 Intensive aquaculture

Intensive aquaculture involves heavy inputs in the form of high protein pelleted feeds and fertilizers, high density stocking, improved pond management, all resulting in high yield per unit area. In this system, supplemental or artificial feeding is required. High level protein rich diet is employed in order to meet up with nutritional requirements of fish. Other management practices are equally made to be intensive, which include medication, constant good-quality water supply (as in flow-through or water recirculatory system) thereby resulting in high yields per unit area. Intensive systems are characterized by dense stocking, stock selection and intensive management and environmental control, partial mechanization of operations and high production per unit area or volume of water. The level of intensity in operations is governed by technical, economic and social factors. Also, in the process of development the level of operations may gradually evolve from an extensive to semi-intensive or intensive level.

Intensive fish farming has promoted the spread of several bacterial diseases, which in turn has led to the increased use of antimicrobials (Song and Sung, 1990; Ajana *et al.,* 2006).

2.2.2 Classification based on species combination or culture integration

Classification of culture system can be based on the number of fish species cultured in a production facility or in combination with crops or livestock/poultry production.

2.2.2.1 Monoculture system

Monoculture is a system of fish production involving the rearing of single species of fish in a culture facility. This practice is most common with the water recirculatory system (Oladosu and Ajasin, 2012). Monoculture system makes farming much more efficient, keeping down costs, better harvest and it improves the growth of the fish. It also allows for huge fish production.

2.2.2.2 Polyculture system

Polyculture system of fish production involves the rearing of two or more species of fish in a single culture production facility. This system of production occurs mostly in semi-intensive earthen pond culture, and it is done for various reasons. For example, the prolific tilapia species can be stocked with the predatory catfish so that the latter will control over-population in the former, while at the same time enjoying the availability of live feed in tilapia seeds. The cost of feeding the catfish is also reduced. Joint stocking of species with different feeding niches e.g., the polyculture of catfish tilapia and heterotis will ensure efficient utilization of all natural food in the water. Hence catfish is a bottom feeder; heterotis and tilapia are surface feeders (Viveen *et al.,* 1985).

2.2.2.3 Integrated system

Integrated system involves the joint production of fish with livestock (like pigs), or with poultry or even with crops (like vegetable, plantain etc). It is commonly targeted at efficient utilization of land space, labor, feed etc., and also provides a broad revenue base and ensures financial sustainability (Webber, 1972; Ajana *et al.,* 2006). Aquaculture operations are often combined with agriculture or animal production activities, and this is considered particularly important and essential in integrated rural development programmes. In addition, there is much to be gained by close collaborations including the sharing of common services (Webber, 1972).

2.3 Fish Farming

There are compelling reasons, economic and political for fish farming to develop into a major industry. At present, West Africa is very dependent upon imports of fish (Hoden and Reed, 1972). Fish farming presents a number of unusual problems to public health and veterinarians trained to combat diseases and enhance production (Ajana *et al.,* 2006).

Fish are very prolific and some, including the favored fish like catfish and tilapia release their eggs by the million. Fish are far more efficient at food conversion than the conventional terrestrial farm livestock. Fish culture is not new; the Chinese and Egyptians are believed to have practiced aquaculture around 2000BC (Ajani *et al.,* 2006). There is little doubt however, that the concentration of large numbers of animals, which is implicated in fish farming (especially intensive), will be readily exploited by micro-organisms, protozoal and metazoal parasites and the repercussion will be the unrelenting danger of disease (Sovenyi and Molnar, 1990).

Global interest in the development of fish farming has led to many attempts to assess the potentials in different countries and enhance fish production. Aquaculture practices range from the propagation of aquatic organisms under complete human control to the manipulation of at least one stage of an aquatic organism life before harvest for the purpose of increasing production (Vaslet *et al.,* 1989).

A pre-requisite to fish farming is a clear conception of the need and the purpose of developing the industry in a particular region of the country. In different regions of the country, aquaculture could be the main source of fish supplies, in some areas it is at present only a supplementary source of aquatic foods despite its great importance (Webber, 1972).

2.3.1 Economics of fish farming

The economics of fish farming, particularly the return on investment, varies considerably depending largely on the efficiency of operations and market conditions. Although readily available information on this aspects is still limited, there have been a number of available information on the economics of aquaculture in recent years (Mitchell and Usry, 1967; Greenfield, 1970; Shang, 1972; Eng, 1973; Pillay, 1973; IPFC, 1975), which reveals that well planned and properly operated aquaculture compares very favorably with similar livestock and poultry production in respect of internal rates of return (Ajana *et al.,* 2006).

Whether fish farming should aim at the production of cheap fish for the common man or high-priced food for the luxury market or export, is a decision to be made with due regard to the prevalent socio-economic development. However, food habits and preferences are very strong among the poor classes and possible changes can only be brought gradually through market promotion. The low-valued species raised by large scale aquaculture as suggested by Dassow

and Steinberg (1973) should be used for processed products in which convenience food value and acceptability of finished products are more important than the species used.

2.3.2 Importance of fish farming

The major reasons for an individual, a community or a country to embark on fish farming are the production of protein and other reasons include the following (Viveen *et al.,* 1985):

i. Improvement of natural stocks in lakes, reservoirs, rivers and the coastal waters by raising the young in hatcheries and restocking them.
ii. Production of sport fish
iii. Production of bait fish for use in industrial fishing
iv. Production of ornamental (as aquarium) fishes
v. Means of recycling waste or effluents from factories and sewage disposal systems.

2.3.3 Value and importance of fish to humans

Fish products are of high economic importance and are used primarily as human food. From fish one obtains not only an abundant caloric value, especially if the fish is fatty, but also higher quality proteins, some fat soluble vitamins and considerable amount of essential elements for the maintenance of a healthy body. Fish protein (like other animal proteins) contain most of the essential amino acids, in particular, the three that are lacking in proteins of plant origin-namely lysine, methionine and tryptophan, and for this reason the protein is described as first class protein and its consumption is highly desirable especially where animal proteins from other sources are lacking (Oladosu and Ajasin, 2012).

Parts of a fish can be used for various purposes

i. Skin of some cartilaginous fishes (*Chondrichthyes*) makes useful leather and polishing materials.
ii. Scales of some fishes yield a substance that when coated on the inside of glass beads make artificial pearls
iii. The lining of the airbladder of sturgeons is used to make isinglass, a shiny powder used as absorbent in the wine industry.
iv. Fish oils besides being used as food, are used in the manufacture of soap.
v. Whole fish not readily acceptable for direct consumption are converted into fish meal for the manufacture of animal feeds.

2.3.4 Table fish production

Table fish production involves the rearing or growing of fingerlings or juveniles to adult fish for human consumption. It lasts for a period of 4-6 months depending on the culture system and adoption of good management and adequate feeding protocols.

2.4 Fish Culture Holding Facilities

Fish are generally cultured in aquatic medium but the culture facilities differ in size and type depending on the choice of the farmer. Common fish holding facilities include (Oladosu and Ajasin, 2012):

2.4.1 Earthen ponds

Earthen ponds are the most common fish production facilities in Nigeria. Earthen ponds are easy to manage and production is faster in this facility because of additional foods to

supplement the feed given to fish. However, fish in earthen ponds are generally prone to predators if not properly managed.

2.4.2 Concrete tanks

These facilities are used for fish production and can be made of blocks with holes filled with concrete or with reinforced concrete slab. It can be rectangular or circular. It is common in urban and peri-urban cities.

2.4.3 Plastic fibre or glass tanks

These facilities are tanks designed and constructed for rearing of fish. This is very common in Nigeria. Moreover, they can be moved from one place to another.

2.4.4 Wooden troughs

These are fish holding facilities constructed with planks. They vary in size and depth. After construction, the tank is lined with tarpaulin or polyethylene material to prevent leakage. It can be moved from one place to another.

2.4.5 Fish cages

Fish cages are enclosure made with net. Therefore, rearing fish in cages mostly in open-waters such as free flowing rivers, reservoirs, lakes and large ponds is called cage culture. Cage culture is still at the experimental stage in Nigeria, only a few farms engage in it. However, it has a high potential to improve local fish supply.

2.4.6 Fish pens

These pens are enclosures installed in open waters that are not too deep for raising fish. The fish has access to the water bottom unlike in cages. Pen culture is not common in Nigeria.

2.5 Fish Production by Culture

Fish farming is presently making significant contribution in the world fish supply. Aquaculture ranges from simple ponds using naturally occurring food sources to highly intensive systems with water control, aeration, and supplemental feeding. Farm size can range from thousands of hectares down to the size of a backyard. The majority of global production recorded lately comes from fresh water aquaculture (58%), followed by mariculture (36%) and brackish water (6 %) (Oladosu and Ajasin, 2012).

Aquaculture represents more than 30% of total food fish production. However, Nigeria aquaculture production is second in Africa after Egypt with Nigeria and Egypt jointly producing a total of 84%, while the rest of Africa contributes the remaining 16% (Ajana *et al.,* 2006). Some common cultured fish in Nigeria are shown in Table 2.1

Table 2.1: Types of fish commonly cultured in Nigeria.

Order	Family	Species	Common names
Chariasiformes	Citharinidae	*Citharinus citharus*	Moon fish
Mormiriformes	Gymnarchidae	*Gymnarchus niloticus*	Trunk fish
Ophiocephaliformes	Channidae	*Channa obscura*	Snake head
Osteoglossiformes	Osteoglossidae	*Heterotis niloticus*	
Perciformes	Lutjanidae	*Lutjanus govensis*	
		Lutjanus agennes	
Siliruformes	Cichlidae	*Oreochromis niloticus*	Nile tilapia
	Bagridae	*Chrysichthysnigro digitatus*	Silver catfish
	Clariidae	*Clarias gariepinus*	Mud catfish
		Clarias anguilaris	Mud catfish
		Heterobranchus bidorsalis	Red mud catfish
		Heterobranchus longifilis	Red mud catfish

Source: Oladosu and Ajasin, 2012

2.6 Fish Health Management

Fish integrates the effects of the many biotic and abiotic variables in the aquatic environment, and reflects the impacts of the numerous stressors to which they are subjected. These include extremes of physico-chemical and biological parameters of water, anthropogenic stressors and stress associated with poor nutrition, husbandry and management practices. With the intensification of fish production to enhance productivity and profitability, the health risk associated with fish production becomes higher (Wood, 1974; Pillay, 1977).

Sources of hazards are increased especially with the biological and chemical complexity of water as the production medium. Fish are cold blooded animals, hence their body temperature and thus their whole metabolism including response to infection is dependent on water temperature. Also pathogens can be transmitted much more easily through water, and since the environment in which fish is being produced is not notobiotic, there is always the presence of different kinds of pathogens including bacteria, fungi, protozoans, and metazoan parasites and viruses (Dorson, 1972; Buchanan *et al.,* 1978; Inglis, 1993; Horinek, 2009).

In addition, the build-up of organic load (heavy metals, domestic and industrial agricultural chemicals) in water could bring about high levels of toxic substances detrimental to the well-being of fish (Solomon, 1972; Arthur *et al.,* 2000). All these and others impact negatively on the aquatic biota including fish, and they portend great implications for fish health in fish farms using surface water as a water source.

Despite all these facts, fish survives so long as the critical parameters are suitable, good husbandry practices that minimize stress and traumas are employed and contamination of production facility by pathogens and toxic substances are avoided (FAO, 1997).

Effective disease control required to contain morbidity and mortality, and stem the economic losses associated with them depends heavily on prompt definitive diagnosis (Sovenyi and Molner, 1990). History of disease occurrence is the genesis of effective diagnosis. This includes source of fish and the management practice employed. Analysis of water quality to detect extremes of water parameters and presence of noxious substances are inevitable to effective diagnosis as the genesis of the problem may be entirely environmental (Roberts, 1989; Abdelkhalek *et al.,* 2008).

2.7 Pathogen Isolation and Identification

Various means of isolation and identification of pathogens include microscopy, primary culture, biochemical, serological and molecular characterization (Boyle and Blackwell, 1991; Bader *et al.,* 2002; Starliper, 2008). These methods of identification of pathogens (bacterial, fungal, viral, protozoans) give opportunity for subsequent antimicrobial sensitivity testing against therapeutic agents, minimizing the development of drug resistant strains. It also provides source of isolates for vaccine development, and molecular characterization for genetic manipulation (Starliper, 2008).

2.7.1 Isolation of bacterial pathogens

There are general bacteriological media and specialized media used in the culture of a variety of potential bacterial pathogens that may be present in infected tissues and target specific bacterial pathogens during enzootic situations. The general media used are nutrient rich and include the brain heart infusion agar (BHIA), tryptic soy agar (TSA) and blood agar base with 5% sheep blood. They are used to culture all bacteria present in the tissue, both pathogenic and non-pathogenic. The isolation of specific agent by the combination of different and selective

approach is to maximize the sensitivity of the culture and prompt diagnosis to speedy control of disease and minimize economic loss (Starliper, 2008).

2.7.2 Isolation of viral pathogens

Virus isolation in cell culture is one principal means of diagnosing viral diseases in fish. Common cell lines used for the isolation of viral pathogens of fish include (Oladosu and Ajasin, 2012):

i. Cell lines from the testis of rainbow trout *(Onchorhynchus myksis)*
ii. Cell lines produced from the caudal part of fat head minnows *(Pimepha lepromelas)*and from the embryo of salmon *(Oncorhynchus tschawytscha)*
iii. Cell lines from the ovary of charmal cat fish
iv. Cell lines produced from the gonad of pike *(Esox lucius)*
v. Cell lines produced from the gonad of walking catfish *(Clarias lazera)*
vi. Cell lines produced from the kidney of walking catfish *(Clarias lazera)*

Cytopathic effects (CPE) represented as plaques in the cell culture over time indicates the presence of viral agent which may further be characterized and identified using:

i. Electron microscopy
ii. Immunoperoxidase
iii. Direct or indirect immunofluorescence
iv. Direct or sandwiched Enzyme-linked immunosorbent assay
v. Polymerase chain reaction

2.7.3 Isolation of fungal pathogen

Fungal organisms affecting the health of fish are believed to be largely secondary invaders, but some species parasitize fish causing morbidity and mortality. Fungal isolation is done routinely, more or less simultaneously with bacterial isolation. They can grow on media used for bacterial isolation. However, there are media that aid good fungal growth. They include: Sabourad Dextrose Agar (SDA), Sweet Wort Agar (SWA) and Czapek Agar (Starliper, 2008).

2.7.4 Unique methods of pathogen identification

Special methods exist for the identification of pathogens (Watts *et al.,* 1996). These methods are rapid, more specific and sensitive than the classical methods. They are readily applicable in the prompt diagnosis of diseases of fish (Abd El-Ghany and El-Ashram, 2008). These methods include immunoperoxidase test, immunofluorescence test and ELISA (serological tests based on antigen and antibody reaction), while PCR is molecular or genetic (nucleic acid) based diagnostic technique (Boyle and Blackwell, 1991; Bader *et al.,* 2002; Suomalainen *et al.,* 2006; Wiklund *et al.,* 2008).

2.8 Disease Prevention and Control in Aquaculture

Because a wide variety of chemicals are currently used in aquaculture production, control measures have been introduced over the years. These include the use of disinfectants e.g., hydrogen peroxide and malachite green; antibiotics e.g., sulfonamides and tetracyclines; and anthelmintic agents e.g., pyrethroid insecticides and avermectins (Rawn *et al.* 2009). However, disease control is an active research field, and alternatives to antibiotic treatments have been explored. The public health hazards related to antimicrobial use in aquaculture include the development and spread of antimicrobial-resistant bacteria and resistance genes and the presence of antimicrobial residues in aquaculture products and the environment.

