

**TETRACYCLINE LEVELS IN FARMED CATFISH (*Claris gariepenus*) FROM
SELECTED PONDS IN EDO CENTRAL, EDO STATE, NIGERIA**

BY

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DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY, EDO STATE

DECEMBER, 2022.

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF ANIMAL AND
ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF
BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR
THE AWARD OF DEGREE OF B.Sc. IN ANIMAL AND ENVIRONMENTAL
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DEDICATION

This project work is dedicated to God Almighty, the giver of Life, for his wisdom, knowledge and understanding throughout this project work and throughout my stay in school.

I would also like to dedicate this work to my family for their great support in my academic pursuit.

CERTIFICATION

This is to certify that this research project work was carried out by CELESTINE GRACE EHIREMEN (Miss.) with matriculation number **LSC1704864** under the supervision of Dr. (Mrs.) I. Tongo, of the department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City.

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ABSTRACT

Antibiotics have been used in fish farms for the treatment of bacterial infections. The indiscriminate use of these antibiotics has however led to the issue of antibiotic residue in food animals, which has emerged globally as a public health risk. Lack of information on antibiotic residue in catfish (*C. gariepinus*) in developing countries such as Nigeria is however a major problem. This study was carried out to evaluate the concentration of tetracycline residues in catfish (*C. gariepinus*) from selected ponds in Edo Central, Edo State, Nigeria. Adult Catfish (*C. gariepinus*) samples were obtained from three ponds in Edo Central and analysed for tetracycline residue using the QuEChERS sample method for residue analysis. Questionnaires were also administered to catfish farmers to evaluate their antibiotic use and knowledge of antibiotic administration. The tetracycline residue present in catfish from the sampled ponds were above the maximum residue limits (MRL). Pond 1 had the highest mean tetracycline residue (1.87 ± 0.03 mg/kg) while Pond 2 had the lowest mean tetracycline residue of the sampled ponds (1.84 ± 0.05 mg/kg). All fish pond owners that were administered questionnaires had some level of education. 33.33% had post-secondary education while 66.67% had other forms of education. All respondents to the questionnaire administered tetracycline to catfish. 100% of the correspondent got the antibiotic from veterinary medicine stores and they were all aware of the risk associated with indiscriminate administration of tetracycline to catfish. All the correspondents had also witnessed the outbreak of diseases in their ponds. Human health risk assessment showed the hazard quotient calculated from both ponds lower than 1 which is an indication that consumption of catfish (*C. gariepinus*) from the sampled ponds might not be harmful due to the concentration of tetracycline residue being low in the fish.

CHAPTER ONE

1.0 INTRODUCTION

Aquaculture in Nigeria has mainly focused on the rearing of catfish. Due to the decline in capture fisheries around the world, it has been surmised that fish stocks may have approached or gone beyond levels of maximum sustainable yield (Add reference). This has therefore placed aquaculture as the only viable alternative aimed at increasing fish production in order to meet the protein need of the people (Adewumi and Olaleye, 2010). Catfishes found in the family Claridae make up most community cultivated fishes in Nigeria. Favoured catfish species in Nigeria include *Clarias gariepinus*, *Heterobranchus bidorsalis*, a *Clarias* and *Heterobranchus* hybrid (Heteroclarias) and *C. nigrodigitatus*. *Heterobranchus* sp. is mostly cultivated in the south eastern parts of Nigeria. The catfish industry in Nigeria used to be plagued by inadequate availability of seed used for stocking and feed but tremendous strides were made and the growth of the aquaculture industry in Nigeria has been steadily boosted by a steady rise in catfish cultivation. Since the practice of culturing *Clarias gariepinus* by hyophysation was introduced in Western Nigeria, the procedure has been quickly adopted across the country and has led to the increase in farm-raised catfishes from the 1980s till date (Oresegun *et al.*, 2007). The increase in fish production came with an increase in different diseases such as abscesses, bleeding, sepsis and skin lesions, increase in morbidity and significant economic loss (Miranda *et al.*, 2013). To prevent these deleterious effects, the use of antimicrobial drugs for the treatment and prevention of diseases was adopted. Use of these antibiotics is fundamental in the treatment of disease and maintenance of the well-being of these animals (Rico *et al.*, 2012).

Antibiotics have been described as substances which possess a direct action on bacteria and are subsequently used for the treatment or prevention of infections or infectious disease. Chemically

they may be described as complex molecules which consist of different functional groups within their formulae and may be divided into different classes according to their mechanism of action. Mechanisms by which antibiotics may act against bacteria include inhibition of protein and nucleic acid synthesis, competitive antagonism and alteration of cell membranes (Kummerer, 2009).

Indiscriminate use of antimicrobials has been reported in Nigeria. The most common antimicrobials used in catfish farming include oxytetracycline, tetracycline, chlortetracycline, sulfonamide, ampicillin and penicillin (Ogbonna and Inana, 2018). However, during the administration of these antibiotics, strict adherence to prescription and withdrawal needs are rarely followed (Oyebanji and Oyebisi, 2018). This consequently leads to the presence of antibiotics residues in the muscles and internal organs of these animals. Antibiotics used in aquaculture farming are not only harmful to the animals to which they are administered. Most of the drugs end up in the environment which can lead to occurrence and prevalence of resistant environmental microflora and resistant genes which are then transferred to humans through direct contact with the fish in the form of food or the water in which the antibiotics have been administered. The indiscriminate administration of veterinary drugs in food animals is usually characterized with inappropriate veterinary supervision, control and regulation. There is however a lack of sufficient data on quantitative risks assessment of antimicrobials usage and very little information on the residual levels of antibiotics in farm fish from ponds in Nigeria (Hernandez, 2005).

1.1 AIM AND OBJECTIVES

This study was therefore carried out to evaluate the levels of tetracycline residues in catfish (*Clarias gariepinus*) farmed in selected ponds in Edo Central, Edo State, Nigeria.

1.2 OBJECTIVES

The specific objectives of this study are:

- (i) Assess the levels of tetracycline residue in catfish (*C. gariepinus*) from ponds in Edo Central, Edo State, Nigeria
- (ii) Compare residual levels of tetracycline in *C. gariepinus* to MRL.
- (iii) Qualitatively assess the perception of farmers towards the use and risk of antibiotics used in ponds.
- (iv) Determine the human health risk assessment of tetracycline residue in the fish samples.

