Occurrence of antibiotic residues in cultured African catfish C. *garienpinus* in selected zones at Enugu Nigeria

ABSTRACT

Antibiotics have been in used in fish farming for several decades in combating diseases but improper application and handling had lead to the occurrence of residues in animal food such as fish . Animal products whose drug residue limit exceeds the maximum residue limit (MRL) pose serious concern such as; allergy, carcinogensis, antibacterial resistance, disruption of intestinal flora, mutagenesis, tetratogenesis among others. The study was thus, conducted to assess the residue concentrations in the selected fish farms in Enugu state Nigeria. The study was conducted in three (3) senatorial zones of Enugu State; Enugu north, Enugu west and Enugu east involving two (2) local governments in each zone respectively; Nsukka and Igbo etiti, Awgu and Ezeagu, Nkanu west and Enugu south. A total of fifty four (n=54) *Clarias garienpinus* organs samples with three replicate (3) were used for the study. The kidney, liver and muscles were obtained from the fish samples and analysed for antibiotics residues using Gas chromatography mass spectrometry (GCMS). The antibiotics residues obtained from the analysed samples includes; tylosin, avilamycin, amoxicillin, chloramphenicol, gentamicin, lincomycin, macrolides and quinolones. Available in this increasing order macrolides > quinolone > lincomycin > chloramphenicol > gentamincin > amoxicillin > avilamycin > tylosin with these mean concentrations respectively; 1.44 ± 0.17, 5.71 ± 0.28, 10.04 ± 0.27, 12.94 ± 0.34, 9.09 ± 0.17, 21.68 ± 0.41, 35.79 ± 0.47, 25.86 ± 0.27µg/kg). Tylosin (liver 1.7 ± 0.50, gills 1.39 ± 0.27; muscles 1.17 ± 0.12µg/kg) had the least concentration in the analysed organs while macrolides (liver 29.44 ± 0.71, liver 49.04 ± 0.31, muscle 28.87 ± 0.31µg/kg) had the highest concentrations in the analysed organs. Also, our results showed that the highest concentration of the drug residue was seen in the gills with these mean values except for chloramphenicol (Tylosin 1.39 ± 0.27, Avilamycin 6.85 ± 0.39, Amoxicillin 11.01 ± 0.34, Chloramphenicol 12.00 ± 0.33, Gentamicin 11.20 ± 0.24, Lincomycin 21.75 ± 0.42, Macrolides 49.04 ± 0.31 and Quinolones 28.40 ± 0.40µg/kg). It also indicated that the values of antibiotics residues were highest at Awgu L.G.A. except for Macrolides; (Tylosin 0.00 ± 0.00, Avilamycin 9.03 ± 0.55, Amoxicillin 11.53 ± 0.31, Chloramphenicol 18.39 ± 0.61, Gentamicin 0.00 ± 0.00, Lincomycin 24.84 ± 0.50, Macrolides 36.12 ± 0.16 and Quinolones 39.05 ± 0.65 (µg/kg)) while Nsukka had the lowest drug residues concentrations except for Tylosin; (Tylosin 3.84 ± 0.30, Avilamycin 2.55 ± 0.20, Amoxicillin 8.99 ± 0.22, Chloramphenicol 13.82 ± 0.50, Gentamicin 6.39 ± 0.10, Lincomycin 21.46 ± 0.20, Macrolides 29.70 ± 0.41 and Quinolones 10.20 ± 0.10µg/kg). It was also observed that across the senatorial zones Enugu west had the highest mean residue concentration; (Tylosin 0.84 ± 0.15, Avilamycin 6.46 ± 0.38, Amoxicillin 11.12 ± 0.36, Chloramphenicol 11.57 ± 0.33, Gentamicin 7.98 ± 0.15, Lincomycin 23.94 ± 0.56, Macrolides 51.58 ± 0.69 and Quinolones 27.99 ± 0.39µg/kg). The study has shown the presence of antibiotic drug residue in fish samples collected from six local government areas under study. The study also indicated disparities in concentrations of drug residues observed in the sample showed the lowest levels of drugs residues whereas, samples from Awgu L.G.A showed the highest levels of drug residues at this significant level $p < 0.05$. Although, the concentrations of these drug residues observed in the samples were below the European Union maximum residue limit, proper monitoring of edible food for pharmaceutical residue is important, also educating farmers on the need to adhere strictly to recommend withdrawal period after the use of products that has these drugs in them is very expedient due to the consequences they pose to human health.

Key words: antibiotic, drug, maximum residue limit, aquaculture, fish

INTRODUCTION

In the recent times, the increasing human population in the face of inelastic production strategies appears to have widened the demand and supply gap of agricultural products, especially protein-based foods (Igwe and Onyekwere, 2007). According to Cheeke, (2002), the global demand for protein-based foods increased by 58 % between 1995-2020 and that consumption raised in the year 2020. This implied that to ensure food nutrition security, there is need to increase the production of protein-based foods. This is more pertinent in developing countries where malnutrition and food insecurity is very common, and this is where production of fish products comes in as a panacea to protein-based nutrition deficiency.

Fishing like other hunting activities has been a major source of food for the human race and has contributed to the reduction of the unsavory outbreak of anaemia, kwashiorkor and other ailments due to malnutrition (Olagunje *et. al*. 2007). This is because fish has a nutrient profile superior to most terrestrial meats (beef, pork and chicken, etc). It is an excellent source of high quality animal protein and highly digestible energy (Kudi *et. al*. 2008). According to Ali *et. al*. (2008), fish is the most important animal protein food available in the tropics. It provides about 40 % of the dietary intake of animal protein of the average Nigerian (Federal Department of Fisheries, FDF, 2007). In addition to its nutritional benefits, fish is important for animal feed, and serves as a source of raw materials for allied industries (Esu *et. al*. 2009). Furthermore, fish farming contributes about one-third of the Gross Domestic Product (GDP) in Nigeria (Amao *et. al.* 2009). The fishery sub-sector provides full-time employment to over 12 million people, which constitutes about 3 % of the active population of the nation; another 11 million people indirectly earn their livelihoods from activities related to fisheries (FAO, 1991; Olagunje *et. al*. 2007).

Over the past two decades, world aquaculture has developed tremendously to become an economically significant industry. The industry continues to grow at an average global annual growth level of 8.8 % year compared with all other animal food production industries (Onada and Ogunola, 2017).

However, despite the huge potentials of fish farming, Nigeria is still one of the largest importers of fish in the developing world. According to the Central Bank of Nigeria (2016), Nigeria spends over

288 billion Naira on annual fish importation. Nigeria is among the largest fish consumers in the world, with over 1.5 million tons of fish consumed annually, of which over 900,000 metric tons are imported, while its domestic fish catch is estimated at 450,000 metric tons/year. This huge gap in the production of fish serves as a motivation for the government and the private sector to put in measures to increase domestic production. This situation has ensured some form of a boost in the aquaculture industry. There are huge prospects and potential for the growth of the Nigerian aquaculture sector, as there are numerous freshwater lakes, rivers, reservoirs, dams, free-flowing boreholes floodplains, etc. available for fish production (Agbelege and Olarewaju, 2010). It has been projected that Nigeria needs an average annual increase of 3.8 % in fish production to keep up with the demands of an ever-increasing population. This might lead to increased production of African catfish in the country because of relatively good knowledge regarding their culture techniques and high market demands. The demand and market price for catfish are higher than those for tilapia or carps (Dauda *et. al*. 2018). Nigeria is often called the land of aquatic splendor. It has networks of abundant natural water resources vis-à-vis rivers, lagoons, creeks, streams, flood plains and coastal waters constituting approximately 25 % of the total landmass of the country.These resources, in addition to 47, 877 ha of swamps are potential biomes for fish farming (Ahmad and Ibrahim, 2016). Nigeria is blessed with over 12.5 million hectares of water surface which a good percentage could be put to use for aquaculture and development (Udo and Dickson, 2017).