The control of pathogenic disease in fish involves the use of a limited number of approved antibiotics and chemotherapeutics (Gorbach, 2001), however, many drugs and chemicals are used globally to control disease in fish due to the increased growth of aquaculture (Primavera *et al.,* 1993; USFDA, 1997; Alderman and Hastings, 1998; Benbrook, 2001). Drugs commonly used in aquaculture include antibiotics, potentiated sulphonamides and chemicals like formalin, potassium permanganate, malachite green, diclorvos (FDA, 1998b; 2001).

Presently emphasis is shifting from chemotherapeutic control of diseases to disease prevention (Marzouk *et al.,* 2003). There have been reports of increased resistance of bacterial pathogens of fish to many antibiotics over the years with the attendant public health implications (McPhearson *et al.,* 1991; Hirsch *et al.,* 1999; Schmidt *et al.,* 2000; MacMillan, 2001; Shears, 2001). Effective preventive methods can sufficiently reduce the devastating effects of disease on fish in terms of growth and yield, as well as the cost of medication which increases the cost of production (IPFC, 1975).

Furthermore, cases of incidence of pathogenic infection of fish especially bacterial infection will still require treatment. It is therefore important to ensure the responsible use of antibiotics by ensuring that antibiotic sensitivity testing is done for adequate selection of drug of choice to control the infection (Muniruzzaman and Chowdhury, 2004).

Biosecurity measures are paramount in fish health management and involve the use of sanitation, quarantine of new fish entrants to farms, restriction of the movement of fish, ensuring safe water source and good water quality, as well as good nutrition for healthy growth. Farm sanitation involves the use of physical and chemical means to reduce pathogen load in rearing facilities, incoming effluent water, materials used for culture, personnel and vehicles.

Production facilities such as earthen ponds and tanks are disinfected in between use with hydrated lime and quaternary ammonium compounds (Oladosu and Ajasin, 2012).

In intensive recirculation system, incoming water is exposed to ultraviolet irradiation to eliminate pathogens that may be in the water. In addition, culture facilities are screened mostly with net from birds and other predators that are potential vectors of fish diseases. Also, the prompt removal and safe disposal of dead fish from culture facility is a pre-requisite for effective disease prevention in aquaculture (Oladosu and Ajasin, 2012).

2.8.1 Controlling diseases using antibiotics

Antimicrobial agents can be defined as substances that have the capacity to kill or inhibit the growth of micro-organisms. After their formal discovery by Fleming in 1928, antibiotics have become essential drugs for human and animal health and welfare. Antibiotics can be derived from natural sources or have synthetic origins. Antibiotics should be safe (non-toxic) to the host, allowing their use as chemotherapeutic agents for the treatment of bacterial infectious diseases.

In addition to their use in human medicine, antimicrobials are also used in food animals and aquaculture, and their use can be categorized as therapeutic, prophylactic or metaphylactic. Therapeutic use of antimicrobials refers to the treatment of established infections, metaphylaxis is a term used for group-medication procedures that aim to treat sick animals while also medicating others in the group to prevent disease, while prophylaxis means the preventative use of antimicrobials in either individuals or groups to prevent the development of infections. In aquaculture, antibiotics at therapeutic levels are frequently administered for short periods of time via the oral route to groups of fish that share tanks or cages. The most common route for

the delivery of antibiotics to fish occurs through mixing the antibiotic with specially formulated feed. However, fish do not effectively metabolize antibiotics and will pass them largely unused back into the environment in feces. It has been estimated that 75 per cent of the antibiotics fed to fish are excreted into the water (Burridge *et al.,* 2010).

2.8.2 The need for disease prevention

The prevention of diseases in fish farming cannot be over-emphasized. The main criterion of disease prevention is the regular monitoring of the fish to detect any changes in their growth rate and condition, parasitic burdens, immune status and the general pattern of their behavior. The implementation of a disease prevention program involves an unremitting, vigilance usually involving the execution of a number of apparently unrewarding tasks since the benefits of an absence of disease are not apparent until outbreaks occur (Anderson, 1974).

It is for this reason that implementation of disease prevention programs are designed and often leave much to be desired (Ajana *et al.,* 2006). Nevertheless, the sequel to an outbreak of disease is probably more serious to those engaged in intensive fish farming than those with conventional terrestrial livestock (Dorson, 1972; Delgado *et al.,* 2003).

In most of the countries with an important aquaculture industry, government agencies exert some controlling actions. For example, in Norway the use of antimicrobials requires a veterinarian's prescription, and hence, their use is therapeutic. They are sold in pharmacies or in feed plants authorized by the Norwegian Medicines Agency. In Norway, it is mandatory to report the amount of antibiotics used and retain records of prescriptions. Intensive fish farming has promoted the growth of several bacterial diseases, which has led to an increase in the use of antimicrobials (Defoirdt *et al.,* 2007; 2011).

Current levels of antimicrobial use in aquaculture worldwide are not easy to determine because different countries have different distribution and registration systems. Nevertheless, Burridge *et al.* (2010) reported that the amount of antibiotics and other compounds used in aquaculture differed significantly between countries. Defoirdt *et al.* (2011) previously estimated that approximately 500–600 metric tons of antibiotics were used in shrimp farm production in Thailand in 1994; he also emphasized the large variation between different countries, with antibiotic use ranging from 1 g per metric ton of production in Norway to 700 g per metric ton in Vietnam. Total prevention of disease in aquaculture system is likely unattainable in practice. Therefore, disease management depends upon good culture practice in combination with chemotherapeutic agents. Some agents are administered (often in feed) on a prophylactic basis (Graslund and Bengtsson, 2001).

2.8.3 Disease control and public health safeguards in fish farming

There are a number of diseases, especially viral infections for which there are no known methods of treatment, and the infected stock must be destroyed in order to prevent their spread (Dorson, 1972). The danger to public health resulting from the consumption of contaminated products which are often eaten raw has to be avoided (Lovell, 1991; Willis *et al.,* 1999).

In many tropical countries public health regulations conflict with the construction and maintenance of ponds and other stagnant or semi-stagnant water bodies used for fish farming. Under certain conditions such water bodies become breeding grounds for mosquitoes that are transmitting agents of malaria, filariasis, yellow fever, dengue and encephalitis (Goldburg and Triplett, 1997; Goldburg *et al.,* 2001). Mutual recognition of the need for fish farming and the

importance of preventing the breeding mosquitoes in aquaculture waters have led to the development of suitable methods for combining mosquito with fish production (Roth, 2000). Another public health hazard associated with fish farming is the transmission of bilharzias through snails that may multiply in fish ponds and other bodies of water used for culture. The introduction of snail-feeding fish in aquaculture waters will serve as a direct means of eradicating snails. These measures will also serve to control the possible transmission of animal diseases like fasciolosis, for which also snails are the intermediate host (Oladosu and Ajasin, 2012).

2.9. Therapy of Fish Diseases

Diseases occur in both natural and cultured fishes and can be categorized into infectious (where the causative agent is bacterial, viral or parasitic) and non-infectious diseases (caused by toxic substances, improper nutrition, poor water quality, physical damage or genetics). Infectious diseases are a major concern in aquaculture both in terms of the potential negative impacts on production and the potential for disease impacts on wild populations (Johnston and Santillo, 2002).

Outbreaks of disease are typically caused by widely distributed opportunistic pathogens. In natural systems they have a low prevalence and low intensity of infection, while in cultured fishes where stresses lower resistance and stocking density facilitates transmission of the impact of disease, outbreaks can be severe (Johnston and Santillo, 2002)

The therapy of fish diseases is still relatively unsophisticated. There have been few concerted research investigations concerned solely with establishing definitive criteria (i.e., toxicity and efficacy) for treatment compounds, far less methods for the application of such chemicals and drugs (Roberts and Shepherd, 1974; Wood, 1974). As with most forms of intensive animal

husbandry, current trends in intensive fish farming lay emphasis on the prevention rather than the treatment of fish diseases, but the majority of outbreaks of disease in cultured fish can usually be attributed to inadequate husbandry or management (Shepherd and Poupard, 1975).

Incorporation of a drug into the food to treat systemic bacteria diseases or gut parasites presents contrasting problems. The physical incorporation of drugs into diet can present practical difficulties. It is very important to achieve an accurate dosage rate in the quantity of medicated feed offered, a balance has to be reached between toxic and therapeutic levels in the blood stream (Anderson, 1974).

Availability of drugs may prove difficult in certain countries and where available they can only be prescribed by a veterinarian who has satisfied himself as to the clinical justification for their use. These restrictions and regulations are of great importance in reducing the risk of development of resistant strains of pathogenic bacteria in both man and animals (Boinapally and Jiang, 2007).

It is imperative to always ensure a strict limitation on marketing of fish following a course of medicated feeding (Smith *et al.,* 1973). There is often no legislative requirement but even so producers are under an important moral obligation not to harvest such fish for at least four weeks after treatment to allow antibiotic to clear from the tissues of the fish; otherwise such fish may be responsible for induction of antibiotic resistance in the pathogenic flora of the individual consuming them (Grave *et al.,* 1999).

2.10 Antibiotics and Chemotherapy

Therapeutic addition of antibiotics and chemotherapeutics to the development of fish is widely practiced and where therapy is limited to a short period of time minimal damage ensues,

although growth may be checked until the normal bacteria flora of the fish, which will also have been eliminated, is restored (Smith *et al.,* 1973). However, where continue therapy is carried out (a very dangerous practice) toxic changes such as depression at haematopoiesis and, especially with sulphonamides, renal tubular necrosis and cast formation will occur (Smith *et al.,* 1973; Frank *et al.,* 1999). The high proportions of antibiotic-resistant bacteria that persist in sediments and farm environments may provide a threat to fish farms because they can serve as sources of antibiotic-resistance genes for fish pathogens in the vicinity of the farms. Because resistant bacteria may be transferred to humans and are capable of transferring their resistance elements to opportunistic human pathogens, the implementation of efficient strategies to contain and manage resistance-gene emergence and spread is critical.

2.10.1 Antibiotics – Mechanisms of action

Antimicrobial drugs may have different types of chemical structures, and they act on different parts of bacterial machinery. In general, antibiotics work by one of two mechanisms (Byarugaba, 2010):

i. A bactericidal effect, i.e., the antibiotic generally kills the bacteria by interfering with either the formation of the bacterium's cell wall or its cell contents. Examples include penicillin, fluoroquinolones, and metronidazole.

ii. A bacteriostatic effect, i.e., the antibiotic stops bacteria from multiplying by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism. Examples include tetracyclines, sulfonamides, chloramphenicol, and macrolides.

Some of the antibiotics that inhibit bacterial cell wall synthesis include Beta–lactams (penicillins, cephalosporins) and glycopeptides (vancomycin, teicoplanin) which work by

binding to the terminal D-alanine residues of growing peptidoglycan chains, thereby preventing the crosslinking steps required for stable cell wall synthesis. Antibacterial drugs that work by inhibiting protein synthesis include macrolides, aminoglycosides, tetracyclines and chloramphenicol.

2.10.2 Resistance mechanisms and transference of mobile genetic elements

The use of antimicrobial drugs in aquaculture has particular differences from their use in terrestrial animals. In aquaculture, antimicrobials are regularly added to the feed, which is then placed in the water where the fish are kept. In some cases, antimicrobials may be added directly to the water. These procedures result in a selective pressure in the exposed environments (usually water). The use of antimicrobials in aquaculture may involve a broad environmental application that affects a wide variety of bacteria (Byarugaba, 2010). Several bacterial species may survive unfavorable conditions or environmental changes after selecting mutations that improve their fitness in the new conditions. Furthermore, bacteria take advantage of mobile genetic elements, such as plasmids and transposable elements. With these elements, bacteria can access a large pool of itinerant genes that move from one bacterial cell to another and can spread through bacterial populations. Some of these genes may provide the ability to resist antibiotic effects. According to Byarugaba (2010), antibiotic resistance takes two forms:

i. Inherent or intrinsic resistance, i.e. the species is not normally susceptible to a particular drug. This may be due to the inability of the antibacterial agent to enter the bacteria cell and reach its target site, or a lack of affinity between the antibacterial and its target (site of action), or the absence of the target in the cell. It has been suggested that some species of bacteria are innately resistant to whole classes of antimicrobial agents. In such

cases, all strains of that bacterial species are resistant to all members of the antibacterial classes (Kumarasamy *et al.,* 2010).

ii. Acquired resistance. This type of resistance represents the major cause for concern because of the transmissible nature of the resistance mechanisms. In this case, the bacterial species are normally susceptible to a particular drug, but some strains express drug resistance. Initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure induced by the use of that agent (Kumarasamy *et al.,* 2010).

2.10.3 Selection of fish pathogens resistant to antibiotics

The major concerns in treating fish with antibiotics are the potential impact of antimicrobials on the aquatic environment, both marine and fresh water, and the wider theoretical risks associated with the development of antimicrobial resistance by fish pathogens. The spread of antimicrobial resistance due to exposure to antimicrobial agents is well documented in both human and veterinary medicine (IOM, 1989; AHI, 2006). Fish pathogens and other aquatic bacteria can develop resistance as a result of antimicrobial exposure (Alderman and Hastingss, 1998; ASM, 2012). When antibiotic resistance occurs, the effectiveness of the antibiotics for treating fish diseases is compromised (Benbrook, 2001; Byarugaba, 2010).

2.11 Antibiotic Usage in Aquaculture

Aquaculture is presently attracting much attention as an alternative for fisheries production in the wild (Delgado *et al.,* 2003). The growing amounts of fish sold in our markets are coming from fish farms (IPFC, 1975). The use of antimicrobial agents in aquaculture as in other intensive farming systems to prevent or treat diseases of fish is not uncommon and sometimes

necessary (Ervik *et al.,* 1994; FDA, 2002; Chitamanat *et al.,* 2006). A major distinction with warm-blooded animals, such as cattle and pigs, is the apparent accumulation and persistence of veterinary drug residues in cold-blooded fish, which is as a consequence of a slower metabolism (Harberer, 2002; Chafer-Pericas *et al.,* 2010a; 2010b; Stevens *et al.,* 2012). In addition, the therapeutic doses in aquatic animals are usually much higher than in warm-blooded and terrestrial animals because of a lower bioavailability of the drugs (JEC, 1990; Samanidou and Evaggelopoupou, 2007; USFDA, 2010). Therefore, drug residues are expected in edible tissues of fish (Calderon *et al.,* 1996; Xu *et al.,* 1996; ASM, 2012; Kjeldgaard *et al.,* 2012).

In the United States all legal drugs used in aquaculture must be approved by the FDA's (Food and Drug Administration) Center for Veterinary Medicine (CVM). Standard information on approved drug uses is presented in the FDAs 'Green Book' (FDA, 1998). This information includes drug ingredients, manufacturers, species route of delivery, dose form, withdrawal times, tolerances, and uses by species including dose rates and limitations. The most common route of delivery of these legal antibiotics to fish occurs through mixing with specially-formulated feed (Benbrook, 2001; FDA, 2001). However, fish do not metabolize antibiotics effectively and will pass them largely unused back into the environment in the feces (Goldburg and Triplett, 1977).

Table 2.2 provides an overview of drugs approved by the FDA for specific aquaculture uses.