CHAPTER TWO

LITERATURE REVIEW

2.1 AQUACULTURE

Fish is an important source of food around the world. It has been considered man's most important source of high-quality protein. Fish has been found to provide about 15% of animal protein consumed worldwide. It is also a specifically important source of protein in regions faced with relative livestock scarcity. FAO reports that fish is responsible for less than 40% animal protein consumed in Nigeria, compared to 10% in North America and Europe, 22% in China and 26% in Asia (Ohen and Abang, 2009). Due to the shortage of protein globally, fish has been widely considered a source of high-quality protein. As a protein source, fish holds a substantial position as a solution to protein deficiency in the country since all food nutrients except carbohydrates have been found to be present in fish. Fish has also been shown to have lower health risks than livestock due to the presence of high cholesterol in meat (Sapkota *et al.*, 2008).

Fish and its products also serve as a source of protein for livestock. They also play an important medicinal role as they replenish the body with vitamins A and D, phosphorus, calcium, amino acids, lysine and sulphur. Due to the attributed benefits of fish consumption, its production has become problematic, as most fishes available are produced from small-scale fisheries in the country (Oresegun *et al.*, 2007). The small-scale fishing industry is however characterized by subsistence farming, inaccessibility to modern fishing gear and remoteness. Hence there is need for the encouragement of more convenient methods of production. To assist in overcoming these problems, the art of fishing has also undergone evolution to become the fastest food producing industry in the world (Kan and Meijer., 2007).

The challenge posed by the gradual increase in the world population and the need to provide nutritional needs by meeting high food demand are important forces in the achievement of the millennium development goals (MDGs). Protein as a food nutrient is very important for a healthy lifestyle and fish has been reported by the Food and Agriculture Organization to be responsible for about 60% of the world's supply of protein. 60% of the developing world also derives over 30% of their annual supply of protein from fish (FAO, 2018). This has led to the development of the aquaculture industry. Global fish production has steadily grown over the years and it serves as a major source of food for humans. The consumption of fish *per capita* in the world which stood at 9.0 kg grew to 20.3 kg in 2016 according to a report by FAO (2018). Global aquaculture production has more than tripled over the past twenty years and the production is expected to double in the next fifteen years as demand for cultured fish increases while wild fisheries approach their biological limits. Worldwide, aquaculture has been responsible for about 46% of total fish food supply in order to meet protein needs of the populace. China, Vietnam, India, Egypt and Bangladesh have been identified as the world leaders in aquaculture supply (Ayinla, 2012).

Aquaculture can be defined as the intentional, rational rearing of fish in a fairly shallow and enclosed body of water where occurring life processes can be monitored and controlled. It is an integral part of the economy of any nation, especially in a time where numerous countries are looking for ways to diversify their economies, in order to remove the dependence on any single product (Donoghue, 2003). It is also an industry responsible for contributing to the food security of a nation, by directly producing fish which can be used for food and indirectly generating employment opportunities, saving foreign exchange and generating foreign exchange through the export of fish and fish products (Adewumi, 2015). However, the high productivity experienced

in the aquaculture industry has been achieved through the employment of intensive farming practices. These practices include growth of huge biomass at high densities of fish per unit of water volume. Practices such as these have led to an increased susceptibility of fish to diseases caused by bacteria, viruses, parasites and fungi (Quesada *et al.*, 2013). Other intensive husbandry practices which have also been implicated in the susceptibility of marine animals in aquaculture to susceptibility to diseases are handling, stripping of broodstock, grading, crowding, antibiotic treatments and starvation. These stressors can also lead to the injury and impaired performance of some marine species such as salmon, which are usually kept in crowded environment, thereby promoting the transmission of infectious pathologies according to studies carried out by Hastein (2004).

The aquaculture industry around the world has continued to grow. FAO (2011) reported that aquaculture production worldwide more than doubled between 1994 and 2004. The report also indicated that Asia accounted for over 80% of the total global production. World aquaculture production in 2010 was estimated to be about 80 million tonnes of which over about 48 million tonnes were produced in China while countries such as Vietnam, India, Indonesia and Thailand made up for the production of 16.6 million tonnes. The aquaculture industry encompasses a wide range of species and methods such as the simple traditional systems whereby fish and other aquatic animals are reared in small ponds and are meant for domestic consumption, and the intensive industrial scale production systems (Rasul and Majumdar, 2017). The development of diseases in the aquaculture industry caused by numerous etiological agents has closely followed the development of aquaculture itself. This has resulted in the resorting of farmers to the use of various remedial measures, chief among them the use of antimicrobials and drugs to control

disease. Studies by Vignesh *et al.* (2011) have shown that frequency of usage of these antibiotics and other chemicals is higher in hatcheries and scientific farms than in traditional farms.

The challenges associated with disease faced by all animal systems is well documented. The best way to solve these problems is often through management practices that are effective. These management practices include management of soil, stock, water, nutrition and most importantly, environment (Ringo *et al.*, 2014). It has also been observed that disease-related problems and the deterioration in environmental quality indicators usually occurs and the final outcome may result in serious economic losses (Balcazar *et al.*, 2006). Therefore, diseases in the aquaculture industry are now regarded as one of the most fundamental, limiting factors (Purivirojkul and Khidprasert, 2009). Studies by Newaj-Fyzul *et al.* (2014) show that the occurrence of infectious diseases in aquaculture systems has remained a significant problem and relevant and proper management approaches should be employed to drastically reduce the effect of pathogens in farmed animals. Recent research conducted has shown that several antimicrobial drugs including antibiotics have been approved and utilised in many countries to treat bacterial diseases affecting aquaculture. However, it has also been observed that the number of antibiotics used in aquaculture systems have been influential in determining the selection of resistant bacteria present in these environments (Ringo *et al.*, 2014).

2.2 AQUACULTURE IN NIGERIA

The annual demand for fish in Nigeria as at 2012 was put at 2.66 million metric tonnes. Of this volume, 1.32 million metric tonnes were supplied. Local production was put at 0.62 million tonnes while 0.7 million tonnes were from important. It is important to note that aquaculture accounted for only 200,000 metric tonnes of the total fish supply. This is an indication of its little contribution to meeting the demands for fish and its products in Nigeria. It has also been

observed that current aquaculture production is a far cry from its potential production (Ayinla, 2012).