The most commonly cultured species of fish in Nigeria include catfish, tilapia and carp. However, many fish farmers in Nigeria focus on catfish (*Clarias gariepinus*) because of how well it adapts to the environment, its hardy nature that allows it to be easily retailed live and its premium market price. Since the culture of *C. gariepinus* through hormonal induction (hypophysation) was initiated in Western Nigeria in 1973, the procedure has been widely practiced throughout Nigeria, thus leading to the increase of farm raised catfish from 1980s till date (Iheke and Nwagbara, 2014). African catfish (*C. gariepinus), Clarias anguillaris*, *Heterobranchus bidorsalis*, *Heterobranchus longifilis* and their hybrids are cultivated for reasons of their high growth rates, disease resistance and amenability of high density culture, related to their air breathing habits (Fagbenro *et. al*. 1993; Miller and Atanda, 2007). Catfish is suitable for stocking in ponds and they tolerate low dissolved oxygen better than other common species in the country. Farm raised catfish is a good source of high quality protein, and it has essentially little carbohydrate and no fiber. The fat content is low

compared to other animal meat. The cholesterol level and caloric value of catfish are also low with other desirable qualities such as fatty acids, mineral and vitamin content which makes the catfish an exclusively desirable recipe for those on fat and calorie controlled diets. Besides, catfish has wide acceptability as food in Nigeria. Despite these considerably high potentials, local fish production has failed to meet the country's domestic demand (Oladimeji, 2017). This has led to the existence of a demand-supply gap of at least 0.7 million metric tons in Nigeria. Increased catfish production in the country, according to Food and agricultural organization (FAO, 2005), can help reduce this worrisome demand supply fish gap in the nation. Ugwumba and Chukwuji, (2010) suggested that greater improvement in catfish production can be achieved with proper analysis that will lead to the knowledge of the level of profitability of catfish farming and the socio-economic features of catfish farmers that constrain maximum production.

Along with the development of aquaculture, diseases caused by various etiological agents followed by mortality of cultured stock have become limiting factors in production. Hence, the farmers and the hatchery operators have resorted to the use of various remedial measures, including use of antimicrobials and drugs for controlling the disease. The frequency of utilizing these antibiotics and other chemicals is more in hatcheries and commercial farms than in home stead farms. Among the drugs employed in agriculture, antibiotics are the most widely used for animal health and management (Levey, 1992). Accordance with a 2008 amendment to the Animal Drug User Fee Act, The U.S. Food and Drug Administration (FDA) released an annual amount of antimicrobial drugs sold and distributed for use in food animals. The grand total for 2009 is 13.1 million kilograms or 28.8 million pounds (US FDA, 1996). The total amount of veterinary antibiotics used in therapeutic purposes and as feed additives was approximately 5000 tons in 2005 (KFDA, 2011). The use of antimicrobials in aquaculture basically started with the work of Gutsell (1946) who recognized the prospective use of antibiotics (sulphonamides for combating furunculosis). The use of antibiotics as food supplements for disease prevention and treatment and as growth promoters, (Pham, 2015) is common practice. However, such use of antibiotics without veterinary control leads to the inevitably to the presence of antibiotic residues in the animal-derived products and by-product (Mensah *et al.*2014). the utilization of antibiotic products in acquaculture is prejudicial to the acquatic environment and aqualife on one hand, and on the other hand, to the fish products consumers due to the toxicity risk of antibiotic residues (Cabello, 2006; Olatoye and Basiru, 2013; Dhaouadi *et. al.*2015). According to Kummerer (2009) antibiotics are naturally occurring or man

made chemicals that can be divided in to different classes such as βlactams, Quinolones, tetracyclines, macrolides and sulfonamides. More antibiotics like chloramphenicol, oxyteteracycline, kanamycin and nifurprazine exist.

After administration of drugs a significant fraction is released into the environment (Zhou *et. al*. 2013). Between 30 to 90 % of all drugs used in humans and animals are excreted unchanged or as active metabolities into the environment through urine and feces (Ijemba 2006 and Lienert *et.al*. 2007). Bacteria resisitance genes are pressing public health problems (UN, 2016). High rates of common infections are caused by resistant bacteria in all WHO regions, including Nigeria (WHO, 2014).Thus, antibiotics resistance has become a serious and growing threat to modern medicine and is considered a leading health concern of the 21st century (UN, 2016). In recognition of the above concern, this study is to determine the occurrence of drug residue in African catfish among cultured fish in six selected local government areas in Enugu State, Nigeria.

Antibiotics have been used in livestock farming for several decades in combating bacterial infections, but lack of proper application and handling can lead to occurrence of residues in the food of animal origin particularly meat, milk, and eggs. Farm animals treated with antibiotics are required to be withheld for the residues in the edible tissues for specific withdrawal period until all residues are depleted to safe level before the animal tissue can be used as food for human consumption (Kukanich *et. al*. 2005).

Different types of antibiotics are used to keep fish free from diseases (Avsever *et. al*. 2010). Among them, oxytetracycline is one of the most popular primarily used antibacterial used in aquaculture production (Erdogdu, 2012). Now it is abundantly used in fish farms to treat disease affected fish and/or as a prophylactic in freshwater aquaculture in Africa (Ali *et. al.* 2016). But antibiotics like oxytetracycline have not always been used in a responsible manner in aquaculture (FAO/WHO 2003). Indiscriminate use of antibiotic could lead to undesirable deposition of their residues in edible tissues which could hamper public health to some extents. Antibiotic residues transferred to humans through food can also alter the intestinal ecology thereby favouring the emergence of resistant microflora (Perrin-Guyomard *et. al.* 2001). Residues of antimicrobials also result in lowering the marketing and export value of aquaculture products (Sapkota *et. al.* 2008; Heuer *et. al.* 2009). So it is important to give attention to this contamination because of the potential hazards associated with these products content in edible tissues. However, in Nigeria indiscriminate

administrations of oxytetracycline in fish culture have been reported by several authors but quantitative risks assessment of antimicrobial residues in fishes is not limited (Muriuki *et. al*. (2001), Erdogdu (2012), Olatoye and basiru (2013), Ali *et. al.* (2016).

Drug residue is defined by CVM (Centre for Veterinary Medicine) as any compound or metabolite of a compound that is present in edible tissues of food animals because of the use of a compound in or on animals (EC European Commision, 2012, Beyene, 2016). Residues can be from the compound itself, its metabolites, or any other substances formed in or on food as a result of the compound's use. CVM has a rigorous program for establishing the safety of residues present in food-animal tissues. Data are required for toxicity testing, residue and metabolism testing, and development of analytical methods. Toxicity testing is used to establish the maximum safe residue concentration in the edible tissues of the target animal. CVM evaluates toxicity with tests designed to monitor acute, short-term, and chronic toxicity over time. Within the scope of these tests, concentrations of drug residues are determined that affect morbidity and mortality as well as reproductive toxicity, teratology, and carcinogenicity (Beyene, 2016).

MATERIALS AND METHODS

Description of Study Area

Geographical Location / Demography of Enugu State Nigeria

Enugu State (figure 1) is in the South East geo-political Zone of Nigeria. It is located at 6^{o} 30' North of Equator, and 7^{o} 30' East of Latitude. It is plus one hour (+1hr) GMT on the World Time Zone. It shares border with the following states: Abia and Imo to the south; Ebonyi to the east, Benue to the north-east, Kogi to the north-west and Anambra State to the west. It covers an area of 7,161 km^2 (2,765sq m), and ranks 29th out of the 36 States of Nigeria in terms of land area. Enugu State has a good climatic condition all the year round. The hottest month is February with about 87.16 ^{o}F (30.64 ^{o}C), while the lowest temperature is recorded in November/December, reaching about 60.54 ^{0}F (15.86 ^{o}C). Lowest rainfall of about 0.16 cubic centimeters (0.0098 cu in) is recorded in February, while the highest rainfall is recorded in July at about 35.7 cubic centimeters (2.18 cu in). With an estimated population of 3,267,837, (1,596,042-males and 1,671,795- females) (NPC, 2006), it ranks 23rd out of the 36 States of the federation. Enugu State is also densely populated, and is rated at 460/km^2 (1,200/sq mi). This is regarded as one of the highest in Africa. Demographers have however, continually put the realistic population figure of Enugu State at six million. Enugu State is basically rural and agrarian, with a substantial number of its working population engaged in fish farming, although trading and services are also important, while trading and services are predominant in the urban area (Department of geography, University of Nigeria, Nsukka).