The reasons for antibiotic uses in aquaculture include the following:

i. For reproduction and growth
ii. For the treatment and prevention of diseases
iii. To control parasites and
iv. For immobilization/tranquilisation

Table 2.2: List of antibiotics used in aquaculture.

Group	Compound	Comments
Sulphonamide	Sulphamerazine Sulphaimidine Sulfadimethoxine[1]	Bacteriostatic agents with broad-spectrum activity against furunculosis in salmonids (trout and salmon)
Potentiated sulphonamide	Co-trimazine/sulfatrim[1,2,3] (combination of trimethoprim and sulfadiazine)	Used for treat diseases in salmon and trout (furunculosis, vibriosis and enteric red mouth).
Tetracyclines	Chlortetracycline Oxytetracycline[1,2.3,4]	Wide use in aquaculture (effective against several fish pathogens and is relatively cheap. Used in salmon, trout, turbot and shrimp farming. Approved for prevention of "red tail" in lobsters in Canada
Penicillins (beta-lactams)	Ampicillin[4] Amoxycillin[2,4]	Used to treat furunculosis in salmon and rainbow trout fry syndrome (RTFS) in Europe.
	Benzylpenicillin[3]	Used for yellow tail and seabream in Japan
Quinolones	Ciprofloxacin	Used in shrimp farms in Asia
	Enrofloxacin	Used in shrimp farms in Asia
	Norfloxacin	Used in shrimp farms in Asia
	Oxolinic acid[2,3,4]	
	Penfloxacin	
	Flumequine[3,4]	
	Sarafloxacin	EU MRL 150 ug/kg fish muscle
Nitrofurans	Furazolidone	Broad spectrum antimicrobial agent. Used in shrimp farms in Asia. Used discouraged as it is a potential carcinogen
Macrolides	Erythromycin[4]	
	Spiramycin	
Aminoglycosides	Gentamycin	
Other antibiotics	Chloramphenicol	Residues in foods may cause aplastic anaemia in man[5]. Used banned in the EU union
	Florfenicol[1,3,4]	Used to treat RTFS and furunculosis in salmon
	Thiamephenicol[4]	
	Tiamulin	
	Nalidixic acid	
	Milozacin	

1 = use permitted in Canada (http://www.syndel.com/msds/canada_approved.htm; 2 = licensed for use in the UK (Alderman and Hastingss, 1998); 3 = use permitted in Norway (Alderman and Hastingss, 1998); 4 = use permitted in Japan (Okamotor, 1992); 5 = Tan (1999).

2.11.1 Problems with antibiotic use in aquaculture

The increasing problems associated with infectious diseases in fish, the limited number of drugs available for treatment and prevention of these diseases, and the rapid increase in resistance to these antibiotics represent a major challenge for fish production (ASM, 1994). Reports by Alderman *et al.* (2001) have raised legitimate public concerns about the safety of antibiotic drug usage in aquaculture. Establishing the exact level of drug use and potential dangers is difficult due to lack of data, fragmented laws, regulations, jurisdictions and interpretation of reporting guidelines (OTA, 1998) and large quantities of aquaculture imports from countries where legal and illegal drug use may escape documentation (FDA, 2001; Benbrook, 2002). In Nigeria, there are no public sources of aquaculture drug use data. Regulators and scientists from agencies such as the National Agency for Food Drug Administration and Control, (NAFDAC), Federal Department of Livestock and Pest Control (FDL&PC), Ministry of Agriculture (MOA), and Ministry of Environment (MOE) who work on fish drug use and the environmental impact of aquaculture have no solid basis to estimate antibiotic drug use.

2.11.2 Contemporary lessons on antibiotic uses in aquaculture

Given the absence or lack of attention to data collection, current disease reporting and aquaculture product quality surveillance systems, it would be likely that short term spikes in antibiotic use would not be detected by regulatory officials and public health experts. This blind-spot in knowledge of antibiotics drug use in aquaculture is serious because the odds of resistant bacteria emerging and spreading beyond farm production sites are greatest during periods of intensive use (Mellon *et al.,* 2001; Gorbal, 2001; Macmillan, 2001; Schmidt, 2000; Benbrook, 2002).

Given the prevalence of disease and the documented need to treat early to prevent the disease from getting established in a pond, it is believed that several fish farmers treat multiple times in many ponds during the course of a year (Mellon *et al.,* 2001).

2.11.3 Use and abuse of antibiotics in fish farming

The fish farming industry is increasingly becoming relevant in the past few years with an explosive growth. This has made the fish farming industry one of the most profitable business ventures globally (Fortt and Buschmann, 2007). Added to these are the sanitary problems of fish farming, mostly the intensive use of a wide range of antibiotics in the production of farmed fish. This practice does not only affect the farmed fish, but also the wildlife that inhabit the surrounding areas of the cages and the human population which ultimately consumes these substances.

The development of fish farming globally has been characterized by the use of antibiotics (Bravo *et al.,* 2005). For instance, in Chile, the use of flumequine, a fluoroquinolone used exclusively for aquaculture purposes, increased in use from 30 to 100 tons between 1998 and 2002 (Bravo *et al.,* 2005; Cabello *et al.,* 2004). The increased use of this drug coincided with the increase in salmon fish production during the same period from 258, 000 to 494, 000 tons (Bravo *et al.,* 2005), this situation made Chile the second largest salmon farming nation in the world after Norway (Soto *et al.,* 2004).

The existence of bacterial diseases with financial implications on fish farming, have required the use of antibiotics for the prevention and treatment of these diseases (Bravo *et al.,* 2005; Cabello, 2003; 2004). As reported globally, the excessive use of antibiotics in fish farming has had negative consequences on animal and human health (Cabello 2004; Bjorlundum, 1990;

Wolf, 2004; Grave *et al.,* 1999), beside the damage to the environment (Cabello 2004; Buschmann *et al.,* 2006; Hektoen *et al.,* 1995; Samuelsen *et al.,* 1992).

Countries such as Norway has completely eliminated the wide spread use of antibiotics for the prevention of diseases in aquaculture (Grave *et al.,* 1999). This was achieved with the purpose of minimizing if not eliminating the harmful effects of the antibiotics and restricting the amount of these substances, while completely eliminating the use of quinolones that have grave effect on human health (Buschmann *et al.,* 2006). In addition to these measures, the regulations also control the use of antibiotics for treatment of diseased fish, through epidemiological control enforced by governmental institutions such as public health and wildlife agencies (Wolf, 2004; Grave *et al.,* 1999; Sorum, 2006).

Furthermore, in other parts of the world, fish farms and cages are surrounded by a wide range of marine ecosystems where artisan farmers live and work, gathering fish and wild fish for human consumption (Buschmann *et al.,* 2006). Some of these wild species feed from the left over pellets and feces of the farmed fish found at the bottom of the ponds or cages (Soto and Norambuena, 2004; Sorum, 2006; Coyne *et al.,* 1997; Kerry *et al.,* 1996). In this way a variety of wildlife are involuntarily being exposed to antibiotics potentially present in the food as well as the feces. Therefore, this antibiotic residue contaminates the flesh of this category of fish which have the potential of passing into the human consumers digestive tracts (Bjorlund, 1990; Samuelsen*et al.,* 1992; Coyne *et al.,* 1997).

The intestinal tracts of healthy fish harbor a microbiota that have been investigated by several authors due to its assumed importance in digestion; nutrition and disease control (Navarrete *et al.* 2008). Studies in germ-free zebrafish have revealed that gut microbiota could be involved in

important processes such as epithelial proliferation, the promotion of nutrient metabolism and innate immune responses (Bates *et al.* 2006). An important aspect of these results was the specificity of the host response, which depends on the bacterial species that colonize the digestive tract (Rawls *et al.* 2004). Possible modifications in gastrointestinal microbiota due to antibiotic treatment could alter this presumably beneficial host-microbiota relationship. Therefore, understanding how antibacterial compounds modify the gastrointestinal microbiota of farmed fish could help to improve the management of hatcheries to reduce antibiotic use and enhance the safety of farmed fish. However, few studies have focused on determining the effects of antibiotic treatment on the microbial ecology of the fish gut. In general, published studies have mainly focused on describing the frequency of antibiotic resistance during and after the use of antibiotics (Kerry *et al.* 1997), the susceptibility of fish pathogens isolated from fish and fish farms to antibiotics (Giraud *et al.,* 2006; Kerry *et al.,* 1997; Akinbowale *et al.,* 2007) and molecular determinants of antibiotic resistance (Miranda *et al.,* 2003; Miranda and Zemelman, 2002; Navarrete *et al.,* 2008; Jacobs and Chenia, 2007; Schnabel and Jones, 1999; Huber *et al.,* 2004; Romero and Navarrete, 2006; Ringø *et al.,* 2004; Jacobs and Chenia 2007; Miranda *et al.* 2003). The presence of bacteria harboring resistance determinants could be related to the widespread use of antibiotics in aquaculture (Cabello, 2006).

The effects of fish farming on bacterial density, biomass, and community structure and the possible connections between these factors and antibiotic resistance have been investigated. In several studies; however, the overall results are still controversial. When an antibiotic treatment begins (usually via medicated feed) the gut microbiota and environmental bacteria can come in

contact with the antibiotics present in fish farm and hatchery wastes (Austin and Al-Zahrani 1988; DePaola *et al.,* 1995; Miranda and Zemelman, 2002).

However, if the use of a given antibiotic in aquaculture is discontinued or if the frequency with which it is used is reduced, it appears likely that the advantage of possessing resistance to the antibiotic would disappear. However, it should be considered that almost 99% of environmental microbes are uncultivable (Amann *et al.,* 1995). A comprehensive understanding of the influence of anthropogenic activities on the environment, for example, the long-term effects of antibiotic use on aquaculture facilities and their surroundings, will require more studies using molecular approaches.

The use of antimicrobial drugs in aquaculture has well-known positive effects on the control of bacterial infections; however, several side effects that affect both the fish and the environment are associated with excessive use. If one takes into account that 70 to 80% of the antibiotics administered to fish as medicated, pelleted feed are released into the aquatic environment via urinary and fecal excretion and/or as unused medicated food (Martinsen and Horsberg, 1995; Smith and Samuelsen, 1996; Samuelsen, 2006), it is not hard to imagine the extent to which antibiotics can affect the aquatic habitat. The effects of antibiotics on the environment are mainly due to the overuse of these drugs by the aquaculture industry and the presence of drug residues in fish products (Saglam and Yonar, 2009).

Unfortunately, there are only a few studies that analyze the side effects of antibiotic use on fish themselves. There is evidence that some antibiotics can induce nephrotoxicity (Hentschel *et al.,*

2005), but the most well documented side effect is immunomodulation (Rijkers *et al.,* 1981; Grondel *et al.,* 1985; Wishkovsky *et al.,* 1987; Tafalla *et al.,* 2002).

It is common to dose farmed fish with antibiotics in their food to protect against disease and, as fish pens are typically located in rivers or lakes, the toxic feces, uneaten food pellets, dead fish, and antibiotic residues are distributed over the entire ecosystem. This is an important point because, as active compounds, antibiotics must be considered potential environmental micropollutants and thus a source of artificial environmental stress for fish. Furthermore, in addition to the presence of the antibiotic itself, the existence of its degradation products is also a cause for concern. Unfortunately, no studies of these problems have been published, and only limited information is available regarding the presence, or absence, of antibiotics (mainly oxytetracycline, florfenicol and oxolinic acid) in the sediment around fish farm nets in a few countries (Carson *et al.,* 2002; Lalumera *et al.,* 2004; Pouliquen *et al.,* 2007).

The use of drugs in aquaculture has different legal constraints in each country, and supervision of compliance with regulations also varies between different countries. It is imperative to investigate how long different antibiotics can persist in the water and whether this time period is sufficient to alter the wellbeing of the farmed fish. This invaluable information will help determine the conditions that promote fish health and survival (Pouliquen *et al.,* 2009).

2.11.4 Worries on antibiotic use in aquaculture

The issue of antibiotic residues present in sea food has been exacerbated by reports of nitrofurans and chloramphenicol detected in shrimps (Johnston and Santillo, 2002). Nitrofurans are drugs whose use in food producing animals and fish is banned in European countries,

because of health concerns, and a possible increase in cancer risk in humans (Johnston and Santillo, 2002). Chloramphenicol a broad spectrum antibiotic has been associated with aplastic anaemia in humans and it has been banned for use in food producing animals and fish (EC, 2002a; 2002b; 2002c). Furthermore, antibiotic residues in cultured fish have increased lately (Willis *et al.,* 1999).

As aquaculture has developed, a range of fish and shellfish diseases have been encountered that have led to major economic losses leading to failure of the viable industry in some parts of the world (Stevens *et al.,* 2012). This has led to the increased use of veterinary drugs and vaccines in intensive production systems to combat disease in favored fish (Stevens *et al.,* 2012).

Antibiotics are used in aquaculture worldwide to treat infections caused by a variety of bacterial pathogens of fish including *Aeromonas hydrophila, Aeromonas salmonocida, Edwardsiella tarda, Pastuerella piscidida, Vibrio anguillarum, Vibrio salomonicida* and *Yersinia ruckeri*. Antibiotics are commonly used as in-feed medications or surface coated onto feed pellets and dispensed in water (Stevens *et al.,* 2012). Although aquaculture production is growing rapidly, disease prevention and treatment practices are not standardized or regulated. When antibiotics are used in aquaculture, the drugs typically remain in the open environment and may flow out of production facilities into open water ways or sewage systems, where they may also interact with other environmental contaminants (Erik, 1994; Gorbach, 2001). The impact of all these factors on the emergence of antibiotic resistance are unknown, because antibiotics typically used in aquaculture are also important in treating human disease and infection (ASM, 1994; MacMillan, 2001; Schmidt, 2000).

Recent studies have demonstrated an increase in resistant bacteria in the intestine of fish receiving antibiotic drugs (ASM, 1994; Ervik, 1994; Rhodes *et al.,* 2000; Benbrook, 2001). The studies have provided information towards understanding the mechanism through which antibiotic resistant bacteria emerging from fish farms can move to humans (Schmidt, 2000; Macmillan, 2001).

There are some barriers such as water and temperature that can lead to the spread of many common bacteria pathogens from fish to humans. When people come contact with fish that has been aggressively treated with antibiotics, they can serve as source(s) of multiple antibiotic resistant bacteria. Because several highly complex environmental scenarios that can facilitate the transfer of bacterial resistance from fish to human can emerge from this interaction (Weber *et al.,* 1994). Attention to these worries on antimicrobial resistant bacteria in aquaculture system is intensifying. Few drugs are approved for use in aquaculture, and the lack of choice increases the potential for abuse and misuse by fish farmers who sometimes use legal drugs, illegal drugs or 'general purpose' chemicals that are not labeled for drug use, or use approved drugs in manner that deviates from the labeled instructions (FDA, 1998).

The identification of antibiotics in cultured fishes has led to extensive coverage of the issue in the international media (Graslund and Bengtsson, 2002). Various other articles have dealt with the issue of antibiotic resistance (Sorum, 1999; 2000). Some articles in the literature provide quantitative data in antibiotic usage in aquaculture (GESAMP, 1997; Johnston *et al.,* 1998).

The accelerated growth of aquaculture has resulted in a series of harmful effects to human health as a result of the spread of antimicrobial resistant pathogens. The widespread, unrestricted use of antibiotics in the aquaculture industry to prevent bacterial infections has

been reported to be a major cause leading to the release of antimicrobial residues in aquatic environment (Samanidous and Evaggelopoulou, 2007). Admittedly, this has resulted in the emergence of antimicrobial resistant bacteria in aquaculture environment and also increased antibiotic resistant pathogens in fish, as well as the transfer of resistance determinants to human pathogens. More so, the use of large amounts of antibiotics is believed to be responsible for the presence of residual antibiotics in fish tissue and fish products (Samanidou and Evaggelopoulou, 2007).