Nigeria has been placed as the African country with the largest aquaculture industry, with its production output placed at over 15,000 tonnes per annum in 2007 which makes up about 4% of the nation's total agricultural GDP. However, the FAO reported that about 560,000 tonnes of fish, worth about \$400 million, were estimated to have been imported while the annual domestic fish supply stood at 400,000 tonnes making Nigeria one of the largest fish importers in the developing world (Emokaro *et al.*, 2010). These values indicate a need for fish and its products in Nigeria; a need that has to be met. To solve this dearth, Nigerians must face the abundant, under-utilized in-land water for improved aquaculture and fish production. However, the expansion of the aquaculture industry in Nigeria has been a relatively slow process due to the encountering of major constraints by the private sector fish farmers. These constraints include lack of quality feed and seed (Akolisa and Okonji, 2005).

In most countries where the aquaculture industry is integral to the economy, government agencies have some exertive power in the control of the administration of antibiotics. In Norway for example, the use of antimicrobials requires a veterinarian's prescription, and hence, use in this form is classified as therapeutic. They are usually sold in pharmacies or in feed plants which have been authorized by the Norwegian Medicines Agency. It is also mandatory to report the amount of antibiotics utilised and the records of prescriptions are usually retained. In the United States, antibiotic administration in aquaculture is under the purview of the Food and Drug Administration (FDA). Control and prevention of diseases in these developed countries is usually carried out by controlling stocking densities of the aquaculture population in order to reduce stress (Balcazar *et al.*, 2007).

As it is in most African countries, the most common cultured species of fish in Nigeria include the imported tilapia, the catfish (*Clarias gariepinus*) and the carp (*Clarias lazera* and *Heterobranchus* spp.). The focus of most farmers is on catfish, due to their market value being two to three times that of tilapia. However, current fish production is at levels too considerably low to meet the requirement of protein for the nation's population (Adeleke *et al.*, 2020).

2.2.1 CHALLENGES ASSOCIATED WITH AQUACULTURE IN NIGERIA

Despite an increase in domestic fish production in Nigeria, the country has yet to bridge the gap that exists between fish production and consumption. This is largely due to challenges plaguing the aquaculture industry such as lack of availability of quality fingerling breeds from hatcheries, high cost of fish feed, availability of arable land and water, proper management of disease and enabling environment for the development and marketing of products from the industry (Adedeji and Okocha, 2011).

2.2.1.1 Fish feed

The rising cost of fish feed has always been a major issue facing the development of the aquaculture industry in Nigeria. Feed has been shown to account for over 50% of the operation cost of aquacultures in Nigeria (Fagbenro and Adebayo, 2005). The high cost of local feed production has led to the dependence of farmers on imported manufactured foods. Although interventions such as credit avenues, subsidies and market liberalization have been employed, production output does not still meet up to the demand of the industry (Udoh and Dickson, 2017).

2.2.1.2 Availability of land and water

Land is very important for the development of any industry; however, the availability of land in Nigeria has severely hampered the development of aquaculture in the country. In the

southeastern part of Nigeria, there has been a competitive use of land for other ventures such as farming of food crops, accommodation purposes and commercial activities (Ugwumba and Chukwuji, 2010). Water pollution has also severely affected fisheries production especially in the Niger-Delta region of Nigeria (Ugwumba and Chukwuji, 2010).

2.2.1.3 Diseases

Awareness level of the impacts of disease on the aquaculture industry is quite low. Although *C. gariepinus* is highly resistant to diseases and is highly valued, there is need for research on diseases the species is vulnerable to (Adedeji and Okocha, 2011). The lack of knowledge of diseases affecting aquaculture is also due to the shortage of skilled veterinarians specializing in aquaculture in the country. This issue is further compounded by the lack of laboratories set up to diagnose fish diseases and the inadequate attention given to fisheries and wildlife education in curriculums in Nigerian tertiary institutions (Adedeji and Okocha, 2011). This has left farmers with the utilization of management practices such as hygienic practices, good water quality, farming disease-resistant species and use of antibiotics (Adewumi, 2015).

2.3 ANTIBIOTICS

Antimicrobials can be defined as substances with the capacity to inhibit or kill the growth of microorganisms (Canada-Canada *et al.*, 2009). Since their discovery by Fleming in 1928, they have become essential drugs employed in the improvement of human and animal health and welfare (Prescott, 1997). Antibiotics can be sourced from natural or synthetic origins. To ensure they are effective, antibiotics have to be safe to the host, allow for their use as chemotherapeutic agents for the treatment of infectious diseases caused by bacteria. In addition to their use in human medicine, antibiotics have also been used in food animals and aquaculture, where their

use can be characterized as therapeutic, prophylactic or metaphylactic. Therapeutic use of antibiotics entails the treatment of already established infections (Boxall *et al.*, 2004). Metaphylaxis involves the employment of group-medication procedures that are aimed at treating sick animals while also medicating others in the populace in order to prevent diseases. Prophylactic use of antimicrobial agents means the preventative use of the agents in either individual species in the populace or the group as a whole to prevent the development of infections (Burridge *et al.*, 2010). Antibiotics used for therapeutic purposes are usually administered to aquaculture for short periods of time via the oral route to fish that are housed in the same tanks or cages (Schwartz and Chaslus-Dancla, 2001).

2.3.1 *Antibiotics Use in Aquaculture*

None of the antibiotics developed and used in the aquaculture industry were made solely for aquaculture. However, the number of antimicrobials used in aquaculture are relatively lower than those used in the treatment of other animals such as chickens. Information on the use and regulation of antimicrobials varies widely across countries (Eckburg *et al.*, 2019). The increase in population usually results in the emergence of diseases and just as it is practiced in other types of animal production such as poultry and pigs, antibiotics are also used in the aquaculture industry in the control of bacterial diseases (Done *et al.*, 2015).

Administration of antibiotics usually depends on the health of the fish in the tanks or on the purpose for administration. Antimicrobials may also be used as growth promoters. Increase in feed efficiency which then leads to increase in weight gain may also be achieved by administration of low doses of antimicrobials, a practice nevertheless questioned by practitioners in the scientific community (Marshall and Levy, 2011).