The map of Enugu state showing the selected local government

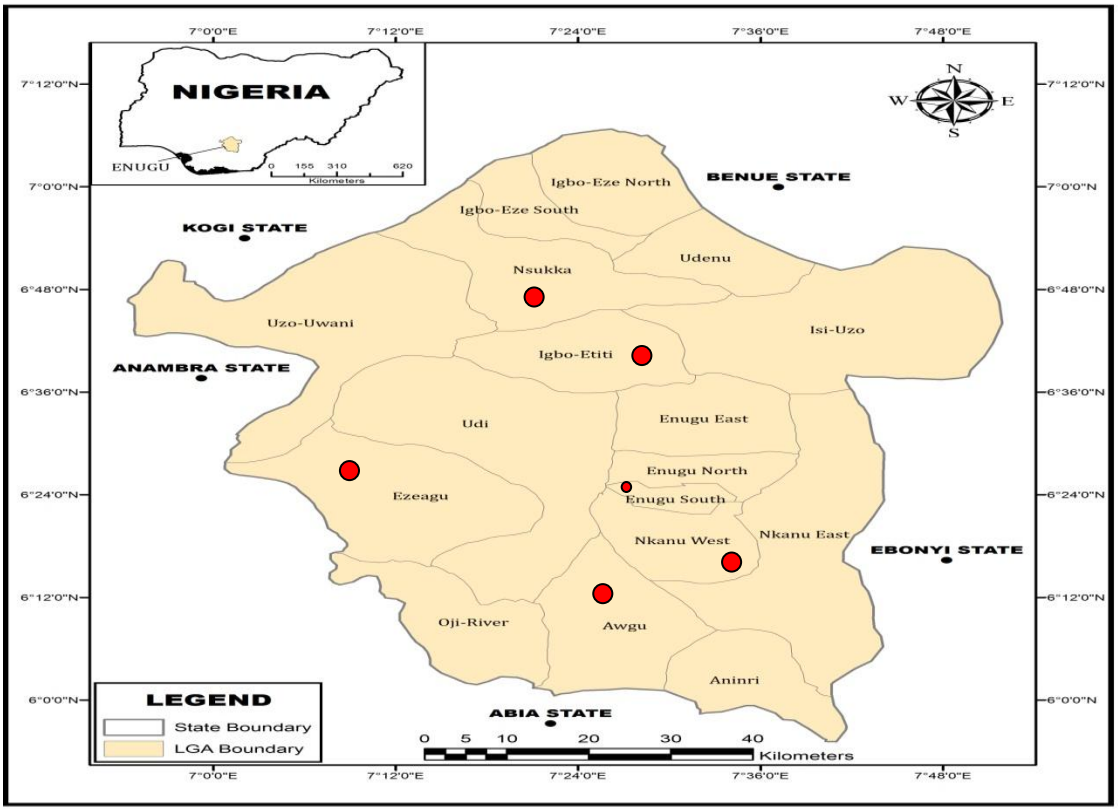

Figure 1: Map showing the study area. A. Map of Nigeria B. Map of the Enugu showing local government under study, study area marked with orange bullet.

Source: Afamefuna *et. al.* (2017)

Ministry of Land surveys, Enugu State.

Description of Method of Sample Collection

The study was conducted in three senatorial zones of Enugu State (Enugu north, Enugu West and Enugu east) involving two (2) local government areas (Nsukka and Igbo Etiti, Awgu and Ezeagu, Nkanu west and Enugu south) in each zone. A total of 18 catfishes were collected from the selected fish farms in these locations while fifty four (n=54) organs (liver, kidney and muscle) of the fish samples were extracted for analysis.

Two fish farms each selected from the three geographical zones in Enugu State;

Enugu North Local Government: Nsukka (Freedom Fishery Farm) and Igbo Etiti (Chukwuwueife farm), Enugu West local Government: Awgu (Diamond Fish Farm)and Ezeagu (God is good farm) Enugu East Local Government: Enugu South (St. Mosco Feed the nation farm) and Nkanu west (Master's skill Acquisition centre, Agbani)

A total of 54 fish organs were extracted for this study from the selected fish.The fish samples were collected from the ponds of the selected fish farms as listed above, in a well labeled plastic bucket with lid. The collected samples were transported in plastic buckets with sufficient amount of ice blocks to prevent deteoriation, and taken to the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology (ESUT), Enugu state, where the organs of interest; liver, gills and muscle were extracted and homogenised in a plastic tube and sent for analysis to the Toxicology Department of Arbovirus Research Centre Enugu, Enugu State.

Description of Method

Instrumentation

The Gas Chromatography Mass Spectrometry (GC–MS) analysis for the different extracts was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film).

Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV).

Pure helium gas (99.995 %) was used as the carrier gas with flow rate of 1 ml/min. the analysis was carried out in the Toxicology Department of Arbovirus Research Centre Enugu, Enugu State.

Preparation of Samples for GC Analysis (AOAC, 1990)

Soxhlet Extraction Method

Ten grams (10g) of the homogenized sample of the fish was mixed with 60g of anhydrous sodium sulphate in agate mortar to absorb moisture. The homogenate was placed in a 500ml beaker and extracted with 300ml of n – hexane for 24h. Crude extract obtained was evaporated using a rotary vacuum evaporator at 40^0c, just to dryness.

Preparation of Sample for GC Analysis

1ml of filtered residue was dissolved in 50ml of chloroform and transferred to a 100ml volumetric flask and diluted to the mark. The chloroform was evaporated at room temperature, 1ml of the reagent (20 % vol. benzene and 55 % vol. methanol) was added, sealed and heated at 40^0C in a water bath for 10 minutes.

After heating, the organic sample was extracted using hexane and water was added to the reaction mixture. The mixture was shaked vigorously by hand for 2mins, a stable emulsion was formed, centrifugation was used to break the emulsion into layers. About half of the top hexane phase was transferred to a small test tube for injection. Adequate care was taken at this point to remove only the organic layer into a tube for injection. Injection directly from the reaction vial is usually discouraged because of the risk in injecting water, for it can ruin GC column.

Fixed Setting of Apparatus:

Generally, gas flows to the columns, the inlets, the detectors, and the split ratio. In addition, the injector and detector temperatures must be set. The detectors are generally held at the high end of the oven temperature range to minimize the risk of analyte precipitation.

Set the oven temp to 180^0c and allow the GCMS to warm up, when the instrument is ready, usually the not ready light will be turned off, and sample now runned. Using a vial, 1 microliter of the sample was injected into the sample injection port.

Preparation of standard

10ul of standard was injected in the chromatography and the retention time compared with retention time of standard.

The GC–MS Analysis

The GC–MS analysis of bioactive compounds from the different extracts was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV).

Pure helium gas (99.995 %) was used as the carrier gas with flow rate of 1 mL/min.

The initial temperature was set at 120 –180 °C with increasing rate of 3 °C/min and holding time of about 10 min.

Finally, the temperature was increased to 300 °C at 10 °C/min.

One microlitre of the prepared 1 % of the extracts diluted with respective solvents was injected in a splitless mode. Relative quantity of the antibiotic residue present in each of the extracts was determined based on peak area produced in the chromatogram.