Cultured fish is characterized by mass medication, and in many cases through water-borne exposure (Burka *et al.,* 1997; Alderman and Hastingss, 1998). In ensuring quality and safety assurance, as in HACCP, GMP and GVP systems, veterinarians, farmers and food producers require tools to guarantee wholesome and safe food from fish (ASM, 2012). Antibiotic residues can allow pathogenic bacteria to proliferate (ASM, 2012). Antibiotics used as growth promoters or to treat disease in fishes can eventually end up in tissue. Antibiotics can potentially have a paradoxical effect that would increase the risk of food borne illness (ASM, 2012; Kjeldgaard *et al.,* 2012). But the ultimate solution to the problem of antibiotics in fish tissue may be harder to achieve. The obvious solution is to eliminate the use of antibiotics as growth promoters and closely monitor its use in fish farming/aquaculture (ASM, 2012; Kjeldgaard *et al.,* 2012).

The addition of selected antibiotics to feeds has been shown to be effective in promoting the growth of livestock. Most livestock can achieve maximum weight in shorter time at less cost when specific amounts of antibiotics have been incorporated into their diet. In addition, benefits have been achieved in the preservation of certain processed and fresh foods by the utilization of antibiotics for the extension of shelf and storage life (WHO, 1963).

In spite of these well-defined non-medical uses of antibiotics, the practice has not been universally adopted. Various reports have expressed concern about the potential hazards of indiscriminate use of antibiotics to public health. Over the years, various food additives have presented health threats and give cause to regret their use. With one-fifth of the world's food supply lost to spoilage and large numbers of the world's population currently underfed, it is clearly the duty of food producers to take advantage of all technological advances for increasing the food supply. Besides, they must be fully aware of the possible hazards, such as antibiotic hypersensitivity, toxicity and the production of bacterial resistance (WHO, 1963).

2.11.5 Issue of antibiotic residues in aquatic products

As in other animal production sectors, antibiotics are used during production and processing in aquaculture to mainly prevent (prophylactic) and treat (therapeutic) bacterial diseases (Arthur *et al.,* 2000). Antibiotics have also been recommended and used as disinfectants in fish handling, and this practice has proved to be ineffective (FAO, 1997). Besides, antibiotics have not been used responsibly in aquaculture. In several situations, control of their use has not provided a proper assurance of the prevention of risks to human (FAO, 2002). The Food and Agriculture Organization (FAO), World Health Organization (WHO), Office International des Epizootics (OIE) and several national governments have raised great concern on the irresponsible use of antibiotics in all production sectors, with particular concern for the potential risks to public health. In addition, several governments around the globe have introduced, changed or tightened national regulations on the use of antibiotics in general and within the aquaculture sector (FAO, 2000).

Most farmers have been using chemicals and antibiotics to treat fish pathogen which leads to the creation of antibiotic resistant pathogens and undesired residues in the fish and the

environment (Chitmanat *et al.,* 2006). Food safety is currently a global concern with the increased detection of antibiotic residues in exported fish products globally (Roberts, 1989; Smith *et al.,* 1994).

2.12 Status of Antibiotics in Foods and Feedstuffs

It is believed that certain antibiotics are capable of stimulating the growth rate of variety of animals including fish. Thus it is a global practice in many parts of the world to feed fish on antibiotic-containing rations. However, there is existence of different opinions as to which antibiotics are the most useful and at what levels they are to be used (WHO, 1963). It is generally accepted that poultry with the exception of ducks and geese, swine, beef cattle, lambs and fish benefit most from the use of antibiotics in feeds (Gustafson and Bowen, 1997; McEwan and Ferdoka-Cray, 2002).

In the early 1950s, the antibiotic levels used in feeds varied from 5-10 ppm. Since then there has been increase in the level of antibiotics in feed. There are reports of levels of 100-200 ppm and even higher. These levels are better suited for the prevention or treatment of infection other than for the purpose of growth promotion (Gustafson and Bowen, 1997; Witte, 1998). The most frequently used antibiotics are the chloramphenicol, penicillin, tetracycline (chlortetracycline, oxytetracycline). Nevertheless, the above named antibiotics are used to a much greater extent than all others combined. However, in some countries other antibiotics such as bacitracin, erythromycin, streptomycin and tylosin are being used (Neu, 1992; USGAO, 1999; WHO, 1997).

Feeding of growth-promoting levels of antibiotics of the order of 20 ppm does not result in detectable levels in meat. However, increased levels of 100-200 ppm result in detectable levels

in the tissues (Troy *et al.,* 2002). There is enough evidence of the efficacy of selected antibiotics in increasing the shelf or storage life of several kinds of processed and fresh foods. However, these procedures have not been fully accepted globally because of the potential public health hazards associated with the practice (IOM, 1989; Gummow, 2003). Presently antibiotics permitted for use in food preservation in certain countries include oxytetracycline, nisin and nystatin (AHI, 2000).

2.13 Common Foods Processed with Antibiotics

Fish and poultry are the two most important foods processed with antibiotics, for which tetracyclines are used (Mellon *et al.,* 2001; McEwan and Ferdoka-Cray, 2002; Call *et al.,* 2008). The method of poultry preservation using antibiotics is by immersing them in slush ice containing the antibiotic. Antibiotics may be applied to fish from the ice in the ship's hold and the antibiotic is being released as the ice melts. More so, tetracyclines may be applied to fish fillets by dipping them in a solution of the antibiotic.

2.13.1 Antibiotic residues in foods

The preservation of fish and poultry using antibiotics results in antibiotic residues in the foods. The chlortetracycline or oxytetracycline residues in poultry and fish are rarely higher than 7 and 5 – 10 ppm respectively. However, most of the antibiotic is degraded or destroyed during cooking processes (McEwan and Ferdoka-Cray, 2002; Call *et al.,* 2008).

2.13.2 Antibiotic residues in aquaculture products

As in other animal production, antibiotics are used in aquaculture during production and processing, mainly to prevent (prophylactic) and treat (therapeutic use) bacterial diseases

(Khachatourians, 1998; USGAO, 1999; AHI, 2000). Antibiotics have also been recommended as disinfectant in fish handling, but this practice has proved to be ineffective (FAO, 2003). Antibiotics have not always been used in a responsible manner in aquaculture and, in a number of reported situations; control of the use of antibiotics has not provided a proper assurance of the prevention of risks to humans (WHO, 1997; Kummerer, 2004).

Food and Agricultural Organization (FAO), the World Health Organization (WHO), the Organization Epizootic Internationale (OIE) and a number of national governments have already raised the issue of irresponsible use of antibiotics in all production sectors, with particular concern for the potential risks to public health. Many governments around the world have introduced, changed or tightened national regulations on the use of antibiotics, in general and within the aquaculture sector (WHO, 1997; AHI, 2000; Call *et al.,* 2008).

2.14 Antibiotics Residues and Public Health Concerns in Aquaculture

Concerns about the consequences of antibiotic use on public health have encouraged the development of strict regulations controlling the use of antibiotics and have led to only a few antibiotics being licensed for use in aquaculture. The use of antibiotics in aquaculture depends on the local regulations, which vary widely between different countries. The emerging view that antibiotics should be used with more care has prompted more strict regulations on the use of antibiotics in aquaculture and on the presence of antibiotic residues in aquaculture products. In some countries, regulations on the use of antibiotics are strict, and only a few antibiotics are licensed for use in aquaculture. However, a large proportion of global aquaculture production takes place in countries that have permissive regulations. Furthermore, many governments have set obligatory Maximum Residue Levels (MRLs) for aquaculture products.

The public health risk associated with antimicrobial residues depends on the quantity of the antimicrobial encountered or consumed, i.e. the exposure. The presence of antibiotic residues in foods of animal origin is a potential health threat (Duran and Marshall, 2005). Antibiotic residues in aquaculture present a risk to public health. It is important to consider that most antibiotics used for treating infections are produced by environmental micro-organisms, meaning that the genes for antibiotic resistance must also have emerged in non-clinical/artificial habitats (Martínez 2008). A better understanding of the ecological role of antibiotics and antibiotic resistance in natural environments may eventually help to predict and counteract the emergence and evolution of resistance and the problem of drug residues.

When consumed directly by humans as medicine, antibiotics may cause adverse side-effects, but these can generally be avoided through adhering to the recommended dose and duration of therapy. However, when antibiotics are unintentionally ingested as residues in food, the amount ingested cannot be quantified or monitored and may cause direct health concerns, such as aplastic anaemia, which is associated with chloramphenicol (IOM, 1989). These direct effects pose significant risks to human health. In addition, the unintentional consumption of antibiotics (through consumption of food animal products) results in the development of antibiotic resistance by pathogenic bacteria considered to be one of the most serious risks to human health at the global level (Byarugaba, 2004; 2005). Antibiotic resistance arises when bacteria acquire resistance to one or more of the antibiotics to which they were formerly susceptible, and when that resistance eventually makes the antibiotics ineffective in treating specific microbial diseases in humans (Quintiliani and Courvalin, 1995; Aarestrup *et al.,* 2001; Byarugaba, 2010).

Antibiotics may cause adverse side effects when consumed directly by humans as medicine, but these are avoided when the recommended dose and duration of therapy are strictly adhered to (FAO, 2002). When antibiotics are unintentionally ingested as residues in food, the amount ingested cannot be quantified or monitored and may lead to direct health concerns, such as aplastic anaemia, which is linked to chloramphenicol. These direct effects pose significant risks to human health (Alderman and Hastingss, 1998).

Furthermore, the unintentional consumption of antibiotics is reported to result in the development of antibiotic resistance in bacteria that are pathogenic to humans, and this is an important problem that is yet to receive adequate attention (Boinpally and Jiang, 2007; Schmidt, 2000; Horinek, 2009). The development of antibiotic resistance by pathogenic organism is reported to be one of the most serious risks to human health globally (USFDA, 2010; Shears, 2001). Antibiotic resistance by pathogenic organisms arises when bacteria acquire resistance to one or more of the antibiotics to which they were formerly susceptible, and when that resistance eventually makes the antibiotics ineffective in treating specific microbial diseases in human (Shears, 2001).

The recognition of the risks associated with the direct and indirect effects on human health of both active and passive consumption of antibiotics has led to bans on the use of certain antibiotics in animal food production (particularly those antibiotics for which no safe residue levels can be determined) and to the establishment of maximum residue limits (MRLs) for those with known risks (Angulo and Griffin, 2000; USFDA, 1997; FAO, 2002).

Bacteria constitute the most economically significant group of pathogenic agents. Bacterial diseases are responsible for heavy mortalities in both cultured and wild fishes globally and most

of the causative micro-organisms are naturally occurring opportunist pathogens which invade the tissue of a fish host susceptible to infection (Roberts, 1989; Muniruzzaman and Chowdhury, 2004). Bacterial infection of fish and fish products may influence human health either directly or indirectly by inducing disease or indirectly through the effect on the human beings by residues of antimicrobial agents used to treat such infections in fishes (Inglis *et al.,* 1993).

Habitual use of antibiotics can lead to problems with bacterial resistance and with unacceptable residues in aquaculture products and the environment (Muniruzzaman and Chowdhury, 2004). The resistant bacterial strains could have a negative impact on therapy of fish diseases or human diseases and environment of the fish farms (Smith *et al.,* 1994). The predominant concerns on microbial resistance due to the use of antibiotics are the possible impact on human health resulting from the emergence of drug-resistant bacteria in animals. This is caused by the prolonged use of low level of antibiotics in animal feed and allows antibiotic residues to persist in sediments for a long time (Sorum *et al.,* 1992; Muniruzzaman and Chowdhury, 2004). This situation has actually brought man to new medical dilemma. In addition to the potential effects on human health, inefficiencies in antibiotic treatment of fish illnesses can lead to significant economic losses.

2.15 Prospects for future Advancement in Preventing Antibiotic Residues in Fish

Antibiotics are of natural or synthetic origin. The drugs have the capacity to kill or inhibit the growth of micro-organisms (Chafer-Pericas *et al.,* 2010b). The presence of antibiotic residues in the environment and especially in farmed fish is increasing due to their extensive use in animal feed, in order to treat animal diseases and promote animal growth (FAO, 2002). Sulfonamides

and tetracyclines are two classes of antibiotic broadly used in aquaculture to treat infection in fish (Romero *et al.,* 2007).

However, these compounds come into contact with humans by means of the food chain (Kan and Meijer, 2007). It is imperative to pay adequate attention to this contamination due to the potential hazards associated with their presence in edible tissues. Thus, there is a global concern about the consumption of aquatic food containing low levels of antibiotics. It is therefore important to secure fish safety by ensuring that fish should not be harvested for food before the antibiotic withdrawal period following treatment (post –treatment period after which the fish is considered drug free) is over (Chafer-Pericas *et al.,* 2010a). Withdrawal periods ranged between 3 and 42 days, depending on the antibiotic, the species and the water temperature. Therefore, it is essential to establish withdrawal periods for each antibiotic before fish are sold (JEC, 1990).

One problem associated with fish farming is the indiscriminate use of antibiotics and antimicrobial agents in the treatment of fish diseases. Fish are prone to various infections that hamper their reproduction, growth, appearance and welfare. Some of these diseases are potential health hazard to humans. Fish diseases are of grave economic concern especially in farmed fish. Antibiotics have been used to treat human as well as domestic animals, livestock, and aquatic animals such as fish and shrimps for many years to prevent or treat infection. Antibiotic resistance is now becoming a problem in not only human environment, but also animal environments (Dietz *et al.,* 2005; Horinek, 2009).

Recent epidemics of pathogenic diseases and infections have shown that the overuse of antibiotics may be harmful and may need to be regulated (Alderman and Hastings, 1998; Dietz *et al.,* 2005). Micro-organisms also have the potential to be resistant to many types of antibodies

(McPhearson *et al.,* 1991; Akinbowale, 2007). Pathogenic bacteria in aquatic animals have been linked to bacteria in humans and it is believed that there should be a concern for safety of human consumption (Schmidt, 2000; Boinapally and Jiang, 2007).

2.16 Status of Antibiotic Use in Aquaculture Industry in Nigeria

Aquaculture is fast gaining increasing relevance in Nigeria. There has been recent increase in aquaculture in Nigeria to boost animal protein through small and large scale production (Amao *et al.,* 2006; Omole *et al.,* 2006). The growing demand for fish for home consumption and at restaurants due to high dietary trend in increase fish production created protein and low fat content has led to intensification and private investment in aquaculture production in Nigeria (Kehinde *et al.,* 2006; FDF, 2007). Therefore, the current increase in fish production created the challenges of fish health conditions, thereby necessitating the use of antimicrobials.

In Nigeria, the administration of veterinary drugs in food animals including aquaculture is characterized by indiscriminate use without appropriate veterinary supervision, regulation and control to protect consumers (Olatoye and Basiru, 2013). The misuse of veterinary drugs as well as violative residues of antimicrobials in Nigeria livestock products have been reported (Dipeolu and Alonge, 2002, Kabir *et al.,* 2004; Olatoye and Ehinmowo, 2010).