The type of antibiotics being used and the method of administration is somewhat monitored in most developed countries. In several European countries, sulphonamides, oxytetracycline, amoxicillin, first generation quinolones and florfenicol are some of the most used antibiotics. Policies and laws have also been put in place to limit the availability of these antibiotics to aquaculture farmers and the populace in general (Rodgers and Furones, 2009).

2.3.2 Antibiotic Regulations

Norway which is the largest producer of salmon in the world has antimicrobial regulation to control the class and volume of the agents in production. Several surveys carried out in the country indicated that extensive use of antibiotics was not only harmful to aquaculture but also to human health and the environment (Fauconneau, 2002). Also, more hygienic management practices were encouraged and effective vaccines against infections were introduced and this contributed to the reduction in the use of antimicrobial drugs by the Norwegian aquaculture industry. Farmers in Chile also reported incidences of disease, drugs that were prescribed for the treatment of said diseases and the method of administration of the (Burrige *et al.*, 2010). However, unlike the Norwegian aquaculture industry, Chilean fish farmers use a large number of antimicrobial drugs in the production of salmon. Smith (2008) reported that Chilean aquaculture used 200 times more antimicrobials than their Norwegian counterparts. This may be explained by the presence of specific pathogens such as *Piscirickettsia salmonis* in Chile (Burrige *et al.*, 2010).

In the United States, florfenicol, oxytetracycline, sulfadimetozone, sulfamiderazin and ormetoprim have been approved by the Food and Drug Administration for use in aquaculture. Only two antimicrobial drugs; oxytetracycline and florfenicol can be used in Brazil (Padua *et al.*, 2012). this kind of reduced regulation on the antimicrobials is unfavourable and may eventually

lead to the irregular use of other antimicrobial compounds. Use of malachite green dye as an alternative to antimicrobials has been practiced by fish producers in Brazil according to Hashimoto *et al.* (2011). Although the dye has produced excellent results against a wide range of microorganisms, it has also been found to be highly toxic to both aquatic and terrestrial animals and its application has been linked with high risks to human health. Fluoroquinones, however have shown promise in the treatment of diseases in other types of livestock such as chicken, pig and cattle and have been adopted in Brazil and countries in the European Union, China and Japan (Quesada *et al.*, 2013).

Asian countries are among the leading producers of fish globally. They are also some of the largest consumers of antimicrobials, with approximately 36 different antimicrobials reported to have been used in aquaculture (Chuah *et al.*, 2016). These antibiotics are classed into several groups such as tetracyclines, beta-lactams, aminoglycosides, sulphonamides, quinolones, macrolides, amphenicol, colistin and inhibitors such as trimethoprim. This is of great concern due to the frequent use of antimicrobials such as colistin which is also used in human medicine. Restrictions have been put upon the use of colistin due to it being a potent antibiotic and its possible high toxicity when present in high doses in humans. Nevertheless, due to an increase in prevalence of resistant bacteria to other drugs, colistin has instead been reintroduced as a therapeutic agent (Poirel *et al.*, 2012).

Natural or synthetic antibiotics are expected to be safe to the host, permitting their use as agents to treat infectious bacterial diseases. Naylor *et al.* (2005) have reported that it has become common for prophylactic antibiotics to be used to supplement the efficiency of the fishes' immune system in order to prevent bacterial colonization and infection. Fish are usually fed with food containing antibiotics and sometimes the antibiotics are injected into the fish. Food that has

not been consumed and fish faeces which contain antibiotic may spread to the sediment at the bottom of raising pens and diffuse into sediments from where they can be washed to distant sites by currents (Gastalho, 2014). Residual antibiotics may remain in the sediment and exert pressure, thereby altering the microfloral composition of the sediment and selecting for bacteria resistant to antibiotics. Factors that have determined antibiotic resistance in microorganisms in such aquatic environments can potentially be transmitted to other organisms such as those on terrestrial environments by horizontal gene transfer, some of which include animal and human pathogens (Sorum, 2006).

Antibiotics are designed to possess inhibitory and cidal capabilities against pathogenic bacteria. They may act in one of three ways: by disrupting cell membranes, by inhibiting enzyme activity or by disrupting protein or DNA synthesis. Compounds that have been selected for antibiotic activity are selected for use in veterinary and human medicine due to their selective toxicity to ribosomal activity, cell membranes or enzyme activity in prokaryotic cells. Nevertheless, there are still outstanding environmental concerns with widespread antibiotic use. Most antibiotics are stable compounds that are usually not degraded in the body, but stay active long after being excreted from the body (Newaj-Fyzul *et al.*, 2014). Presently, antibiotics considerably contribute to the growing problem of active medical substances circulating in the environment. Globally and particularly in developing countries, there is a large, unchecked usage of copious amounts of antibiotics in the fish industry used to cure infection (Vignesh *et al.*, 2011). The heavy and continuous use of antibiotics in the aquaculture industry poses severe hazards to the health of both aquatic and human ecosystems. Antibiotics utilized in aquaculture for remedial or prophylactic purposes often accumulate in the tissues of aquatic animals. The presence of antimicrobial drug residues in edible tissues can cause changes in the intestinal micro-flora,

allergies, acquisition of drug resistance and toxic effects. Residue of chloramphenicol found in food consumed by humans have been reported to result in aplastic anaemia which leads to severe bone marrow diseases. Antibiotics containing nitrofurans have also been shown to cause cancer and so many other diseases (Heuer *et al.*, 2009).