Statistical Analysis

The data obtained from the study were statistically analysed using the statistical package for social science (SPSS) version 20.0, (Chicago USA). Analysis of variance would be used to check for the significant mean difference between the detected drug residues followed by Post Hoc Duncan test to measure specific differences between pairs of mean. Values were presented as mean = standard deviation and level of significance set at < 0.05.

RESULTS

Antibiotics Residue in Liver, Gills and Muscles of fish (*Clarias garienpinus*) Samples

The result for the analysis of antibiotics residue in fish organs was represented in Table 1. It was observed that drug residues: such as tylosin, avilamycin, amoxicillin, chloramphenicol, gentamicin, lincomycin, acrolides, and quinolone were present in all the organs investigated (liver, gills, and muscles). The result showed the mean drug residue concentrations were higher in the order; macrolides > quinolone > lincomycin > chloramphenicol > amoxicillin > gentamincin > avilamycin > tylosin. Tylosin showed the least concentration in these organs while macrolides had

the highest concentration in the organs. In the liver, the concentration of tylosin, chloramphenicol and quinolone were highest with mean concentrations of 1.77 ± 0.50, 14.68 ± 0.51and 25.93 ± 5.33 µg/kg respectively. In the gills, concentrations of avilamycin, amoxicillin and gentamicin were observed to be highest with mean concentrations of (6.85 ± 0.39, 11.01 ± 0.34 and 11.20 ± 0.24 µg/kg) respectively. Equal concentrations of lincomycin was observed in the liver, gills and muscles of the fish samples with mean concentration of 21.68 ± 0.43 µg/kg. The muscles of the samples showed the least concentrations of most of the drug residues except the concentration of chloramphenicol which was observed to be higher in the muscle 12.12 ± 0.36 µg/kg than in the gills 12.00 ± 0.33 µg/kg of the samples. Also, the mean concentration of chloramphenicol in the muscles (12.06 ± 0.35 µg/kg) were observed to be equal to the concentration observed in the gills. The concentrations of macrolides residues were observed to be highest amongst the analysed organs with mean concentration of (35.78 ± 0.44 µg/kg) compared to the other antibiotics investigated.

Table 1: Concentration of drug residues in *Clarias garienpinus* from the selected Local Government Areas

Location	Tylosin	Avilamycin	Amoxicillin	Chloramphenicol	Gentamicin	Lycomycin	Macrolides	Quinolones
Nsukka	3.84±0.30^{a}	2.55±0.20^{a}	8.99±0.22^{a}	13.82±0.50^{a}	6.36±0.10^{a}	21.46±0.20^{a}	29.70±0.41^{a}	10.20±0.10^{b}
Igbo Etiti	2.15±0.31^{a}	5.44±0.40^{b}	9.23±0.10^{a}	12.99±0.55^{a}	16.13±0.40^{b}	18.88±0.12^{b}	30.17±0.55^{a}	27.14±0.22^{a}
Awgu	0.00±0.00	9.03±0.55^{c}	11.53±0.31^{b}	18.39±0.61^{b}	0.00±0.00	24.84±0.50^{a}	36.11±0.16^{b}	39.05±0.65^{c}
Ezeagu	1.68±0.30^{b}	3.88±0.21^{a}	10.70±0.40^{b}	4.74±0.05^{c}	15.96±0.30^{b}	23.03±0.61^{a}	67.05±1.22^{c}	10.92±0.12^{b}
Nkanu	0.42±0.05^{c}	7.59±0.22^{c}	9.93±0.30^{a}	12.77±0.12^{a}	16.07±0.21^{b}	23.73±0.60^{a}	26.69±0.44^{d}	36.93±0.22^{c}
Enugu South	0.60±0.05^{c}	5.75±0.12^{b}	9.83±0.30^{a}	14.90±0.21^{b}	0.00±0.00	18.15±0.40^{b}	24.98±0.30^{d}	30.90±0.30^{c}
MRL (EUL, 1990; 2008)	100	200	50	0.2	100	100	50	100

Results are in mean±SE. MRL – Maximum Residue Limit. Same alphabets within a column are not significantly different ($p<0.05$)

Antibiotics concentration in the liver of *C. garienpinus* samples from the selected L.G.A in Enugu State

The concentrations of tylosin, avilamycin, amoxicillin, chloramphenicol, gentamicin, lincomycin, macrolides and quinolone were investigated in the liver of fish samples collected from different local government areas in Enugu State. Avilamycin, gentamicin and quinolone were not observed in the liver of fish samples from Nsukka. Tylosin and gentamicin were not observed in the liver of fish samples from Awgu L.G.A. Also, gentamicin was not observed in the liver of fish samples from Enugu South L.G.A (Table 2). The entire drug residues investigated were observed in the liver samples from Igbo Etiti, Ezeagu and Nkanu L.G.A. The concentration of tylosin was observed to be highest in the samples from Nsukka (4.17 ± 0.90 µg/kg) and lowest in samples from Enugu South (1.19 ± 0.90 µg/kg). Avilamycin concentration was observed to be highest in liver samples from Awgu L.G.A (9.08 ± 0.80 µg/kg). The concentrations of amoxicillin, chloramphenicol, lincomycin, macrolides and quinolone were observed to be highest in liver samples from Awgu L.G.A (17.30 ± 0.40, 18.92 ± 0.80, 25.32 ± 0.40, 36.21 ± 0.40 and 38.96 ± 0.40 µg/kg respectively). Liver sample of fishes from Enugu South had the lowest concentrations of lincomycin and macrolides (18.47 ± 0.50 and 25.17 ± 0.40 µg/kg respectively). Liver sample from Nsukka L.G.A had the lowest concentration of amoxicillin (9.37 ± 0.90 µg/kg). The lowest concentration of chloramphenicol was observed in liver samples from Nkanu L.G.A (12.74 ± 0.40 µg/kg). Liver samples from Igbo Etiti had the lowest concentrations of gentamicin (16.10 ± 0.40 µg/kg) and quinolone (16.18 ± 0.40 µg/kg). Lincomycin and macrolides concentrations were lowest in samples from Enugu South L.G.A (18.47 ± 0.50 and 25.12 ± 0.40 µg/kg respectively) as shown in (Table 2).

The concentration of tylosin, avilamycin, amoxicillin, gentamicin, lincomycin, macrolides and quinolone in the liver of all the samples investigated from the different Local Government Areas were below the maximum residue limit set by the European Union Legislation (1990; 2008); 100, 200, 50, 100, 100, 50 and 100 µg/kg respectively. The concentration of chloramphenicol in all the liver samples from the different L.G.As were above the EU maximum residue limit (0.2 µg/kg).

Table 2: Concentration of drug residue in the liver of *C. garienpinus* samples from the selected Local Government Areas in Enugu State

Location	Tylosin (µg/kg)	Avilamycin (µg/kg)	Amoxicillin (µg/kg)	Chloramphenicol (µg/kg)	Gentamicin (µg/kg)	Lincomycin (µg/kg)	Macrolides (µg/kg)	Quinolone (µg/kg)
Nsukka	4.17±0.90^{a}	0.00±0.00	9.37±0.90^{a}	14.23±0.55^{a}	0.00±0.00	21.74±0.90^{a}	29.73±0.37^{a}	0.00±0.00
Igbo Etiti	2.28±0.40^{b}	5.81±0.90^{a}	9.55±0.80^{a}	13.07±0.40^{b}	16.10±0.40^{a}	19.16±0.90^{b}	31.39±2.27^{b}	16.18±0.40^{a}
Awgu	0.00±0.00	9.08±0.40^{b}	17.30±0.40^{b}	18.92±0.90^{c}	0.00±0.00	25.32±0.40^{c}	36.21±0.40^{c}	38.96±0.40^{b}
Ezeagu	1.69±0.40^{c}	5.90±0.40^{a}	10.03±0.89^{c}	14.21±0.40^{a}	16.14±0.40^{a}	23.07±0.40^{a}	27.06±0.40^{a}	32.76±0.40^{c}
Nkanu	1.27±0.40^{c}	7.69±0.55^{c}	10.07±0.40^{c}	12.74±0.40^{b}	16.17±0.40^{a}	23.69±0.40^{a}	27.07±0.40^{a}	36.83±0.40^{b}
Enugu South	1.19±0.90^{c}	5.79±0.45^{a}	10.20±0.90^{c}	14.92±0.40^{a}	0.00±0.00	18.47±0.50^{b}	25.17±0.40^{d}	30.87±0.40^{d}
MRL (EUL, 1990; 2008)	100	200	50	0.2	100	100	50	100