2.17 Premi®Test

Premi®Test is a broad spectrum antimicrobial residue screening test developed to detect a large number of the most widely used antimicrobial substances in fresh fish, meat, meat products, kidney and eggs. The test is based on the inhibition of the growth of *Bacillus stearothermophilus*, a bacterium very sensitive to many antibiotics and sulpha compounds. Commercially available Premi®Test was released to monitor meat from cattle, pigs, poultry and

aquaculture for the presence of compounds of antibacterial residues (Alderman and Hastingss, 1998). This screening test was developed to detect as many as possible veterinary relevant antibacterial compounds within a short period of three hours excluding time needed for sample preparation (Geijp *et al.,* 1998). It is a relatively fast and easy-to-use test suitable for use by meat inspectors and meat processors, and in slaughter houses and the retail trade (Haard, 1995; Geijp *et al.,* 1998). The test consists of an array of tubes containing a test organism immobilized in agar, while the tubes are organized in trays of 10 by 5 tubes.

Drugs of farm animals, when administered either by injection or through feed will lead to residues in the muscles, kidney or liver for a certain period of time (Haard, 1995). Antibiotics are applied as medications, or as growth promoters (Meyer, 1991). Growing concerns over consumer health-related issues are associated with the intake of these residues (Burka *et al.,* 1997). An increase in the number of drug resistant bacteria has led to an increased demand for reliable test methods (Geijp *et al.,* 1998).

The Premi®Test antibiotic screening test is important in the prevention of fish containing antibiotics above the required/legal limits from gaining entrance into the food chain. It is a fast sensitive, reliable, easy-to-use and cost-effective test covering a broad range of antibiotics. Where conventional tests require overnight incubation, Premi®Test gives a reliable result in less than 4 hours, which allows for quick decision to be taken during processing of fish.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Kaduna State is located in the northwestern geographical zone of Nigeria. It lies between Latitudes 09^{o} 02''N and 11^{o} 23''N and Longitude 06^{o} 15''E and 08^{o} 60''E and shares boundaries with Katsina, Kano and Zamfara States to the North, Plateau and Bauchi States to the East, Nasarawa State and the Federal Capital Territory to the South and Niger State to the West. The State covers an area of approximately 45,567 km^2, and has an estimated human population of 6,066,562 people according to the 2006 census figures, it has more than six (6) million people, with three (3) senatorial zones and 23 Local Government Areas (KDSG, 2008).

Climatic conditions in Kaduna State are tropical with well defined wet and dry seasons, the dry windy season and the rainy (wet) seasons. On the average, the State enjoys a rainy season of about five (5) months. There is heavy rainfall in the southern parts of the state like Kafanchan and northern parts like Zaria with an average rainfall of about 1,016 mm (KDSG, 2008).

The State extends from the tropical grassland known as Guinea Savannah to the Sudan Savannah in the North. The grassland is a vast region covering the southern part of the State to about Latitude 1100'' north of the equator. The prevailing vegetation of tall grasses and big trees are of economic importance during both the wet and dry season. Agriculture is the main stay of the economy of Kaduna state with about 80% of the people actively engaged in farming. Another major occupation of the people is animal rearing and poultry farming; however, aquaculture is emerging. The animals reared include cattle, sheep, goats and pigs (KDSG 2008).

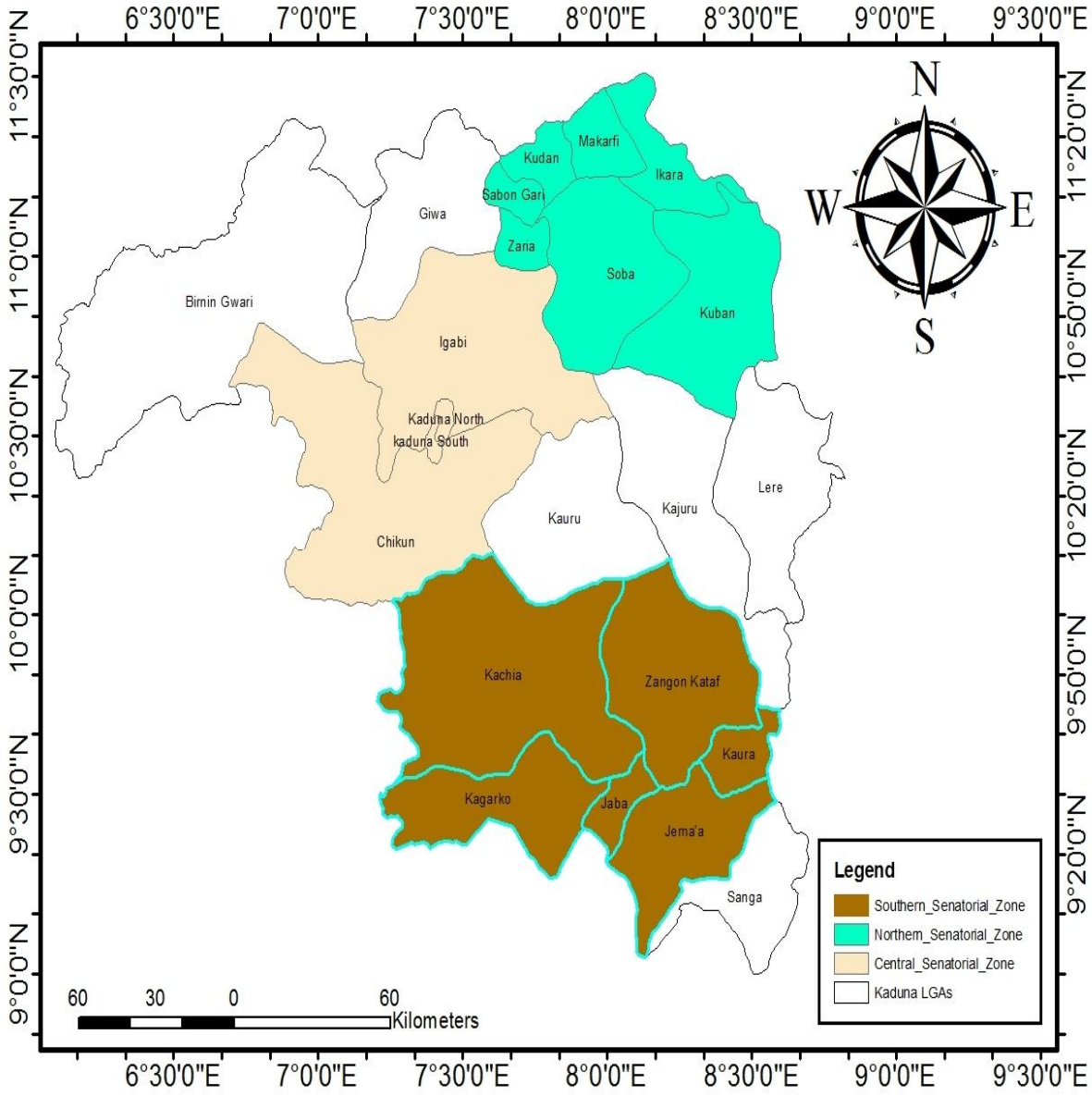


Figure 3.1: Map of Kaduna State showing the three Senatorial Districts and sampled Local Government Areas (Source: Adapted from administrative map of Kaduna)

3.2 Sampling Design and Sample Size

A cross sectional descriptive study was conducted. Sample size estimation for the fish was determined using the formula as described by Thrusfield (2005);

$$n = \frac{z^2 pq}{d^2}$$

Where:

n = sample size

z=desired confidence 1.96

p=prevalence

q=1- p

d=allowable error 5%

3.3 Sampling Technique

A probability sampling procedure using convenience sampling technique was employed for the three senatorial zones, so that each zone is adequately represented in the sampling. The calculated size was 314 fish samples; however, 300 fish were sampled. Ten (10) fish farms were sampled in each of the senatorial zone while a minimum of ten (10) fish between the ages of 3-6 months-old (500 g – 1 Kg) were collected per farm. Fish were sampled from earthen ponds, concrete ponds and plastic tanks.

3.4 Sample Collection

Fish samples were collected from the ponds by conventional techniques, which is by using scoop net and transported to the Department of Veterinary Public Health and Preventive

Medicine Laboratory A.B.U. Zaria in black 50 litre jerry cans (10 fish per 50 litre jerry can) so as to reduce stress.

3.5 Determination of Fish Morphometric Indices

The weights of the fishes were determined by table scale in grams and kilograms, while the total length (length between the head and tip of tail) was taken by measuring tape. The total length was recorded in centimeters.

3.6 Determination of the Presence of Antibiotic Residues in Raised Fish

Live fish samples were screened for antimicrobial drug residues using a microbial inhibition-based broad spectrum antimicrobial residues screening test kit (Premi®Test kit, DSM Netherlands**).** The Premi(R)Test kit was purchased directly from the manufacturer and stored as directed until use.

The principle of the Premi®Test is based on the inhibition of the growth of *Bacillus strearothermophilus*, a micro-organism very sensitive to many antibiotics and sulphonamide residues. A standardized number of spores are imbedded in an agar medium with selected nutrients. When meat fluid is added to the Premi®Test and heated at 64^{o}C, the spores will geminate. The germinated spores will multiply and form an acid when no inhibitory substances are present in sufficient amount (above the detected level) no growth will occur and the colour will remain purple.

Muscle tissues at the dorsum of the fish close to the gills were collected from the fish samples. The fillet (muscle sample) of each individual fish was processed separately so as to reckon with antimicrbial-positive fish. Each sample was tested following the manufacturer's instructions as follows: Muscle tissues were incubated at 64.5^{o}C for 15 min to facilitate the production of

liquor. After incubation, liquor was collected by compressing the fish muscle in a juicer. Until testing, liquors were stored at a temperature of -20^{o}C.

The Premi®Test consists of an array of tubes containing a test organism immobilized in agar. The tubes were organized in trays of ten by five tubes. One hundred micro litres of the fish fillet liquor was added to each test tube. The test tubes were pre-incubated at ambient temperature for 15 to 20 minutes. Incubation was continued at 64^{o}C for approximately three hours and the color was monitored as follows:

i. A clear colour changing from purple to yellow indicated that the antibiotic present are below the Premi®Test detection limits.

ii. A purple colour indicated the presence of antibiotics at or above the detection limit of the test.

The very moment the blank test samples started changing from purple into yellow, all test tubes were read.

- Hands were thoroughly washed before starting the test procedures
- The required number of ampoules were cut out without damaging the aluminum foil of the ampoules and then the foils were carefully removed.
- Approximately 2 cm^{3} of muscle tissues at the dorsum of the fish close to the gills was cut and a meat press used to extract approximately 250 ul of meat fluid per sample.
- A new disposable tip on the syringe for each sample was used.
- 100 ul of the fluid was pipetted onto the agar in the ampoule and then allowed to stand at room temperature for 20 minutes for pre-incubation.
- The meat juice was flushed away by gently filling and emptying the test ampoules twice

with demineralized (demi) water.

- The ampoules were washed with demineralized water only.
- The last wash water was carefully removed from the test ampoule.
- The test ampoules were incubated in the Premi®Test incubator and water bath (64°C + 0.5°C).

3.7 Determination of the Pattern and Conditions of Antimicrobial Drug use and the Level of Awareness amongst key Stakeholders in Fish Production

The pattern of use of antibiotics in fish farms and the level of public awareness on the deleterious

effects of antibiotic residues in fish and fish by-products were studied using a structured questionnaire which was pretested and adjusted to correct limitations identified during pretesting (Appendix I). The questionnaire gathered information on demographic characteristics of respondents involved in fish production including fish farmers, veterinarians, veterinary assistants and other respondents. Information on fish production and management practices, fish health and drug usage were obtained. Additional questions were also asked, when necessary to shed more light on some issues raised during questionnaire administration.

3.8 Data Analyses

The data generated were analyzed by descriptive statistics and cross tabulation using SPSS (version 17). Chi-square test was used to determine association between categorical variables at a significant level of $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Prevalence of Antimicrobial Drug Residues in Fishes

Out of the 300 fishes tested, antibiotic residue were detected in 221 (73.70 %) with overall prevalence of 63 %, 74 % and 84 % in Kaduna North, Central and South senatorial districts respectively. There was also a statistically significant association ($\chi^2 = 11.38$, df = 2, $p<0.05$) between senatorial district and detection of antibiotic residue in the fishes (Table 4.1). All the fishes were table size ready for market with a mean weight and total length of 0.66 ± 0.01 kg (range 0.2 – 1.2 kg) and 35.06 ± 0.34 cm (range 18-50 cm) respectively.

4.2 Pattern of Antimicrobial Drug use in Fish Farms in Kaduna State

Out of the 100 questionnaires distributed to investigate the pattern of use of antimicrobial drugs in fish farms in the study area, 88 of the questionnaires were retrieved and evaluated.

4.2.1 Demographic characteristics

The demographic characteristics of the fish farmers indicated that majority (81.8%) were between the ages of 20-59, while 5.70 % and 12.5 % respectively were less than 20 years and 60 years and above (Table 4.2). About 66.67 % of the farmers were males and 33.33 % were females. Regarding the highest level of education attained by the farmers, majority (68.60 %) had tertiary education, 27.90 % had secondary education, 2.30 % had primary education while 1.16 % had no formal western education. The primary occupation of the respondents included; civil servants (27.27 %), farmers (25.00 %), business men/women (35.23 %) and artisans 12.50 % (Table 4.2).

4.2.2 Farm management practices

Intensive system of farming was practiced by 89.53 % of the farmers while 8.14 % and 2.33 % practiced semi-intensive and extensive farming systems respectively. Commercial purpose was the reason for fish farming among 90.70 % of the fish farmers, subsistence by 4.65 % and both commercial and subsistence by 2.31 % (Table 4.3).

Catfish (*Clarias gariepinus*) was the major (72, 84.70 %) fish species kept by the farmers. *Tilapia* was kept by only a small proportion (4, 4.71 %) while 9 (10.59 %) kept both catfish and *Tilapia*. Forty-nine (56.98 %) kept only fish in the farms, 27 (3.40 %) kept both fish and poultry, while 10 (11.63 %) kept fish and other livestock. A major percentage of the farmers; 76.74 % and 67.01 % respectively had other fish farms surrounding theirs and do not allow other animals access into their fish ponds (Table 4.3).

Seventy one (88.75 %) of the farmers had between 1-10 fish ponds in their farms with 55 (63.95 %) having a fish holding capacity of between 500-2000 fishes. Concrete, earthen and plastic ponds were commonly used by 28 (32.56 %), 13 (15.12 %) and 20 (23.26 %) respectively of the farmers for rearing fishes while 25 (29.07 %) use a combination of the three different types of ponds (Table 4.3).

4.3 Antimicrobial Drug use by Farmers in Fish Production

Fifty one (59.30 %) of the fish farmers reported they had 2-5 outbreaks of disease in their farms in the last 6 months, while 7 (8.14 %) had not encountered any disease outbreak in the last 6 months. The farmers (7, 8.33 %) reported that when they encounter such episodes of disease, it usually occurs frequently and 60.27 % (44) said it affects less than half of the fishes.

Seventy nine (91.86 %) of the farmers have used antibiotics/drugs in the fish ponds for disease treatment (86.75 %), growth promotion (3.61 %), prophylaxis (4.82 %) and another 4.82 % for the triad of treatment, prophylaxis and growth promotion (Table 4.4). Tetracycline was the most commonly (63.41 %) used antibiotic in the fish ponds by the farmers and they applied antibiotics/drugs once weekly (7.32 %), twice weekly (14.69 %), once monthly (43.90 %), yearly (15.85 %) and occasionally (18.29 %) into the fish ponds (Table 4.4).