2.4 TYPES OF ANTIBIOTICS USED IN AQUACULTURE

2.4.1 *Quinolones*

Antibiotics classified in the quinolone class are widely utilised in the aquaculture industry, whereby traces have been found in the water column, aquaculture effluents and sediments surrounding or nearby aquaculture facilities (Canada-Canada *et al.*, 2009). Concentrations of quinolones found in these enclaves are high enough to impose selective pressure on aquatic bacteria. These bacteria can then go on to mix with species of different origins, leading to favourable gene exchange and the spread of antibiotic resistance. Quinolones as antibiotics are broad-spectrum and also possess action against mycobacteria and anaerobes. They carry out their functions by the inhibition of nucleic acid synthesis through disruption of the enzymes, DNA gyrase and topoisomerase IV and by breaking bacterial chromosomes. Oxolinic acid, a widely used quinolone, is used to treat aquaculture that has been infected with gram-negative fish pathogens. Quinolones are usually administered to fish orally (Romero *et al.*, 2012). Mechanisms by which resistance to quinolones occurs, provides mutations in the bacterial genes, as mutations in the genes encoding the topoisomerase IV targets and DNA gyrase, or other genomic alterations modify quinolone or reduce drug accumulation by reducing drug absorption or

increasing drug outflow. Quinolone resistance may also result from plasmid gene uptake from the environment or from other strains with resistance (Tello *et al.*, 2012).

2.4.2 Tetracyclines

Tetracyclines inhibit protein synthesis in bacteria by preventing association of aminoacyl tRNA with the bacterial ribosome (Angulo and Griffin, 2000). Resistance to tetracycline could occur via three mechanisms: limited access of tetracycline to the ribosome, alteration of the ribosome to prevent effective binding of tetracycline and production of tetracycline-inactivating enzymes. Tetracyclines are widely used in both veterinary and human medicine. Tetracycline has been used in the treatment of both internal and external infections in fish. Disorders such as cottonmouth disease, gill disease, body slime and pop-eye have been treated using tetracyclines (Olatoye, 2013). Furushita *et al.* (2003) reported that bacteria from aquaculture fish and those from a clinical facility showed a high similarity for genes encoding for tetracycline resistance, implying that they might have originated from the same source. Tetracycline resistance genes have also been identified in marine sediments from resistance genes in bacterial plasmids from marine sediments that possessed high identity with plasmids or transposons from human pathogens. The same resistance gene profile has been identified in both fish bacteria and human clinical isolates (Santos and Ramos, 2018).

2.4.3 β -Lactams, Fosfomicin, Florfenicol, Colistin, Chloramphenicol and Macrolides

β -lactam antibiotics act by inhibiting the last step in peptidoglycan synthesis by acylating the transpeptidase involved with cross-linking peptides to form peptidoglycans. β -lactams usually target penicillin-binding proteins. This binding interrupts the terminal transpeptidation process to induce loss of viability and lysis (Eckburg *et al.*, 2019). There is also the plasmid-associated

resistance to colistin mediated by the *mcr-1* gene which appears to be another transmissible antibiotic resistance determinant that might have originated from the aquaculture industry (Tomova *et al.*, 2015). Although the administration of chloramphenicol has been banned in most countries, the substance and drugs containing the compound has been used to treat the growth of gram-negative anaerobic bacteria (Shalaby *et al.*, 2006).

2.5 EFFECT OF ANTIBIOTICS ON ENVIRONMENTAL BACTERIA

Upon the start of an antibiotic treatment, the gut microflora of the fish and environmental bacteria may come in contact with the antibiotics present in the fish farm. Oxytetracycline is one of the most commonly used antimicrobial drug and it has been found to be poorly absorbed through the intestinal tract of fish and it also has to be administered at a high dosage (100-150 mg per kg fish per day for 10-15 days). In this concentration, large amounts of the antibiotic are slowly excreted, thereby increasing the selective pressure which might then lead to selection of bacteria which are oxytetracycline resistant in the gut (Boxall *et al.*, 2004).

Oxytetracycline use in fish rearing has been shown by Tafalla *et al.* (2002) to be related to an increase in oxytetracycline-resistant microorganisms. The prevalence of oxytetracycline resistance in microorganisms isolated from freshwater salmon farms has been investigated by Miranda and Zemelman (2002), who discovered that the highest number of resistant bacteria were present in effluent samples.

Fluoroquinolones in the environment may also promote co-selection of broad-spectrum beta-lactamases gene (Wellington *et al.*, 2013). Some antimicrobials, even when only used in human medicine or veterinary medicine, may also promote cross-resistance to several antibiotics.

Although, enrofloxacin is uniquely used in veterinary medicine, it may promote cross-resistance to ciprofloxacin, which is used in the treatment of human infections. Cross-resistance may also occur across different classes of antibiotics as observed in the case of oxytetracycline which its cross-resistance has been promoted by oxolinic acid (Holmstrom *et al.*, 2003).

Most studies therefore have surmised that the continuous use of antimicrobials in aquaculture will invariably result in increased levels of antibiotic resistance. There is the need for a clear-cut and quantitative analysis of resistance-conferring genes using a culture-independent method. Furthermore, analysis of the antimicrobial, tetracycline, has indicated that no sample contained therapeutic concentrations at any sampling time suggesting that prevalence of genes that confer resistance to tetracycline may have been as a result of the preservation of these genes during periods of lack of selection pressure (Rasul and Majumdar, 2017).

Molecular approaches and the use of large-scale sequencing technology could be significant tools used to determine the diversity of antibiotic-resistance genes present in the environment. Concepts such as the resistome concept has been used to explain the diversity of antibiotic resistance found naturally in a particular environment. The resistome of aquatic ecosystems have however been poorly described. Observations have been made showing that a single antibiotic may co-select for a diversity of resistances. In order to assess and understand this risk, studies should be carried out to focus on the ability of different aquaculture antibiotics to co-select for multiple resistances (Grigorakis and Rigos., 2011).

It is important to note however the sparsity of studies focusing on determining the effects of antibiotic use on the microbial ecology of the fish gut. Published studies have focused their attention on antibiotic resistance frequency during and after the administration of antibiotics,

susceptibility of pathogens of aquatic species isolated from fish farms to antibiotics and the molecular determinants of antibiotic resistance (Miranda *et al.*, 2003).

2.6 ANTIBIOTIC RESIDUE

The difficulty in being able to calculate the appropriate dose of antibiotic that should be administered to aquaculture has posed a serious threat to both animal and human health and environmental safety (Pham *et al.*, 2015). Antibiotics are usually combined with animal feed but sometimes, during disease outbreaks, farmers may apply antibiotics using other routes. It has been widely reported that antibiotics have not always been correctly administered (Jacoby, 2005). The use of antibiotics in this unchecked manner has created concerns for the development of antibiotic resistant pathogens and deposition of residues in the food chain. It is very important to pay attention to contamination that occurs in this manner as some potential hazards may be associated to the presence of these antibiotic residues in edible tissues. Public health risks that have been associated with consumption of edible tissues containing antibiotic residue include mutagenic, teratogenic and carcinogenic effects (Miranda *et al.*, 2013). Aplastic anemia has also been associated with chloramphenicol. Urinary vestibular damage caused by aminoglycoside, hypersensitivity caused by penicillin and auditory functions impediment have also been recognised in studies as other side effects brought on by consumption of edible tissues containing antibiotic residue (Ezenduka *et al.*, 2011).