Results are in mean ± SE; MRL – Maximum Residue Limit. Same alphabets within a column are not significantly different ($p<0.05$)

Antibiotics concentration in the gills of *C. garienpinus* samples from the selected L.G.A in Enugu State

The concentrations of all the drug residue investigated (tylosin, avilamycin, amoxicillin, chloramphenicol, gentamicin, lincomycin, macrolides and quinolone) were observed in the gills of fish samples from Nsukka and Igbo Etiti L.G.A. as shown in (Table 3). Tylosin and gentamicin were not observed in the gill samples from Awgu L.G.A. Likewise, tylosin was not observed in gill samples from Nkanu L.G.A. Chloramphenicol and quinolone were not observed in the gill samples from Ezeagu L.G.A. while gentamicin was not observed in the gill samples from Enugu South L.G.A. The concentrations of tylosin and gentamicin were highest in gill samples from Nsukka L.G.A (3.68 ± 0.40 and 19.07 ± 0.50 µg/kg respectively). The concentrations of avilamycin, amoxicillin, chloramphenicol, lincomycin and quinolone were high in the gill samples from Awgu L.G.A (9.18 ± 0.50, 17.30 ± 0.40, 18.42 ± 0.41, 25.42 ± 0.50 and 39.07 ± 0.51 µg/kg respectively). Macrolides concentration was observed to be highest in the gill samples from Ezeagu L.G.A (147.06 ± 1.20 µg/kg). Gill samples from Nsukka L.G.A had the lowest concentrations of amoxicillin (8.87 ± 0.50 µg/kg) and quinolone (30.59 ± 0.40 µg/kg). Igbo Etiti L.G.A gill samples had the lowest concentration of avilamycin (5.18 ± 0.40 µg/kg). The lowest concentration of gentamicin was observed in the gill samples from Ezeagu L.G.A (15.87 ± 0.13 µg/kg). Gill samples from Awgu L.G.A had the highest concentration of chloramphenicol (18.42 ± 0.41 µg/kg). The lowest concentrations of tylosin, lincomycin and macrolides were observed in the gill samples from Enugu South L.G.A (0.61 ± 0.39, 17.99 ± 0.02 and 24.88 ± 0.12 µg/kg respectively).

All the drug residue investigated were observed to be below the EU maximum residue limit (tylosin 100 µg/kg, avilamycin 200 µg/kg, amoxicillin 50 µg/kg, gentamicin 100 µg/kg, lincomycin 100 µg/kg, macrolides 50 µg/kg and quinolone 100µg/kg) except for macrolides in the gills of samples from Ezeagu (147.06 ± 0.40 µg/kg) which was above the EU maximum residue limit (50 µg/kg). The concentration of chloramphenicol observed in all the samples were also above the EU maximum residue limit (0.2 µg/kg)

Table 3: Concentration of drug residue in the gills of *C. garienpinus* samples from the selected Local Government Areas in Enugu State

Location	Tylosin (µg/kg)	Avilamycin (µg/kg)	Amoxicillin (µg/kg)	Chloramphenicol (µg/kg)	Gentamicin (µg/kg)	Lincomycin (µg/kg)	Macrolides (µg/kg)	Quinolone (µg/kg)
Nsukka	3.68±0.40^{a}	7.66±0.40^{a}	8.87±0.40^{a}	13.02±0.50^{a}	19.07±0.50^{a}	21.29±0.45^{a}	29.68±0.32^{a}	30.59±0.40^{a}
Igbo Etiti	2.24±0.36^{b}	5.18±0.40^{b}	9.26±0.51^{a}	13.07±0.40^{a}	16.10±0.40^{b}	18.76±0.50^{b}	29.56±0.44^{a}	32.75±0.40^{a}
Awgu	0.00±0.00	9.18±0.50^{c}	17.30±0.40^{b}	18.42±0.41^{b}	0.00±0.00	25.42±0.50^{c}	36.21±0.41^{b}	39.07±0.51^{b}
Ezeagu	1.79±0.50^{b}	5.74±0.26^{b}	11.12±0.20^{c}	0.00±0.00	15.87±0.13^{b}	23.17±0.50^{a}	147.06±0.40^{c}	0.00±0.00
Nkanu	0.00±0.00	7.57±0.43^{a}	9.86±0.15^{a}	12.74±0.40^{a}	16.17±0.40^{b}	23.84±0.55^{a}	26.84±0.16^{d}	37.03±0.60^{c}
Enugu South	0.61±0.38^{c}	5.74±0.40^{c}	9.65±0.35^{a}	14.76±0.24^{c}	0.00±0.00	17.99±0.02^{b}	24.88±0.12^{d}	30.98±0.50^{a}
MRL (EUL, 1990; 2008)	100	200	50	0.2	100	100	50	100

Results are in mean ± SE; MRL – Maximum Residue Limit. Same alphabets within a column are not significantly different ($p<0.05$)

Antibiotics concentration in the muscles of *C. garienpinus* samples from the selected L.G.A in Enugu State

The result in Table 4 showed the highest concentration of tylosin was observed in the muscle of samples from Nsukka L.G.A (3.12 ± 0.38 µg/kg) and the lowest concentration was observed in samples from Ezeagu L.G.A (1.40 ± 0.27 µg/kg). Tylosin was not observed in muscle samples from Awgu, Nkanu and Enugu South L.G.A. The concentration of avilamycin was observed to be highest in muscle samples from Awgu L.G.A (8.84 ± 0.16 µg/kg) and lowest in muscle samples from Igbo Etiti L.G.A (5.32 ± 0.55 µg/kg). Avilamycin was not observed in the muscle samples from Nsukka and Ezeagu L.G.A. Muscle samples from Ezeagu L.G.A had the highest concentration of amoxicillin (10.96 ± 0.04 µg/kg) and muscle samples from Nsukka had the lowest concentration of amoxicillin (8.73 ± 0.27 µg/kg). Amoxicillin was not observed in muscle samples from Awgu L.G.A. Gentamicin concentration was observed to be highest in muscle samples from Igbo Etiti L.G.A (16.20 ± 0.50 µg/kg) and lowest in muscle samples from Ezeagu L.G.A (15.87 ± 0.13 µg/kg). Gentamicin residue was not observed in the muscle samples from Nsukka, Awgu and Enugu South L.G.A. Lincomycin was observed in all the samples from all the different local government areas. The highest concentration of lincomycin was observed in samples from Nkanu L.G.A (23.65 ± 36 µg/kg) while the lowest concentration was observed in samples from Enugu South L.G.A (17.99 ± 0.15 µg/kg). Also, the macrolides residue was observed in the all the samples from all the Local Government Areas investigated. The highest concentration of macrolides was observed in samples from Awgu L.G.A (35.90 ± 0.10 µg/kg) and the lowest concentration was observed in samples from Enugu South L.G.A (24.88 ± 0.12 µg/kg). The concentration of quinolone was observed to be highest in samples from Awgu L.G.A (39.12 ± 0.34 µg/kg) and lowest in samples from Enugu South L.G.A (30.34 ± 0.36 µg/kg). Quinolone residue was not observed in samples from Nsukka and Ezeagu L.G.A (Table 4).