From the results obtained, veterinarians, animal scientists, livestock superintendents, fellow farmers and the farmers were responsible for recommending (11.90 %, 16.67 %, 2.38 %, 1.19 % and 67.86 %, respectively) and administering (27 %, 39.29 %, 8.33 %, 13.10 % and 11.90 %, respectively) the antimicrobial drugs (Table 4.5).

Water was reported as the major (66.00 %) route of administering for these antibiotics/drugs, followed by feed (14.00 %) and a combination of water and feed by 20 % of the farmers (Figure 4.1).

Table 4.1: Prevalence of antimicrobial drug residues among fish samples from the three senatorial districts of Kaduna State, Nigeria.

Senatorial district	Total number of fish samples	Samples positive for drug residue (%)
North	100	63
Central	100	74
South	100	84
Total	**300**	**73.70**

$\chi^2 = 11.38$, df=2, $p<0.05$

Table 4.2: Relationship between farm management practices and detection of antimicrobial drug residues in farmed fish from Kaduna State, Nigeria.

Management Practices	Number of Positive Farms (%)
Water Quantity	
Adequate	22 (81.48)
Low	1 (3.70)
Moderate	4 (14.81)
Water Quality	
Low	13 (48.12)
Good	14 (51.85)
Feed Type	
Pelleted	17 (62.96)
Extruded	9 (33.33)
Pelleted and Extruded	1 (3.70)
Feeding Characteristic	
Overfeeding	12 (44.44)
Normal	14 (51.85)
Underfeeding	1 (3.70)
Stocking Density	
High	16 (59.26)
Adequate	8 (29.63)
Low	1 (3.70)
Overstocking	2 (7.41)

Table 4.3: Demographic characteristics of the stakeholders in the fish industry in Kaduna State, Nigeria.

Demographic variables	Frequency (%)
Age	
< 20	5 (5.70)
20-59	72 (81.80)
60 and above	11 (12.50)
Sex	58 (66.67)
Males	29 (33.33)
Females	
Educational status	2 (2.30)
Primary Education	24 (27.90)
Secondary Education	59 (68.60)
Tertiary Education	1 (1.16)
None	
Primary Occupation	24 (27.27)
Civil Servant	22 (25.00)
Farmer	31 (35.23)
Business man/woman	11 (12.50)
Artisan	

Table 4.4: Farm management practices among fish farmers in Kaduna State, Nigeria.

Variables	Frequency (%)
Farming system	
Intensive	77 (89.53)
Semi-intensive	7 (8.14)
Extensive	2 (2.33)
Purpose of fish farming	
Commercial	78 (90.70)
Subsistence	4 (4.65)
Both commercial and subsistence	2 (2.31)
Fish species kept	
Tilapia	4 (4.71)
Catfish	72 (84.70)
Mixed	9 (10.59)
Type of livestock kept	
Fish only	49 (56.98)
Fish and poultry	27 (31.40)
Fish and other livestock	10 (11.63)
Presence of other fish farms around the farm	66 (76.74)
Yes	20 (23.26)
No	
Access by other animals into fish pond	
Yes	28 (32.94)
No	57 (67.01)
No of ponds in the farm	
1-10	71 (88.75)
11-20	6 (7.50)
>20	3 (3.75)
Pond type	
Concrete	28 (32.56)
Earthen	13 (15.12)
Plastic/fibre tanks	20 (23.26)
Combination of the above	25 (29.07)
Pond holding capacity	
< 500	20 (23.26)
500-2000	55 (63.95)
2001-4000	9 (10.47)
>4000	2 (2.33)
Marking outlets	
Hotels and restaurants	4 (4.71)
Markets	35 (41.18)
Households	7 (8.21)
Combination of the above	39 (45.88)

Table 4.5: Pattern of disease outbreak and antimicrobial drug use in fish farms in Kaduna State, Nigeria.

Variables	Frequency (%)
Disease episode in farm in the last 6 months	
No episode	28 (32.56)
2-5	51 (59.30)
Never	7 (8.14)
Frequency of disease outbreak	
Frequently	7 (8.33)
Occasionally	53 (63.10)
Rarely	16 (19.05)
Never	8 (9.52)
Population of fish affected	
All	3 (4.11)
Majority	8 (1.10)
Half	18 (24.66)
Less than half	44 (60.27)
Ever used antibiotic/drug in fish ponds	
Yes	79 (91.86)
No	7 (8.14)
Type of antibiotic/drug used	
Tetracycline	52 (86.75)
Sulphonamides	2 (2.44)
Other (Fish salt, perflox)	28 (34.15)
Purpose of antibiotic/drug use	
Disease treatment	72 (86.75)
Promote growth	3 (3.61)
Prophylaxis	4 (4.82)
All of the above	4 (4.82)
Frequency of antibiotic/drug use	
Once weekly	6 (7.32)
Twice weekly	12 (14.63)
Once a month	36 (43.90)
Yearly	13 (15.85)
Occasionally	15 (18.29)

Table 4.6: Personnel responsible for recommending and administering antimicrobial drugs for fish farmers in Kaduna State, Nigeria.

Variables	Frequency (%)
Who recommends the antibiotic/drug to be used?	
Veterinarian	23 (27.00)
Animal scientist	33 (39.29)
Livestock superintendent	7 (8.33)
Fellow fish farmer	11 (13.10)
Self	10 (11.90)
Who administers the antibiotic/drug?	
Veterinarian	10 (11.90)
Animal scientist	14 (16.67)
Livestock superintendent	2 (2.38)
Fellow fish farmer	1 (1.19)
Self	57 (67.86)

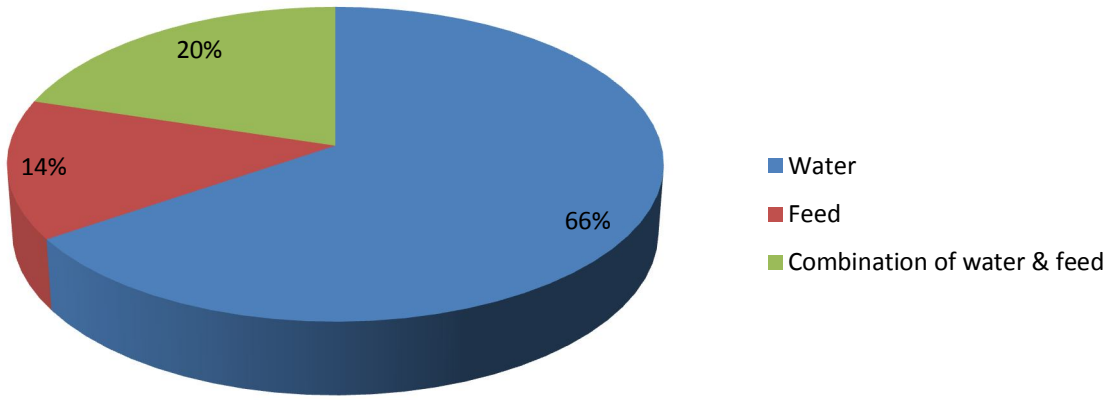


Figure 4.1: Common routes of antimicrobial drug administration by farmers in fish farms in Kaduna State, Nigeria.

4.4 Awareness on Antimicrobial Drug Residue in Food and its Potential Dangers

Majority (67.00%) of the fish farmers were not aware nor have they heard of antibiotic/drug residue in food (Figure 4.2). Twenty seven per cent each of the respondents heard of drug residue from veterinarians, friends and print media, 13 % got this information from the school environment while 3 % each got this information from fellow farmers and the electronic media (Figure 4.3). There was no statistically significant association ($p > 0.05$) between sex of the respondents ($\chi^2 = 0.563$, $p = 0.755$), primary occupation of the respondents (Fisher's exact test $p = 0.649$) and awareness of drug residue (Table 4.7).

Sixty four per cent of the respondents were not also aware of the dangers associated with antimicrobial residue while 36% were aware (Figure 4.4). Sixteen (48.48 %), 8 (24.24 %) and 9 (27.27 %) of the respondents that knew the dangers, reported drug resistance and hypersensitivity and a combination of drug resistance and hypersensitivity respectively as the dangers associated with consumption of antibiotics residues in food (Table 4.8).

Majority (58.62 %) of the respondents were not sure if antibiotics/drug residues can be ingested with fish, 31 (35.63 %) were sure while 5 (5.75 %) said it can't be consumed with fish. Twenty six (30.23 %), 7 (8.14 %) and 53 (61.63 %) of the respondents think, don't think and not sure respectively that drug residue in fish is a serious health problem (Table 4.8). Also, 28 (35 %), 19 (23.75 %), 30 (37.50 %) and 3 (3.75 %) of the respondents sell out their fishes for consumption during treatment with antibiotics, a few days after treatment with antibiotics, more than one week after treatment with antibiotics and a combination of the above (Table 4.8).

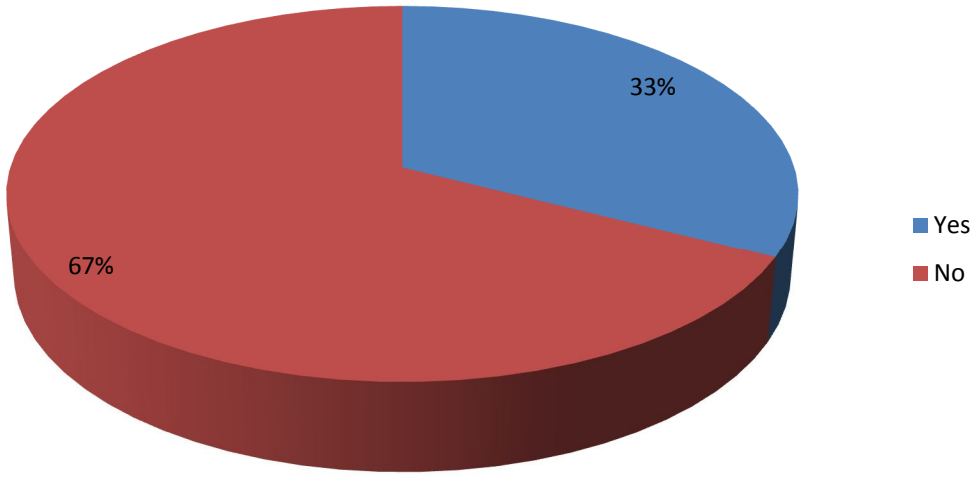


Figure 4.2: Awareness of antimicrobial drug residues among fish farmers in Kaduna State, Nigeria.

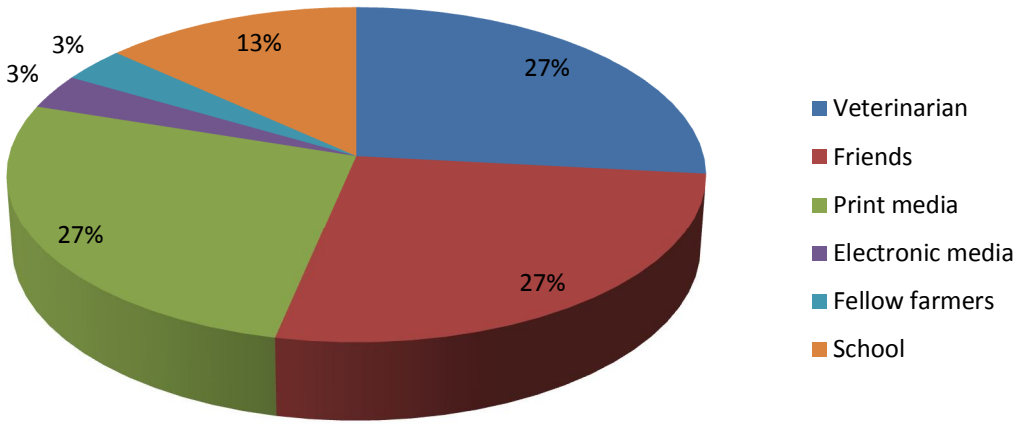


Figure 4.3: Respondents source of awareness on antimicrobial drug residues in fish farms in Kaduna State, Nigeria.

Table 4.7: Relationship between awareness of antimicrobial drug residues among fish farmers and demographic characteristics

Demographic variables	Aware	Not aware	χ^2 value	*P value*
Sex				
Male	18	39	0.563	0.755
Female	10	19		
Age				
< 20	0	5		
20-59	25	46		
60 and above	3	7		
Level of Education				
None	0	1		
Primary	1	1		
Secondary	2	21		
Tertiary	25	33		
Primary Occupation				0.649*
Civil servant	9	15		
Farmer	8	14		
Business man/woman	7	22		
Artisan	4	7		

*Fishers Exact test

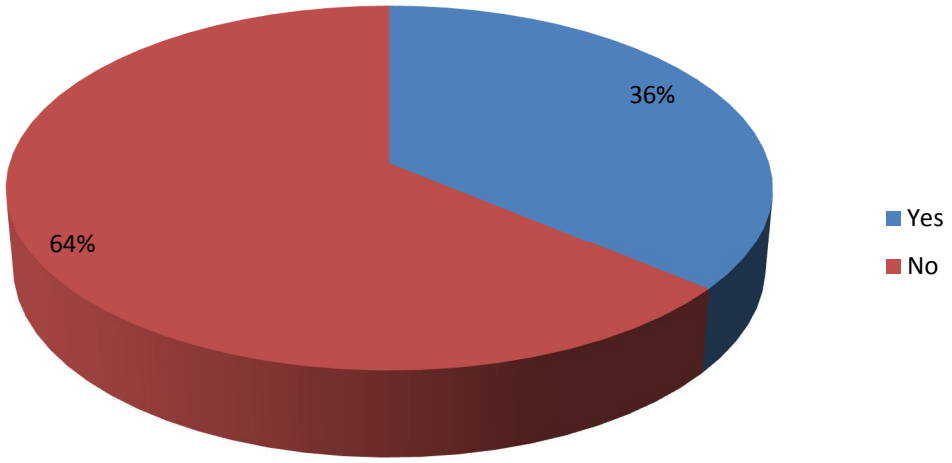


Figure 4.4: Awareness on the dangers associated with antimicrobial drug residues among fish farmers in Kaduna State, Nigeria.

Table 4.8: Beliefs and practices associated with antimicrobial drug residues among fish farmers in Kaduna State, Nigeria

Variables	Frequency (%)
Dangers associated with consumption of antibiotic residue in food	
Drug resistance	16 (48.48)
Hypersensitivity reactions	8 (24.24)
Combination of the above	9 (27.27)
Can one ingest antibiotic/drug via consumption of fishes?	
Yes	31 (35.63)
No	5 (5.75)
Not sure	51 (58.62)
Do you think antibiotic/drug residue in fish is a serious health problem?	
Yes	26 (30.23)
No	7 (8.14)
Not sure	53 (61.63)
How long before you sell you fishes after treatment with antibiotic?	
During treatment	28 (35.00)
Few days after treatment	19 (23.75)
More than one week after treatment	30 (37.50)
Combination of the above	3 (3.75)

CHAPTER FIVE

DISCUSSION

This study was aimed at the assessment of antimicrobial drug use and the occurrence of residues in farmed fish in Kaduna State, Nigeria. In the present study, the overall prevalence of antimicrobial drug residues from farmed fish in Kaduna State was 73.70% from the 300 fish samples screened, with the prevalence of 63%, 74% and 84% from Kaduna North, Kaduna Central and Kaduna South respectively. This shows that antibiotic residues are present in farmed fish in Kaduna State. The overall prevalence obtained in this study is higher than 52.5% reported by Olatoye and Basiru (2013) in Ibadan. Olatoye and Basiru (2013) screened only catfish (*Clarias gariepinus*) from farms and restaurants. In this present study both catfish and *Tilapia* were screened and all were from farms. In both studies, Premi$^{(R)}$ Test was used for the screening, but Olatoye and Basiru (2013) used the high performance liquid chromatography (HPLC) to specifically screen for tetracycline.