Presence of antibiotic residue above permitted levels in the muscles and liver of fish has been reported by Chuah *et al.*, (2016). According to Codes Alimentarius Commission, the limit for antibiotic residue in fish liver and muscle should not exceed 600 and 200 µg/kg, respectively (Olatoye and Basiru, 2013). When tissues containing antibiotic residues are consumed by humans, it may result in the development of resistance to antibiotics by human intestinal

microflora (Heuer *et al.*, 2009). When administration of treatment using high antibiotic doses is prolonged, it might result in toxicity to the fish and accumulation of the residues. Subsequently, administration of antibiotics at low doses for a short period of time may contribute to the development of antibiotic resistance in bacteria that were not initially killed. Antibiotics such as quinolones and tetracyclines in aquaculture is of particular concern as they are also used to treat various infections in humans (Rico and Van den Brink, 2014).

2.7 ANTIBIOTIC USE IN NIGERIA

Studies carried out by Olatoye and Ehinmowo (2010) examined the presence of oxytetracycline in cattle tissues in Akure, Nigeria but these studies only focused on tetracycline, chlortetracycline, oxytetracycline and doxycycline in chicken and catfish. Few studies have been carried out on the presence of antibiotics in catfish generally but there have been no studies examining levels of tetracycline and epimers residues in catfish and catfish products in Nigeria (Onipede *et al.*, 2021). Studies carried out by Onipede *et al.* (2021) on the presence of antibiotic residue in catfish revealed that tetracycline, one of the most commonly used antibiotics in Nigeria, had concentrations below the detection limits. Concentration of doxycycline in catfish were also found to be below limit of detection for all the samples analysed. The presence of both of these antibiotics in levels below detection was however not taken as evidence that the antibiotics were not administered on the farms but, probably, there had been an adequate withdrawal period practiced by the fishery farmers in the study. The antibiotic, chlortetracycline, was however found in concentration above the limit of detection, although it was obtained in only one of the samples used in the study. Onipede *et al.* (2021) concluded from their studies that there was common administration of antibiotics in fisheries but there was proper adherence to an adequate period of withdrawal which differed from country to country.

In studies carried out by Adelowo and Okunlola (2020), it was recognised that due to the recent growth of the aquaculture industry in Nigeria, it was hectic to gather data showing the quantitative use of antibiotic in aquaculture from different countries. However, it was gathered from their studies that the farmers used poultry waste either as feed supplement or for pond fertilization, and poultry waste contained residue of antibiotics. Antibiotics found to be used in the study were found to be used in other animal production industries such as swine and poultry (Amaechi, 2014; Oluwasile *et al.*, 2014; Adebowale *et al.*, 2016). Tetracyclines were also reported to be the most commonly used antibiotic in the studies by Adelowo and Okunola (2020). The main method of drug administration was found to be feeding and then water. These methods of administration have however been found to be risky as these antibiotics have been found to be persistent in aquatic sediments (Martinez, 2009).

The findings of Oyebanji and Oyebisi (2018), indicated that antibiotics were administered for treatment of animals which were sick, for the prevention of secondary infections by bacteria and as a prophylactic measure on healthy animals. They however found oxytetracycline to be the most commonly used antibiotic by farmers. The studies also showed that farmers most commonly got their information about drugs and its related matters through the media.

The findings reported above all point to the lack of substantial information on the proper use and administration of drugs and drug administration. To tackle this issue, there should be enough public awareness through the utilization of media to educate farmers and people in general on the risks associated with the consumption of livestock products containing illegal levels of antibiotic residues. Legislation should be mandated by the State and Federal Government regarding use of drug and control of veterinary drug residues. Basic facilities should also be provided nationwide

to monitor residue nationwide and periodically survey antibiotic residues in edible tissues at all levels (Oyebanji and Oyebisi, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The survey was conducted in Edo Central, Edo State, Nigeria.