The concentration of all the drug residue investigated except for chloramphenicol (0.2 µg/kg) were observed to be below the EU maximum residue limits (tylosin 100 µg/kg, avilamycin 200 µg/kg, amoxicillin 50 µg/kg, gentamicin 100 µg/kg, lincomycin 100 µg/kg, macrolides 50 µg/kg and quinolone 100µg/kg).

Table 4: Concentration of drug residue in the muscle of *C. garienpinus* samples from the selected Local Government Areas in Enugu State

Location	Tylosin (µg/kg)	Avilamycin (µg/kg)	Amoxicillin (µg/kg)	Chloramphenicol (µg/kg)	Gentamicin (µg/kg)	Lycomicin (µg/kg)	Macrolides (µg/kg)	Quinolone (µg/kg)
Nsukka	3.68±0.38[a]	0.00±0.00	8.73±0.27[a]	14.22±0.48[a]	0.00±0.00	21.35±0.51[a]	29.68±0.32[a]	0.00±0.00
Igbo Etiti	1.94±0.06[b]	5.32±0.55[a]	8.88±0.13[a]	12.83±0.18[b]	16.20±0.50[a]	18.73±0.47[b]	29.56±0.44[a]	32.49±0.41[a]
Awgu	0.00±0.00	8.84±0.16[b]	0.00±0.00	17.82±0.50[c]	0.00±0.00	23.78±0.22[c]	35.90±0.10[b]	39.12±0.34[b]
Ezeagu	1.40±0.27[b]	0.00±0.00	10.96±0.04[b]	0.00±0.00	15.87±0.13[a]	22.84±0.16[a]	27.03±0.37[c]	0.00±0.00
Nkanu	0.00±0.00	7.52±0.37[b]	9.86±0.14[b]	12.82±0.48[b]	15.88±0.12[a]	23.65±0.36[c]	26.17±0.50[c]	36.93±0.50[c]
Enugu South	0.00±0.00	5.66±0.34[a]	9.65±0.35[b]	15.02±0.50[a]	0.00±0.00	17.99±0.02[b]	24.88±0.12[d]	30.84±0.36[a]
MRL (EUL, 1990; 2008)	100	200	50	0.2	100	100	50	100

Results are in mean ± SE; MRL – Maximum Residue Limit. Same alphabets within a column are not significantly different ($p<0.05$)

Comparison of Drug Residue in *Clarias garienpinus* Samples from the Selected Local Government Areas in Enugu State

The levels of drug residues observed in catfish collected from the six Local government Areas investigated were summarized in Figure 2. It was observed that samples from Awgu L.G.A (tylosin 0.00 ± 0.00, avilamycin 9.03 ± 0.55, amoxicillin 11.30 ± 0.31, chloramphenicol 18.39 ± 0.61, gentamicin 0.00 ± 0.00, lincomycin 24.84 ± 0.50, macrolides 36.11 ± 0.16, quinolone 39.05 ± 0.65 µg/kg) had the highest levels of all the drug residue investigated except for macrolides (67.05 ± 1.22 µg/kg) which was highest in samples from Ezeagu. Also, tylosin and gentamicin was not observed in the samples collected from Awgu L.G.A. Samples from Ezeagu LGA (tylosin 1.68 ± 0.30, avilamycin 3.88 ± 0.21, amoxicillin 10.70 ± 0.40, chloramphenicol 4.74 ± 0.50, gentamicin 15.96 ± 0.30, lincomycin 23.03± 0.61, macrolides 67.05 ± 1.22 and quinolone 10.92 ± 0.12 µg/kg) had the second highest levels of drug residue. All the drug residues investigated were present in the samples collected from Ezeagu L.G.A. Samples collected from Nkanu L.G.A (tylosin 0.42 ± 0.05, avilamycin 7.59 ± 0.22, amoxicillin 9.93 ± 0.30, chloramphenicol 12.77 ± 0.12, gentamicin 16.07 ± 0.21 , lincomycin 23.73 ± 0.60, macrolides 26.69 ± 0.44, quinolone 36.93 ± 0.22µg/kg) had the presence of all the drug residues investigated. Although the concentrations of these drug residues were observed to be below the concentrations observed in Awgu and Ezeagu samples; but higher than the concentrations observed in Igbo Etiti (tylosin 2.15 ± 0.31, avilamycin 5.44 ± 0.40, amoxicillin 9.23 ± 0.10, chloramphenicol 12.99 ± 0.55, gentamicin 16.13 ± 0.40, lincomycin 18.88 ± 0.12, macrolides 30.17 ± 0.55, quinolone 27.14 ± 0.22 µg/kg), in Nsukka (tylosin 3.84 ± 0.30, avilamycin 2.55 ± 0.20, amoxicillin 8.99 ± 0.22, chloramphenicol 13.82 ± 0.50, gentamicin 6.36 ± 0.10, lincomycin 21.46 ± 0.20, macrolides 29.70 ± 0.41, quinolone 10.20 ± 0.10 µg/kg), and Enugu South L.G.A (tylosin 0.60 ± 0.05, avilamycin 5.75 ± 0.12, amoxicillin 9.83 ± 0.30, chloramphenicol 14.90 ± 0.21, gentamicin 0.00 ± 0.00, lincomycin 18.15 ± 0.40, macrolides 24.98 ± 0.00, quinolone 30.90 ± 0.30 µg/kg). The entire drug residue investigated were observed in samples collected from Igbo Etiti L.G.A. It was observed that the concentrations of tylosin and chloramphenicol in samples from Igbo Etiti were higher than the concentration observed in samples from Nkanu L.G.A. But the concentrations of avilamycin, amoxicillin, gentamicin, macrolides and quinolone observed in samples from Igbo Etiti L.G.A were lower than the concentrations observed in samples from Nkanu west. Samples from Nsukka L.G.A had the lowest concentrations of the drug residues investigated compared to other Local Government Areas. The entire drug residue investigated was observed in the samples from Nsukka L.G.A but were the lowest concentrations except for tylosin. The concentration of

tylosin observed in samples from Nsukka L.G.A was the highest compare to the concentration of tylosin observed in samples from the other Local Government Areas.