The presence of antimicrobial residues in farmed fish in the present study concur with other reports of antimicrobial residues in food animal production in Nigeria (Dipeolu and Alonge, 2002; Kabir *et al.,* 2004; Fagbamila *et al.,* 2010; Ezenduka *et al.,* 2011; Olatoye and Saraye, 2012) and other countries (Okerman *et al.,* 2004; Stead *et al.,* 2004; Bagumire *et al.,* 2010; Chafer-Pericas *et al.,* 2010). It is assumed that the high prevalence of antimicrobial drug residues observed in this study may be due to the indiscriminate use of these drugs by fish farmers (Choo, 1995), and also the non-observance of drug withdrawal period after treatment and before the fish are sold to consumers as observed in the questionnaire responses of the farmers in the present study and reported by Nisha (2008).

Besides, a low prevalence of antimicrobial drug residue was obtained from Kaduna North as compared to that obtained from other senatorial districts. It is presumed the presence of a Veterinary Teaching Hospital and a Veterinary Clinic in Kaduna North may have influenced the use of antimicrobial drugs by farmers in this area. The farmers in this zone may have routinely sought for professional advice and have access to veterinary services.

Furthermore, Kaduna South in the present study had the highest prevalence (84%) of antimicrobial drug residues in farmed fish. The high prevalence recorded in this senatorial zone could be attributed to the non-availability of potable water for their ponds and less number of veterinarians that may have rendered veterinary service to their farms. Thus, farmers resorted in the use of the readily available contaminated water. Consequently, this may lead to increased disease incidence in ponds (Oladosu and Ajasin, 2012) due to increased occurrence of pathogenic organisms (Goldburg and Triplett, 1997; Dietz *et al.,* 2005). Hence, fish farmers are obliged to use antimicrobials frequently. Further still, the low number of veterinarians in Kaduna South may have limited the access of fish farmers to professional veterinary services, which culminated in farm management practices that are not in tandem with recommended standard practices in the use of antimicrobials.

According to Hernandez (2005) and Cabello (2006), several classes of antimicrobial drugs are commonly used in large quantity in fish farming especially in developing countries including Nigeria where the use of these drugs is not regulated. Similarly, when antimicrobials are mixed with fish feed as growth promoters (Svobodova *et al.,* 2006; Kan and Meijer, 2007), residues may be deposited in the meat and consumers may inadvertently consume fish containing these residues, which may lead to changes in their microbial flora (Cerniglia and Kotarski, 1999; Perrin-Guyomard *et al.,* 2001), thus altering the intestinal ecology and favouring the emergence

of resistant microflora (Perrin-Guyomard *et al.,* 2001) and drug allergies such as hypersensitivity reactions and other human hazards (Dayan, 1993; Berends *et al.,* 2001).

In the present study, 66.7% of stakeholders interviewed were male while 33.33% were female. The mean age of fish farmers in this study concur with that reported by Olatoye and Basiru (2013) in Ibadan. However, the average years of fish production experience differ in both studies. The numbers of fish farmers (91.86%) who engaged in routine use of antimicrobial drugs to control and prevent diseases in their ponds were slightly higher than 90% reported by Olatoye and Basiru (2013) in Ibadan.

Aquaculture is a rapidly growing business in Nigeria and Kaduna State (Omole *et al.,* 2006; Amao *et al.,* 2006; Kehinde *et al.,* 2009; Oladosu and Ajasin, 2012). Fish farming is attracting the attention of wide range of people with different educational qualification and occupation. Therefore, the increasing growth of fish farming in Nigeria and Kaduna State could lead to pollution of the aquatic environment with huge amounts of antimicrobial drug and consequently, their residues in fish and fish products (Hirsch *et al.,* 1999).

The most frequently used antimicrobial agent found in this study was oxytetracycline, which is in agreement with previous reports (Choo, 1995; Miriuki *et al.,* 2001; Perrin-Guyomard *et al.,* 2001; Okerman *et al.,* 2004). Oxytetracycline being a broad spectrum and relatively cheap antibiotic is widely used by farmers in both fish and livestock production. Thus it may be right to presume that tetracycline is the most misused antimicrobial drug in aquaculture.

The number of fish farmers (67.80%) practicing self administration of antimicrobials in Kaduna State is higher than those reported in Ibadan (Olatoye and Basiru, 2013). Additionally, observance of drug withdrawal period by farmers was low in Kaduna State. Similarly,

awareness on the potential hazards of antimicrobial drug residues in humans was low. Majority (67 %) of farmers in the present study were ignorant on the presence and public health implication of antimicrobial drug residues in fish (Bagumire *et al.,* 2010). Despite their high educational attainment (68.60 %), most fish farmers in Kaduna State, surprisingly, still engaged in self-medication and misuse of antimicrobial drugs as a routine practice without proper diagnosis. Furthermore, they did not observe withdrawal period after treatment. This may be another reason that may be attributed to the high prevalence obtained in this study. Also, farm management practices as observed in this study can influence the presence of residues in fish and the consequence of resistant pathogens.

Admittedly, the high prevalence of antimicrobial drug residues obtained in this study using Premi$^{(R)}$ Test as the screening method for antimicrobials in fish confirms the suitability and further validate the sensitivity of the test as argued by Okerman *et al*. (2004), Stead *et al*. (2004) and Olatoye and Basiru (2013). Premi$^{(R)}$ Test has been reported to be sensitive for detection of several groups of antimicrobial agents (Olatoye and Basiru, 2013). Further still, Okerman *et al*. (2004) argue that the efficient control of drug residues in foods of animal origin requires good screening tests, which must be cheap, easy to perform, allow for simultaneous analyses of large numbers of samples and give rapid results (Van den Bogaard *et al.,* 1994; Okerman *et al.,* 1998a; 1998b; Stead *et al.,* 2004).

Unfortunately, there are no available information on the survey, monitoring, quantitative analyses of antimicrobial drug residues in farmed fish in Kaduna State. Admittedly, the poor enforcement of regulations on veterinary drug handling and use in Nigeria could perhaps account for the high level of antimicrobial drug residues obtained in farmed fish in Kaduna State.

Therefore, the use of antimicrobial drugs in aquaculture should be a great concern as it may lead to antimicrobial drug resistant pathogens and residues in food chain. The results obtained in the present study have confirmed that there may be misuse of antimicrobial drugs in farmed fish in Kaduna, Nigeria.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has established the following:

i. Antimicrobial drug residues are present in farmed fish in Kaduna State, Nigeria with overall prevalence of 73.70 %.
ii. Kaduna South had the highest prevalence (84 %) of antimicrobial drug residues, followed by Kaduna Central with 74 % and Kaduna North with 63 %.
iii. Fish farmers in Kaduna State are engaged in uncontrolled and unsupervised administration of antimicrobial drugs in aquaculture production (91.86 %).
iv. Awareness on the dangers and public health implication of antimicrobial drug residues in fish is low amongst fish farmers in Kaduna State (63 %), thus may be contributory factor for the high level of antimicrobial drug residue.

6.2 Recommendations

i. Proper use of antimicrobial drug, vaccination, the use of immunostimulants and probiotics for disease control and prevention in aquaculture should be encouraged.
ii. Fish farmers should be educated on the need to always observe withdrawal periods after treatment and before sale of treated fish to consumers.
iii. Government should enforce strict regulation on the use of veterinary drugs in fish farming; educate fish farmers on the dangers inherent in antimicrobial drug residues in

fish in order to safeguard the general populace from hazards associated with the consumption of antimicrobial drug residues.

iv. Routine antimicrobial drug residue screening of fish meant for human consumption is advocated.

v. Use of antimicrobial drugs for the purposes such as growth enhancement, preservation e.t.c. other than treatment should be discouraged.

vi. Further studies to determine the specific antimicrobial drug residues in farmed fish in Kaduna State are required.

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APPENDICES

Appendix 1: Research Open-ended Questionnaire

DEPARTMENT OF PUBLIC HEALTH AND PREVENTIVE MEDICINE, FACULTY OF VETERINARY MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

Research Questionnaire Survey On: Determination of Antibiotic Residues in Fish Farms using Premi®Test, and its Public Health Significance in Kaduna State, Nigeria.

INSTRUCTION: Kindly read and answer the following questions correctly. The information given will be used solely for academic purpose. All information obtained will be treated confidentially. Thank you for the anticipated cooperation and time spared as you kindly fill or tick the appropriate box or space provided.

Serial No.:……

Section A: Biodemographic Data

1. Age
   a) < 20 years [ ]
   b) 20 - -50 years [ ]
   c) 60 years and above [ ]
2. Sex
   a) Male [ ]
   b) Female [ ]
3. Level of education
   a) Tertiary/university [ ]
   b) Secondary [ ]
   c) Primary [ ]
   d) Informal education [ ]
4. Primary occupation
   a) Civil servant [ ]
   b) Farmer [ ]
   c) Business [ ]
   d) Others (specify) ……..
5. how long have you been into fish farming
   a) < 2 years [ ]
   b) 2- 5 years [ ]
   c) 6 – 9 years [ ]
   d) 10 years and above [ ]
6. What is the size/number of your household? ……..persons/people

Section B: Fish Farm Location

7. Senatorial district
   a) Kaduna south [ ]
   b) Kaduna central [ ]
   c) Kaduna north [ ]
8. Local Government Area …….
9. Village/Town ……Longitude …… Latitude …….

Section C: Farm Management

10. Type of fish farm practiced
    a) Fish only [ ]
    b) Fish and poultry [ ]
    c) Fish and other animals [ ]
    d) Others (specify) ……..
11. How many ponds are in your farm? ……..
12. Are there other fish farms around you?
    a) Yes [ ]
    b) No [ ]
13. Do chickens, ducks or any other animals have access to your ponds?
    a) Yes [ ]
    b) No [ ]
14. What are the species of fish in your farm?
    a) Tilapia [ ]
    b) Catfish [ ]
    c) Mixed [ ]
15. Type of fish Ponds in your farm
    a) Concrete tanks [ ]
    b) Earthen ponds [ ]
    c) Wooden trough [ ]
    d) Plastic/fiber tanks [ ]
16. What is your fish pond holding capacity
    a) < 500 [ ]
    b) 500 – 1000 [ ]
    c) 1100 – 1500 [ ]
    d) 1600 – 2000 [ ]
    e) 2100 – 3000 [ ]
    f) 3100 – 4000 [ ]
    g) 4000 and above [ ]
17. Fish farming systems
    a) Intensive [ ]
    b) Semi-intensive [ ]

c) Extensive [ ]

18. Fish production system
    a) Fish seed [ ]
    b) Brood stock [ ]
    c) Partial flow through [ ]
    d) Stagnant green water system [ ]
19. Purpose of fish farming
    a) Commercial [ ]
    b) Subsistence [ ]
    c) Others (specify) …….
20. What is/are your market outlet (s)
    a) Hotels and restaurant [ ]
    b) Market [ ]
    c) Household [ ]
    d) More than any above [ ]
    e) Others (specify) ……

Section D: Diseases and Antibiotics/Drug Administration

21. What is the history of disease in your farm within the last 6 months
    a) Had no episode [ ]
    b) 2 – 5 episode [ ]
    c) Never had disease [ ]
22. What is the nature/description of disease/ailment observed in your fish farm in the last 6 months?
    a) Frequently [ ]
    b) Occasionally [ ]
    c) Rarely [ ]
    d) Never [ ]
23. What population of fish were affected
    a) All [ ]
    b) Majority [ ]
    c) Half [ ]
    d) Less than half [ ]
24. Have you ever used antibiotics/drugs in your fish ponds
    a) Yes [ ]
    b) No [ ]
25. If yes, which antibiotic/drug
    a) Tetracycline's [ ]
    b) Sulphonamide [ ]
    c) Others (specify) ……..
26. For what purpose did you used the antibiotics in your fish ponds

a) To treat disease [ ]
b) To promote fish growth[ ]
c) Others (specify) ……

27. How often do you use antibiotics/drugs in your fish ponds
a) Once a week [ ]
b) Twice a week [ ]
c) Once a month [ ]
d) Yearly [ ]

28. Who diagnose disease(s) in your fish farm
a) Veterinary doctor [ ]
b) Animal scientist [ ]
c) Livestock superintendent [ ]
d) Fellow farmer [ ]
e) Self [ ]
f) Others (specify) ……

29. What are the patterns of antibiotics/drugs use in your fish farm
a) Frequently [ ]
b) Occasionally [ ]
c) Rarely [ ]
d) Never [ ]

30. Who recommends the antibiotics/drug to use in your farm
a) Veterinary doctor [ ]
b) Animal scientist [ ]
c) Livestock superintendent [ ]
d) Fellow farmer [ ]
e) Self [ ]
f) Others (specify) ……

31. Which antibiotic/drug were recommended after disease diagnosis
a) Tetracycline's [ ]
b) Sulphonamide [ ]
c) Others (specify) ……

32. Who administers the antibiotics/drugs
a) Veterinary doctor [ ]
b) Animal scientist [ ]
c) Livestock superintendent [ ]
d) Fellow farmer [ ]
e) Self [ ]
f) Others (specify) ……

33. How was the antibiotics/drugs administered?
a) Water [ ]

b) Feeds [ ]
c) Injection [ ]
d) Others (specify)……

34. Which antibiotic/drug do you use commonly in your fish ponds
    a) Tetracycline's [ ]
    b) Sulphonamide [ ]
    c) Others (specify) ……

Section E: Antibiotics/Drug Residue Effects Awareness

35. Have you ever heard of antibiotic/drug residue before?
    a) Yes [ ]
    b) No [ ]
36. If yes, where?
    a) Media (TV, Radio) [ ]
    b) Newspaper [ ]
    c) Others (specify) ……
37. Are you aware of the dangers associated with antibiotic/drug residues?
    a) Yes [ ]
    b) No [ ]
38. If yes, which of them?
    a) Drug resistance [ ]
    b) Hypersensitivity reaction [ ]
    c) Shock [ ]
    d) Others (specify) …….
39. Do you know any who have experienced any of the option in Q38
    a) Yes [ ]
    b) No [ ]
    c) Not sure [ ]
40. Do you think antibiotics/drug residue in fish is a serious health problem?
    a) Yes [ ]
    b) No [ ]
    c) Not sure [ ]
41. Do you think fish can/could be responsible for any of the option in Q38
    a) Yes [ ]
    b) No [ ]
    c) Not sure [ ]
42. If yes, should the indiscriminate use of antibiotics/drugs in fish ponds be discourage?
    a) Yes [ ]
    b) No [ ]
    c) Can't say [ ]



Appendix II: A picture showing the research sampled fish (*Clarias* spp) after collection.



Appendix III: Fish in a weighing balance before tissue sample was collected.



Appendix IV: Determination of Morpometric indices such as the standard length of fish was measured using a measuring tape.