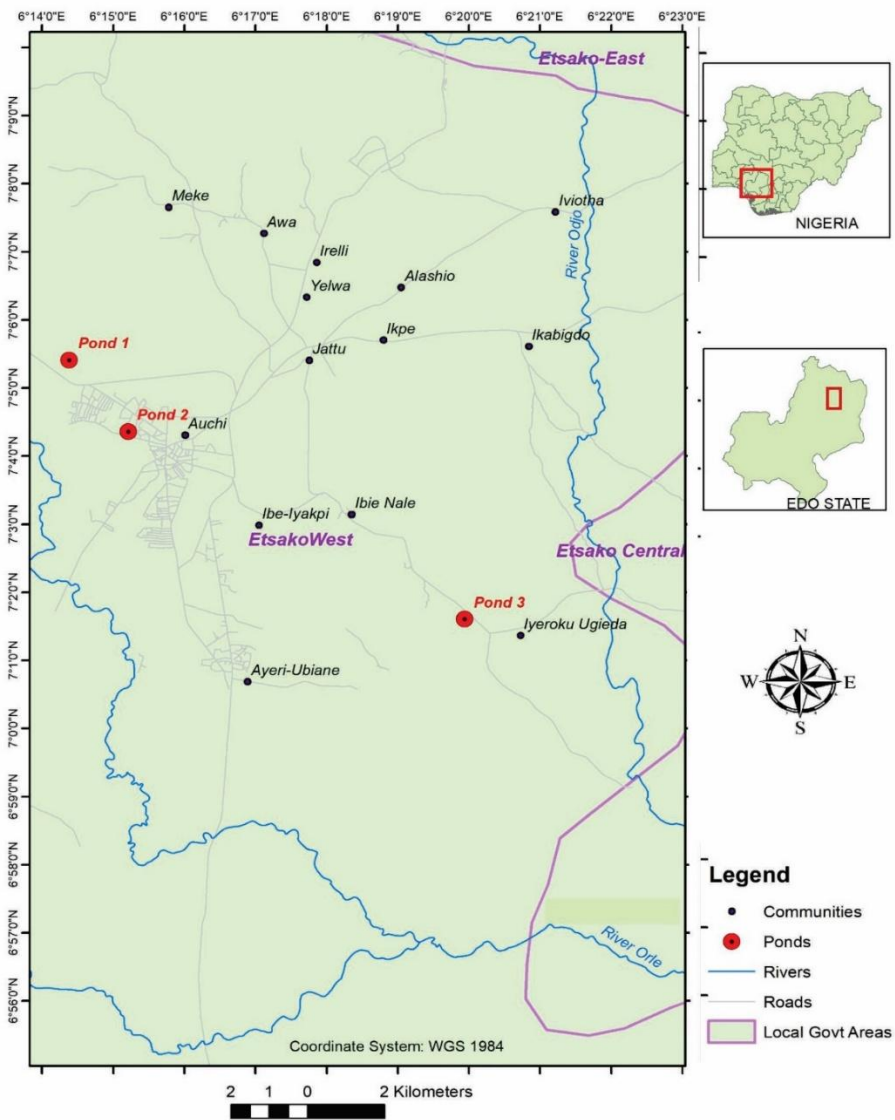


Figure 1: Map of the study area showing the sampling ponds in Edo Central, Edo State, Nigeria

3.2 Questionnaire survey

Semi-structured questionnaire was randomly administered to aquaculturists engaged in fish farming randomly selected across Auchi, Etsako Local Government Area, Edo State. The questionnaire was made up of sections on socio-demographic characteristics, fish farming operations, practices of antibiotic usage and period of withdrawal in their fishes as well as their attitude to antibiotic resistance and residues.

3.3 Collection of fish samples

Adult catfish (*Clarias gariepinus*) were purchased from three (3) commercial catfish ponds in Edo Central, Edo State, Nigeria. The length of the catfish ranged from 50 to 60 cm while the weight ranged from 750 to 1000 g. Catfish purchased from commercial ponds were without skin lesions or sunken eyes. Each sample was transported in appropriate containers and taken to the laboratory within four (4) hours of collection. Each catfish was sacrificed, the body muscles were harvested and approximately 150 g of liver and muscle samples were randomly collected from the catfish and thereafter stored in the freezer (-20°C) until time for subsequent extraction and residue analysis (Olatoye and Basiru, 2013).

3.4 Ethical statement

The animals were treated based on laid down guidelines.

3.5 Sample Preparation

25 g of each sample was homogenized and deproteinized with 3 volumes of 1 N hydrochloric acid after which 8 ml of the homogenate was thoroughly swirled with 32 ml acetonitrile and allowed to stand for 5 minutes. The supernatant was decanted through a glass wool and 20 ml of

the filtrate was vigorously shaken with 20 ml petroleum ether and 20 ml methylene chloride. The aqueous layer containing the analyte was then collected for HPLC analysis.

3.6 Laboratory Analysis

Two extraction techniques were adopted for this research: QuEChERS and separating funnel. A recovery study was performed in order to evaluate the efficiency of each extraction procedures that used. One method was used for the fish and soil while the other was used for the water extracts.

QuEChERS extraction was followed by solid phase extraction (SPE). Approximately 2 g of air-dried fish was weighed and placed in a glass tube. The samples were then spiked with a mixture of antibiotics at a concentration of 50 ng/g (dw) (sulphonamides, tetracycline, ampicillin) (European Commission, 2010). The spiked samples were placed in a 50 mL polypropylene tube. 5 mL of double distilled deionized water is added and the mix was vortexed for 30 seconds then it was followed by the addition of 10 mL of acetonitrile (ACN) with the subsequent vortex for 1 minute. Then, the QuEChERS extraction composed of magnesium sulphate 6g and sodium acetate 1.5g was added and the mix was hand-shaken for 1 min. Samples were then centrifuged for 5 min at 10,000 rpm. The ACN layer was then transferred to tube containing the sorbents (primary secondary amine (PSA). In order to carry out a SPE, the sample was vortexed again for 1 min and then centrifuged for 10 min at 5000 rpm. Finally, 6 mL of the extract was taken and evaporated to dryness. It was then reconstituted in 1 ml of methanol (MeOH) at -20°C until its analysis. The detection and quantification of the target compounds was carried out with HPLC.

Recoveries were calculated by comparing the concentration measured in the samples after the analytical procedure with the initial spiking concentrations. The concentration that was measured

in each sample was determined by using internal sample calibration. The internal standard curve was in the range of 0.01 to 50 ng/g (Kummerer, 2009).

3.7 Statistical analysis

Mean, standard deviation, standard error and t-test of obtained results were calculated using the Statistical Package for Social Sciences (SPSS v.22) software. T-test was used to calculate if there was significant difference within the concentration of tetracycline between the sampled ponds. .

CHAPTER FOUR

4.0 RESULTS

4.1. QUALITATIVE ASSESSMENT OF ANTIBIOTIC USAGE

Qualitative data obtained from the owners of the selected fish ponds that were sampled in Edo Central are shown in Table 4.1.

Table 4.1: Socio-economic characteristics of sampled pond owners

		Pond owners n (3)
Gender	Male	3 (100%)
	Female	0
Educational status	None	0
	Primary	0
	Secondary	0
	Post-secondary	1 (33.33%)
	Vocational	0
	Other	2 (66.67%)
Farming experience	0 – 5 years	1 (33.33%)
	5 – 10 years	2 (66.67%)
	11 – 20 years	0
	> 20 years	0

All respondents were male and they had some form of education with one respondent possessing post-secondary education. 2 of the respondents had at least 5 years of experience in catfish (*C. gariepinus*) rearing while the other respondent had less than 5 years' experience in the industry.

Table 4.2 shows the response of owners of selected catfish ponds to questions regarding antibiotic use. All respondents admitted to using tetracycline in their fish ponds, purchasing the tetracycline from a veterinary medicine store and experiencing the outbreak of disease in their ponds. Antibiotic administration was done mostly through feed (2 respondents) and through feed and water bath (1 respondent). 2 respondents employed only the use of quality fish feed as best management practice in catfish rearing while the other respondent employed various management practices such as water re-use, adequate aeration and circulation of the ponds, moderate stocking levels and use of quality fish feed. All respondents were aware of the risks associated with the indiscriminate use of antibiotics in ponds.