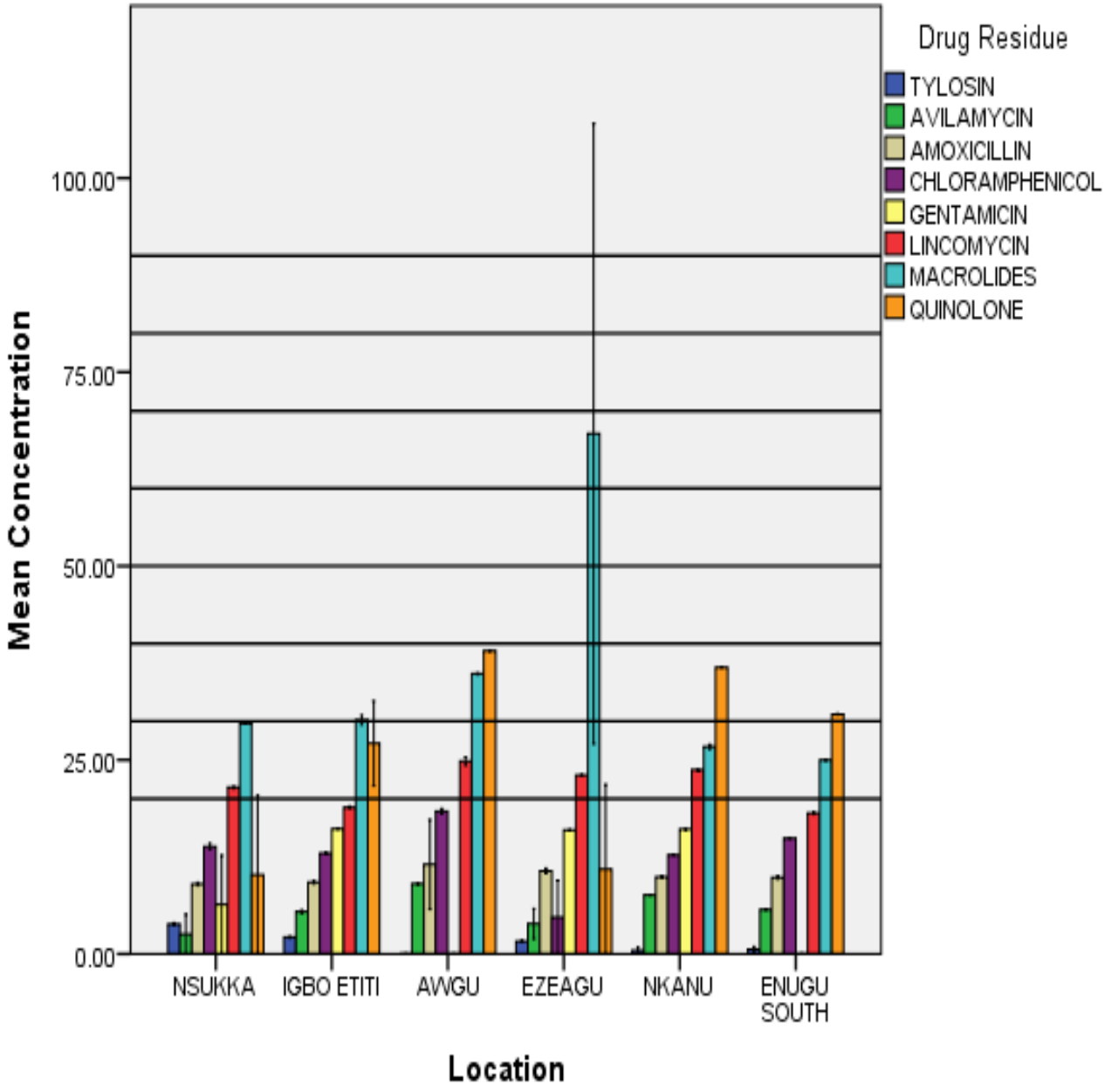

Figure 2: Concentrations of drug residues in *Clarias garienpinus* from the selected Local Government Areas under study

Table 5: Comparison of the mean drug residues concentration in *C. garienpinus* from the selected Local Government Areas

Location	Tylosin	Avilamycin	Amoxicillin	Chloramphenicol	Gentamicin	Lycomycin	Macrolides	Quinolones
Nsukka	3.84±0.30[a]	2.55±0.20[a]	8.99±0.22[a]	13.82±0.50[a]	6.36±0.10[a]	21.46±0.20[a]	29.70±0.41[a]	10.20±0.10[b]
Igbo Etiti	2.15±0.31[a]	5.44±0.40[b]	9.23±0.10[a]	12.99±0.55[a]	16.13±0.40[b]	18.88±0.12[b]	30.17±0.55[a]	27.14±0.22[a]
Awgu	0.00±0.00	9.03±0.55[c]	11.53±0.31[b]	18.39±0.61[b]	0.00±0.00	24.84±0.50[a]	36.11±0.16[b]	39.05±0.65[c]
Ezeagu	1.68±0.30[b]	3.88±0.21[a]	10.70±0.40[b]	4.74±0.05[c]	15.96±0.30[b]	23.03±0.61[a]	67.05±1.22[c]	10.92±0.12[b]
Nkanu	0.42±0.05[c]	7.59±0.22[c]	9.93±0.30[a]	12.77±0.12[a]	16.07±0.21[b]	23.73±0.60[a]	26.69±0.44[d]	36.93±0.22[c]
Enugu South	0.60±0.05[c]	5.75±0.12[b]	9.83±0.30[a]	14.90±0.21[b]	0.00±0.00	18.15±0.40[b]	24.98±0.30[d]	30.90±0.30[c]
MRL (EUL, 1990; 2008)	100	200	50	0.2	100	100	50	100

Results are in mean±SE. MRL – Maximum Residue Limit. Same alphabets within a column are not significantly different ($p < 0.05$)

The Comparison of Mean Drug Residue Concentration Amongst the Organ Samples

The result in Table 5 showed that tylosin, amoxicillin and lycomycin were highest in the liver. Also, avilamycin, gentamicin, macrolides and quinolone were observed to be the highest in the gills of *C. gariepinus* analysed. The result revealed that the muscle had the least level of drug residue except for chloramphenicol which had the highest residue level in the muscle. The drug residue level in the gills were the highest. All the drug residue levels were observed to be below the EU maximum residue level except for chloramphenicol which has (0.2 µg/kg) as EU maximum limit.

Table 6: Comparison of the mean concentration of the organs across the selected Local Government Areas under study

Organ	Tylosin	Avilamycin	Amoxicillin	Chloramphenicol	Gentamicin	Lycomycin	Macrolides	Quinolones
Liver	1.70±0.50^{a}	5.71±0.45^{b}	11.09±0.72^{b}	14.68±0.51^{b}	8.06±0.20^{a}	21.91±0.58^{a}	29.44±0.71^{a}	25.93±0.33^{b}
Gills	1.39±0.27^{a}	6.85±0.39^{b}	11.01±0.34^{b}	12.00±0.34^{a}	11.20±0.24^{b}	21.75±0.42^{a}	49.04±0.31^{b}	28.40±0.40^{c}
Muscles	1.17±0.12^{a}	4.56±0.23^{a}	8.01±0.01^{a}	12.12±0.16^{a}	7.99±0.13^{a}	21.39±0.29^{a}	28.87±0.31^{a}	23.31±0.27^{a}

Results are in mean±SE. MRL – Maximum Residue Limit. Same alphabets within a column are not significantly different ($p < 0.05$)

Comparison of Mean Drug Residue in *Clarias garienpinus* Organ Samples from the Three (3) Senatorial Zones in Enugu State.

The result in table 6 showed the mean levels of drug residues of the analysed samples from the three senatorial zones. The result showed that tylosin and gentamicin are the highest level of residues in Enugu north zone while avilamycin, amoxicillin, lycomycin, and quinolone had the least in this zones.

Amoxicillin, lycomycin and macrolides had the highest drug residue levels in Enugu west zone. Also, avilamycin, chloramphenicol, and quinolone had the highest drug residue levels in Enugu east senatorial zones.

The results showed that Enugu north had the least of drug residue analysed while Enugu east and Enugu west had the highest level of drug residues.

All the drug residues analysed were observed to be below the EU maximum residue limit (Tylosin 100, Avilamycin 200, Amoxicillin 50, Chloramphenicol 0.2, Gentamicin 100, Lycomycin 100, Macrolides 50, Quinolides 100 µg/kg).

Table 7: Comparison of the mean drug residues concentration in *C. garienpinus* for the three senatorial zones

Location	Tylosin	Avilamycin	Amoxicillin	Chloramphenicol	Gentamicin	Lycomycin	Macrolides	Quinolones
Enugu North	2.96±0.31[b]	3.95±0.30[a]	9.11±0.16[a]	13.41±0.53[a]	11.25±0.25[b]	20.17±0.16[a]	29.44±0.35[b]	18.67±0.16[a]
Enugu West	0.84±0.15[a]	6.46±0.38[b]	11.12±0.36[b]	11.57±0.33[b]	7.98±0.15[a]	23.94±0.56[b]	51.58±0.69[c]	27.99±0.39[b]
Enugu East	0.51±0.05[a]	6.57±0.17[b]	9.38±0.30[a]	13.84±0.17[a]	8.04±0.11[a]	20.94±0.50[a]	25.84±0.57[a]	33.92±0.26[c]
Total	**4.43±0.51**	**17.12±0.85**	**30.11±0.82**	**38.82±1.03**	**29.27±0.51**	**65.25±0.22**	**107.36±1.41**	**77.58±0.81**
Mean	**1.44±0.17**	**5.71±0.28**	**10.04±0.27**	**12.94±0.34**	**9.09±0.17**	**21.68±0.41**	**35.99±0.47**	**25.36±0.27**

Results are in mean±SE. MRL – Maximum Residue Limit. Same alphabets within a column are not significantly different ($p<0.05$)

Discussion

The outcome of this research work reviewed that the following antibiotics were present in the analysed African catfish organs in this order; macrolides > quinolone > lincomycin > chloramphenicol > amoxicillin > gentamicin > avilamycin > tylosin. This is in line with Huerta *et. al*. 2018, who observed the presence of antibiotics residues of which most were antileptics and antidepressants drug residues in the fillets of wild fish samples collected from polluted river sites in the USA. Likewise, Rafati *et. al*. 2018, observed antibiotics oxytetracycline residue in the livers and fillets of *Oncorhynchus mykiss* collected from water discharge in Nahavand, Iran.