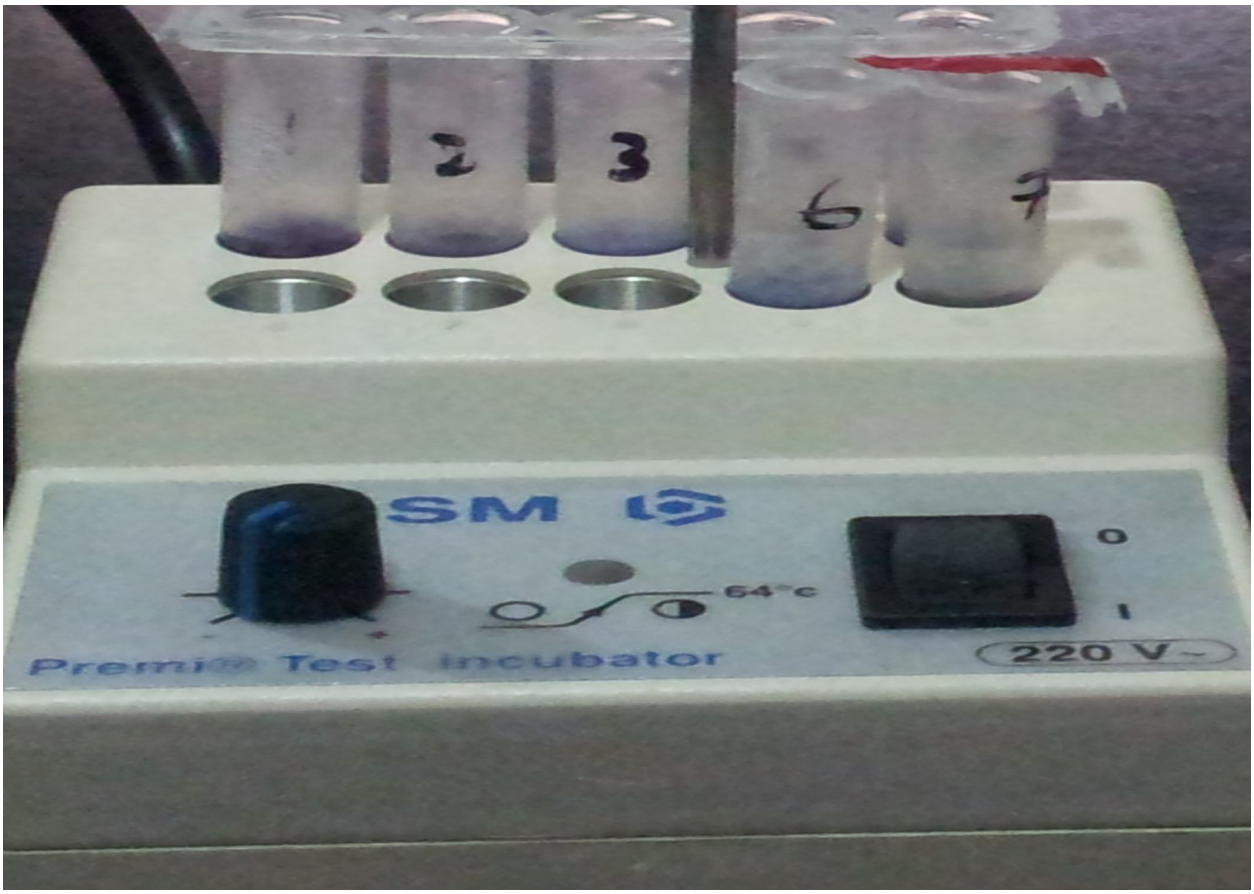
Appendix V: A picture showing the process of tissue sample collection from fish prior to antimicrobial drug residues determination.



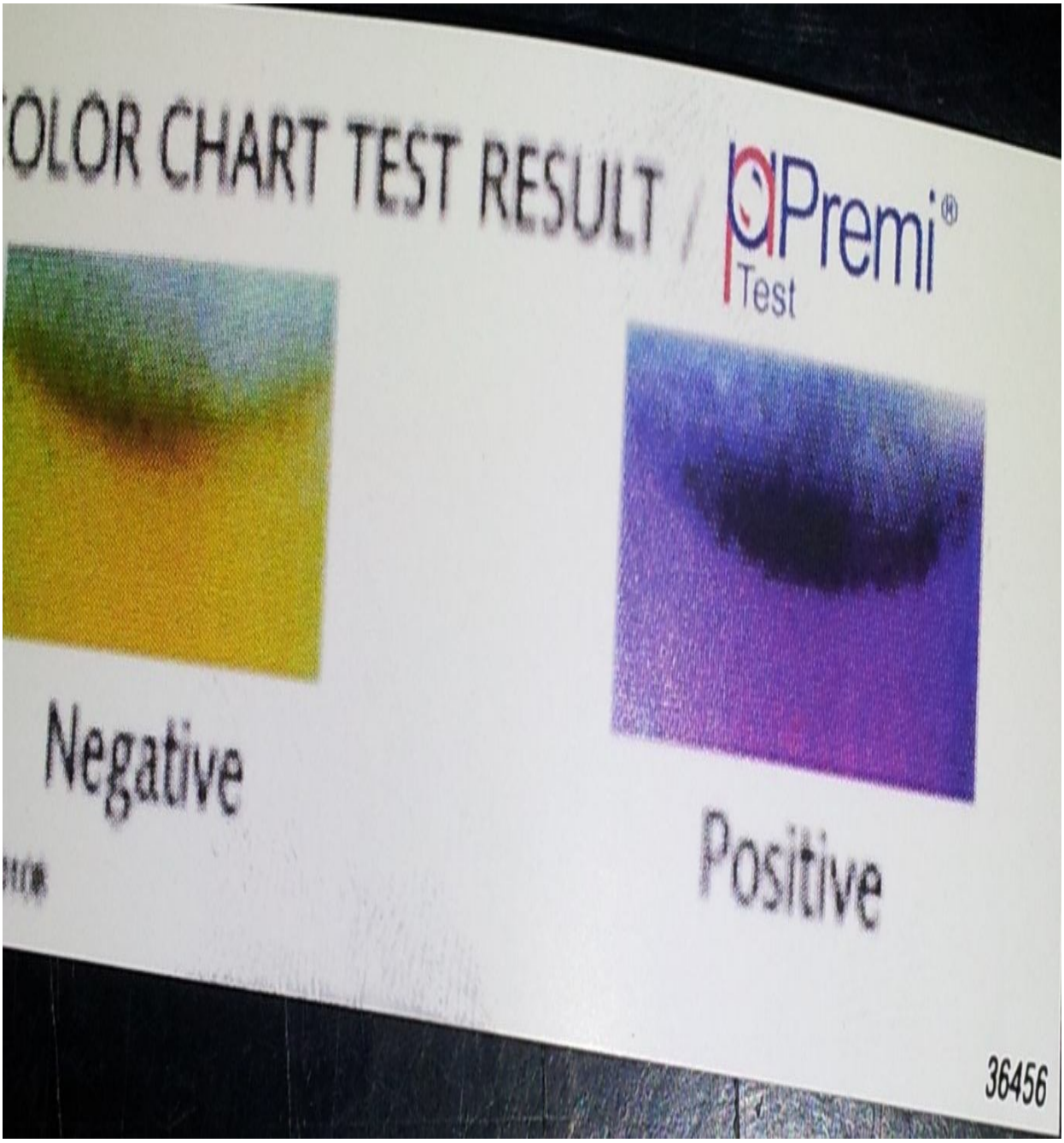
Appendix VI: Broad spectrum antimicrobial screening test (Premi® test) kit obtained from the manufacturers (DMS, Netherlands).



Appendix VII: Muscle tissue juicer compressor and measuring tape used to compress, collect muscle juice as well as measure fish sample



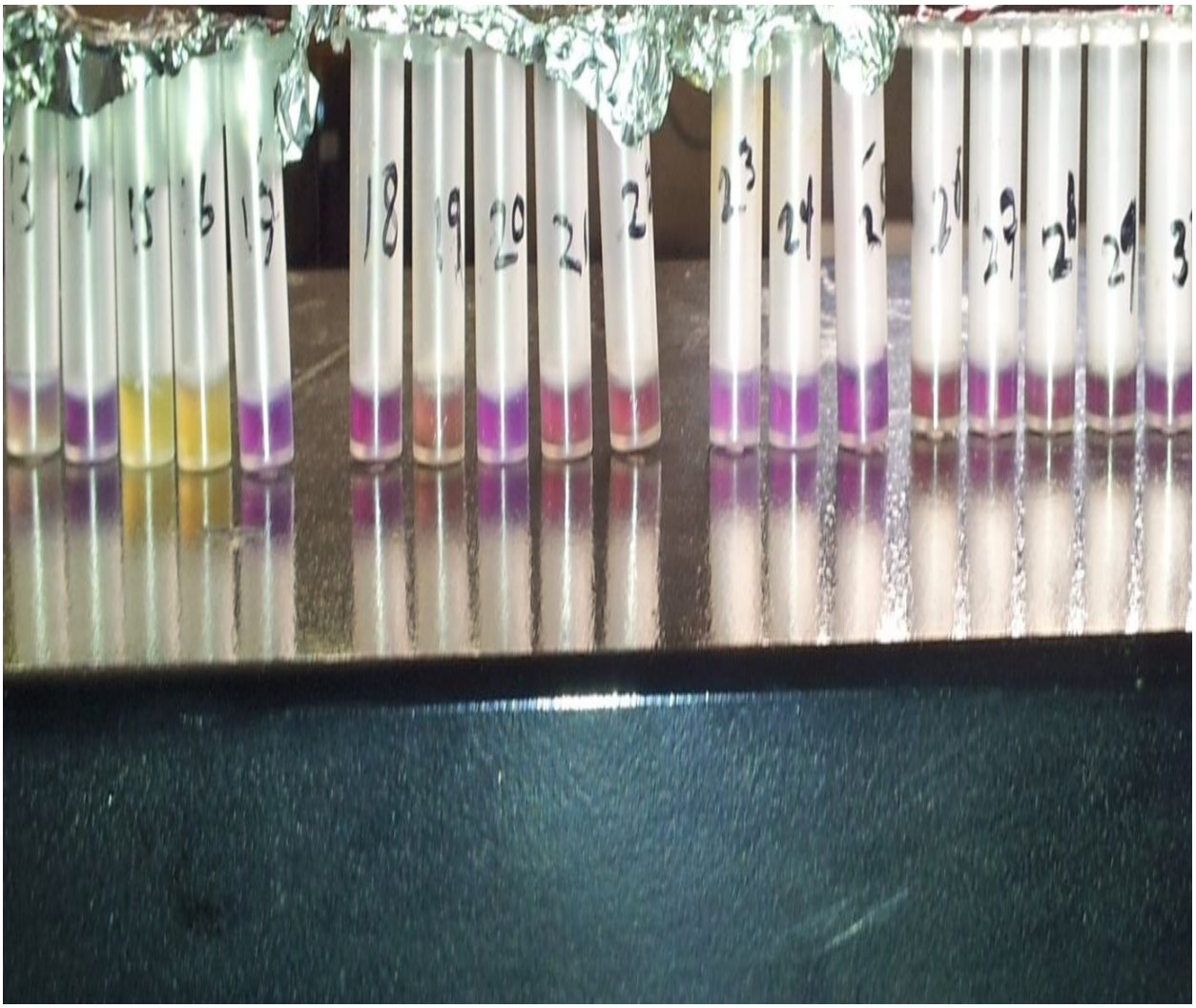
Appendix VIII: Premi$^{(R)}$Test and water bath incubator for the determination of presence of antimicrobial drug residues in fish



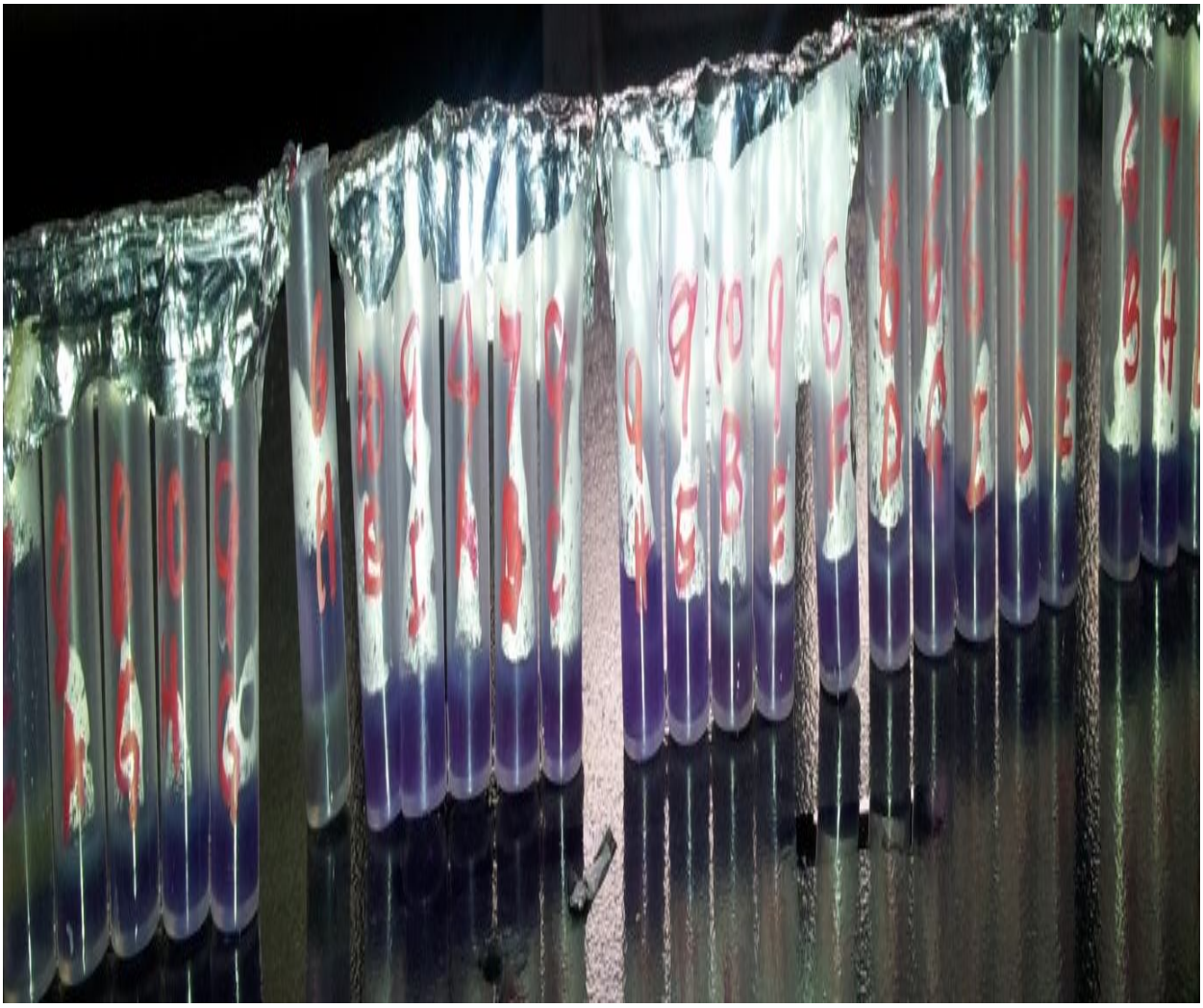
Appendix IX: A Premi$^{(R)}$Test colour chart used for identification of a positive or negative presence of antimicrobial drug residue in fish



Appendix X: Samples of meat juice in test tubes with yellow colour indicating absence of antimicrobial drug residues in fish samples



Appendix XI: Samples of meat juice in test tubes with both yellow and pink colour indicating absence and presence of antimicrobial drug residues in fish samples



Appendix XII: Samples of meat juice in test tubes with pink colour indicating presence of antimicrobial drug residues in fish samples