Table 4.2: Antibiotic use by fish pond owners

Parameter	Response	Pond owners: n (3)
Use of antibiotic	Yes	3 (100%)
Type of antibiotic	Tetracycline	3 (100%)
	Doxycycline and oxytetracycline	2 (66.67%)
	Penicillium and ampicillin	1 (33.33%)
	Flouroquinolone	2 (66.67%)
	Enrofloxacin	2 (66.67%)
	Metronidazole	1 (33.33%)
Source of antibiotics	Vet. medicine store	3 (100%)
Method of antibiotic administration	Feed	2 (66.67%)
	Feed and water bath	1 (33.33%)
Reason for antibiotic use	Treatment of diseased fish	1 (33.33%)
	Treatment of diseased fish and disease prevention	2 (66.67%)
Experienced any disease outbreaks	Yes	3 (100%)
Best management practices that have been employed	Water re-use	2 (66.67%)
	Adequate aeration and circulation of ponds	
	Moderate stocking levels	
	Use of quality fish feed	
	All of the above	1 (33.33%)
Awareness of risks associated with indiscriminate use of antibiotics in ponds	Yes	3 (100%)

4.2. TETRACYCLINE RESIDUAL LEVELS IN *C. gariepinus* FROM SELECTED FISH FARMS IN EDO NORTH, EDO STATE, NIGERIA

The concentration of tetracycline in catfish (*C. gariepinus*) collected from selected ponds in Edo Central, Edo State, Nigeria, is presented in Table 4.2. The highest mean tetracycline residue was recorded in Pond 1 (1.87 ± 0.03 mg/kg).

Table 4.3: Residual values (mg/kg) of tetracycline in catfish (*C. gariepinus*) from selected ponds in Edo Central, Edo State.

	Replicate		MRL ($\mu\text{g/kg}$)	Mean \pm S.D
	Sample (mg/kg)	(mg/kg)		
Pond 1	1.89	1.85	100	1.87 ± 0.03
Pond 2	1.81	1.88	100	1.84 ± 0.05
Pond 3	0.00	0.00	100	0.00

The residual levels (mg/kg) of tetracycline in catfish (*C. gariepinus*) from pond 1 ranged from 1.85 mg/kg to 1.89 mg/kg with a mean of 1.87 ± 0.03 mg/kg while the values is Pond 2 ranged from 1.81 mg/kg to 1.88 mg/kg with a mean of 1.84 ± 0.05 mg.

Table 4.4: Estimation of risk assessment by hazard quotient for mean concentrations of tetracycline residues in catfish (*C. gariepinus*)

Pond	EDI ($\mu\text{g}/\text{kg}/\text{day}$)	ADI ($\mu\text{g}/\text{kg}/\text{day}$)	Hazard Quotient
Pond 1	4.36	30	0.15
Pond 2	4.29	30	0.14

The mean concentration of tetracycline residue in catfish were 1.87mg/kg and 1.84mg/kg respectively.

*Estimated daily intake (EDI $\mu\text{g}/\text{kg}/\text{day}$) was calculated by the following equation:

$$\text{Fish consumption} \times \text{mean concentration of residue in catfish/ body weight (per 60kg)}$$

*Data for acceptable daily intake of tetracycline was derived from FAO (2011).

The calculated hazard quotient calculated from both ponds was lower than 1 which is an indication that consumption of catfish (*C. gariepinus*) from the sampled ponds might not be harmful due to the concentration of tetracycline residue being low in the fish.

CHAPTER FIVE

DISCUSSION

The use of antimicrobials in the treatment of diseases affecting animals has been a major issue in recent times. This issue has been emphasized in developing countries such as Nigeria where indiscriminate administration of these antimicrobials are a common occurrence (Jafari *et al.*, 2001). Although these antimicrobials abound, few have been modified specifically for fish. Around the world, the common antibiotics used in aquaculture include sulfonamides, quinolones, tetracyclines, emamecin, and macrolides (Fauconneau, 2002).

This practice of administering drugs meant for different groups of animals and a shortage of well-trained veterinarians specializing in fish medicine has led to the proliferation of fish harbouring antibiotic-resistant organisms. Consumption of such fish by humans has far-reaching consequences (Ramirez *et al.*, 2007).

The residual levels of tetracycline obtained in this study ranged between 1.81 mg/kg to 1.89 mg/kg. These values are higher than values obtained by Adelowo and Okunlola (2015). High residue of antibiotics in catfish can be harmful to both the environment and those who consume the fish (Boxall *et al.*, 2004).

The hazard quotient expresses the risk posed to human health by consumption of milk having residues and also present the intensity of the hazardous effect. Results revealed the estimated daily intakes (EDI) were much lower than the acceptable daily intakes (ADI) for tetracycline. Less per capita fish consumption of Nigerians contributes to lower exposure to residues found in catfish (Adewumi, 2015). The hazard quotient values below one proved that detected levels of

residues in catfish had no significant toxicological effects on the health of consumers in the study area.

This study shows that catfish farmers still use antimicrobials in the treatment of aquaculture. The drugs that were administered to the catfish by the farmers were not specifically indicated to be administered to only fish. This study also supported previous study that tetracycline, is still administered directly to fish or included in their meal, with the intention of preventing diseases. Of the three ponds from which catfish samples were taken, two showed the presence of tetracycline residue in the fish. This corroborates studies by Alarape and Adeymo (2017), Olatoye and Basiru (2013), and Ashiru *et al.* (2011) who all found tetracycline in catfish samples, in varying concentrations. **In conclusion the results** obtained in this study revealed that appreciable quantity of tetracycline residues can be found in catfish from ponds in Edo Central, Edo State. The consistent misuse of antibiotics by aquaculture farmers can be attributed to a lack of knowledge of basic veterinary practices. Although antibiotics are useful in the aquaculture, regulating their use and educating personnel in the industry on proper use can go a long way in reducing residues in catfish and invariably reduce the risk associated with the consumption of catfish with antibiotic residue.

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