The presences of these residues could be attributed to the following reasons; farmers not following recommended label directions or dosage (extra-label usage); not adhering to recommended withdrawal times, administering too large a volume at a single injection site, use of drug-contaminated equipment, or failure to properly clean equipment used to mix or administer drugs, dosing, measuring, or mixing errors, allowing animals access to spilled chemicals or medicated feeds, animal effects- age, pregnancy, congenital, illness, allergies, chemical interactions between drugs, variations in water temperature for fish species, environmental contamination. This is in agreement with the reports of Van Dresser and Wilke, 1989 and Kukanich *et. al. (*2005), who reported that the high levels of veterinary drugs in food was due to failure to observe and adhere to the recommended withdrawal periods. Sundlof, 2000 suggested that the improper maintenance of treatment records or failure to identify treated animals

adequately can also lead to their omission. McCaughey *et. al*. 1990, was of the opinion that faecal recycling, where the drug excreted in faeces of treated animals contaminates the feed of untreated animals, can be the cause of residues of certain antimicrobial groups. This is in line with Elliott *et. al.*1994, who said housing of un-medicated pigs in boxes where pigs had previously been treated orally with sulfamethazine resulted in residues in urine, kidney and diaphragm. Kaneene and Miller, 1997 and Higgins *et. al.*1999, argued that high drug residues can also occur as a result of improper use of a licensed product or through the illegal use of an unlicensed substance or extra-label dosages and use. Residues can also occur in calves fed milk and/or colostrum from cows receiving antimicrobials as suggested by Guest and Paige, 1991. In most countries β-lactams are widely applied in mastitis therapy and are consequently the major reason for the presence of inhibitory substances in milk as purported by Sternesjö and Johnsson, 1998. The disease status of an animal and the way in which drugs are administered also influence the potential for residues as they affect the pharmacokinetics of the drugs, metabolism, or the presence of infection and/or inflammation may cause the drug to accumulate in affected tissues as suggested by Kaneene and Miller 1997. Subcutaneous and intramuscular administrations increase the potential for residues at the injection sites as suggested by Kaneene and Miller (1997) and Berands *et. al*. (2001). Secondary drug concentration peaks in plasma have been detected after subcutaneous injections of benzathine procaine penicillin. Contamination of feeding stuffs could also be an important source of unintended application of antimicrobials as suggested byMcEvoy, 2002. In a survey carried out in Northern Ireland antimicrobials were detected in 44 % of feeds declared by the manufacturers to be free of medication (Lynas *et. al*. 1998). Residual quantities of medicated feed may be retained at various points along the production line, contaminating subsequent batches of feed as they are processed according to Kennedy *et. al.* 2000. Data from a sulfamethazine residue programme suggested that 25 % of violations were due to inadequate cleaning of feed mixers (Guest and Paige, 1991).

Amongst the local governments within the study areas it was discovered that Awgu local government as well as Enugu west senatorial zone had the highest drug residue level, this could be linked to farmers from this Awgu LGA and Enugu West senatorial zone possessing a limited knowledge of drugs residues in aquatic animals and its implication on the food safety and consumer health. This could also be tied to socio-demographic characteristics, pattern of application and level of awareness on dangers of use of antimicrobial agents. This supports the ascertion of Abiola *et. al.* (2020), that awareness level of most farmers are low resulting in drug residue occurrence. It also lend taught to Banrie, (2013), who suggested farmers have limited

knowledge of antibiotics and their decision- makingprocessfarm owners depended on consultations fromsellers and manufacturers for antibiotics, who may encourage them to use antibiotics indiscriminately to make profit.Olufemi Olatoye and Basiru Afisu, (2013) reported that misuse of antibiotics in aquaculture production without veterinary prescription and control coupled with lack of awareness of the food safety consequences were the contributing factors for the high level of residue violation. Samwel Limbu *et. al.* (2020), suggested that the rearing of fish in intensive systems reduced their immunity leading to eruption of diseases, consequently prompting the use of antibiotics. Similarily, Okoacha *et. al.* 2020, highlighted that farmers with secondary and tertiary education were more likely to produce fish that contained antibiotics residues than those with primary education, while fish farms managed by men were about three times more likely to contain residues than those managed by women. Also, Idowu *et. al.* 2010 suggested that two- third of farmers were not adhering to the recommendation of drug use and thus allowing drug residues in egg. Olatoye *et. al.* 2010 reported that the high level of drug residue was as a result of the indiscriminate and misuse of veterinary drugs as commonly practiced among livestock producers and marketers without observing withdrawal period prior to slaughter. Beyene, 2016, argued that the most likely reason for drug residues maybe as a result human management, such as improper usage, including extra-label or illegal drug applications.
Contrary to the above views is Esther *et. al.* 2005, who argued that other practices such as manure use and untreated waste disposal may contribute to antibiotic resistance on fish farms in Ghana not use of antibiotics.

Amongst the analysed organs, the gills had the highest, followed by the liver and then the muscle Table 7, this could be attributed to the gills being the site for drug action in fish, as well as the muosa.This also confirmed the opinion of Banrie (2013), who noted that the gills and gut mucosa are sites of drug action where there is high rate of blood circulation. Also, the fish gills serve as a multi-functional organ in that it serves for gaseous exchange, play other role which includes; osmotic and ionic regulation, acid-base regulation and excretion of nitrogenous waste. This was in agreement with David *et. al.* (2003), who suggested that the gill epithelium is the site of many processes that are mediated by the renal epithelia in the terrestrial vertebrates. Beyene *et. al.* (2016), was of the opinion that veterinary drug residues usually accumulate in the liver or kidney rather than other tissues. He also argued that the different residue levels can be found in the different tissue positions such as site and routes of administration. Mensah *et. al.* 2019 and Rafati *et. al.* 2018, also observed higher concentrations of oxytetracycline in the liver of *O. mykiss samples* collected from Nahavand, Iran. In contrast to these findings, Mensah *et. al. 2019,*

observed high concentrations of tetracyclines (about 11. 1 %) in the muscles of *C. garienpinus* and *O. niloticus* samples collected from Benin. They noted that drug residues were not observed in any other fish tissue other than the muscles.

Conclusion

The study has shown the presence of antibiotic drug residue in fish samples collected from six local government areas in Enugu State. The study indicated disparities in the concentrations of drug residues observed in the samples from the different Local Government Areas. The muscle of the fish sample had the lowest levels of drug residue compared to the liver and gills. Samples from Nsukka L.G.A had the lowest levels of drug residues; whereas, samples from Awgu L.G.A had the highest levels of drug residues. Amongst the senatorial zones Enugu North L.G.A. had the lowest levels of drug residues; whereas, samples from Enugu West and Enugu East L.G.A. had the highest levels of drug residues. Although the concentrations of these drug residues observed in the samples were below the European Union maximum residue limit, it is important to control and monitor the contamination of edible food source by antibiotics residues in order to prevent the consequences it poses to human health.

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