

# Prevalence of oxytetracycline residues in farmed African catfish (*Clarias gariepinus*) in Sokoto State, Nigeria

Bello Abdulrashid<sup>1\*</sup>, Usman Mohammed Abubakar<sup>2</sup>, Shehu Umar<sup>2</sup>, Usman Garba Rambo<sup>3</sup>, Saulawa Mahmud Abdullahi<sup>4</sup>, Mohammed Jibrin Salihu<sup>3</sup> and Mohammed Fouad<sup>5</sup>

<sup>1</sup>Ministry of Animal Health and Fisheries Development Sokoto State, Nigeria.

<sup>2</sup>Nigeria Police Veterinary Unit, Abuja FCT, Nigeria.

<sup>3</sup>Veterinary Teaching Hospital, Usmanu Danfodiyo University Sokoto, Nigeria.

<sup>4</sup>Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Bayero University Kano, Nigeria.

<sup>5</sup>ECOWAS Regional Animal Health Center, Parc de Sotuba, BP: E2165, Bamako, Mali.

Corresponding author email: [resheedtambuwal@gmail.com](mailto:resheedtambuwal@gmail.com); Tel: +2348035377779

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Research Article

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### ABSTRACT

*Intensification of aquaculture has led to the increasing use of drugs and other chemicals in order to reduce mortality and increase yield and production. Antimicrobial regimens are being employed prophylactically and therapeutically to combat the challenges of fish health condition and also growth promotion in aquaculture. A number of these chemoprophylactic and chemotherapeutic agents are used in feeds or directly into the culture after lowering the level of water. Oxytetracycline, a broad-spectrum antibiotic is widely used in aquaculture as a therapeutic or prophylactic agent because of its broad spectrum activity and its low price. This study was carried out to determine the prevalence of Oxytetracycline (OTC) residues in cultured African catfish (*Clarias gariepinus*) in Sokoto State, Nigeria. One hundred and eighty (180 table size and ready-for-market catfish were sampled from the selected fish farms across three senatorial zones in Sokoto State for detection of Oxytetracycline residue using quantitative ELISA. The results showed that forty-nine (27.22%) catfish samples contains average Oxytetracycline residue concentration of 349.0925 ppb, which is more than the recommended Maximum Residue Level of 200 ppb. There was no significant statistical association between Oxytetracycline residues detected between three agricultural zones.*

**Keywords:** African Catfish, *Clarias gariepinus*, ELISA, Nigeria, Oxytetracycline Residue, Sokoto State



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### INTRODUCTION

Fish are generally vertebrates which belong to the phylum Chordata and order Siluroformes. It is an important source of food, income, employment and recreation for people around the world. Fish is a very important source of animal protein for both man and livestock in developed

and developing countries (Nelson, 2006). In Nigeria, humans consume approximately 80% of the catch, and the remaining 20% goes to the manufacturing of products such as fish oil, fertilizer, and animal feeds (Ozigbo *et al.*, 2014). Catfish are a diverse group of fish, representing

more than 3,000 species, 478 genera and 36 families. They constitute 1/3 of the freshwater fish fauna of the globe (Lalronunga and Tluanga, 2011). African catfish, *Clarias gariepinus* is a freshwater species found in the Middle East, and throughout West, Central and Southern Africa (Wang *et al.*, 2006). It inhabits natural lakes, fishponds, streams and natural ponds in both deep and shallow water (Turan and Turan, 2016). *Clarias gariepinus* and *Clarias anguillaris* remain the two catfish species most farmed in Africa, even though more than 100 species populate African waters. *Clarias gariepinus* is also commonly referred to as African Catfish, Sharp-tooth Catfish, Common Catfish, Mudfish, Barbel, Sharp-toothed Catfish and North African Catfish (Olayemi and Akinwade, 2013).

Intensification of aquaculture has led to the increasing use of drugs and other chemicals in order to reduce mortality and increase yield and production. Antimicrobial regimens are being employed prophylactically and therapeutically to combat the challenges of fish health condition and also growth promotion in aquaculture. A number of these chemoprophylactic and chemotherapeutic agents are used in feeds or directly into the culture after lowering the level of water.

Oxytetracycline, a broad-spectrum antibiotic discovered in the 1940s is active against a wide variety of bacteria (Rafati *et al.*, 2018). It is widely used in aquaculture as a therapeutic or prophylactic agent because of its broad spectrum activity and its low price. It was the first antibacterial approved by United States Food and Drug Administration for use in finfish aquaculture (USFDA, 2009). The indiscriminate use of antibiotics for veterinary purposes has increasingly become a matter of public health concern. This indiscriminate use of antibiotics could lead to undesirable deposition of their residues in edible tissues offered for human consumption which could pose a health risk to the consumers. The associated public health risk due to indiscriminate use of drugs include; acute cumulative allergic, toxic, mutagenic, teratogenic, carcinogenic effects (Olatoye and Basiru, 2013). Due to the risks associated with the indiscriminate use of antibiotics, it has become necessary to carry out research to determine the presence of antibiotics residues in fish from commercial fish farms, and also create awareness on the deleterious effects of antibiotic residues when they exceed the maximum acceptable limit. The aim of this study was to determine the prevalence of Oxytetracycline residue in cultured catfish in all the three senatorial zones (namely; Southern, Northern and Eastern Senatorial zones) of Sokoto State.

## MATERIAL AND METHODS

### Study Area

The study was conducted in Sokoto State, Nigeria. Sokoto State is located in the Northern Sudan Savanna ecological zone in Northern Nigeria. With a land area of 28,232.37sq kilometers, the state is located between longitudes 11° 30' East and 13° 50' East and between latitude 4° North and

6° North (Umar, 2013). It is bordered in the North by the Niger Republic, Zamfara State to the East and Kebbi State to the South and West (Figures 1 and 2). The population of Sokoto State based on 2016 population estimates by the National Population Commission is 4,998,100. The annual rainfall of Sokoto State stands around 600mm, while mean annual minimum and maximum temperatures of 21.5°C and 34.9°C respectively have been recorded (Umar, 2013). The dry season starts from October and lasts up to April in some parts and may extend to May or June in other parts. The wet season, on the other hand, begins in most parts of the State in May and lasts up to September or October (Adegboye *et al.*, 2016).



Figure 1: Map of Nigeria Showing Sokoto State



Figure 2: Map of Sokoto State, Nigeria

### Study Design

The epidemiological study method used in this study is cross sectional. The study was conducted between January and August 2025.

### Sampling Techniques

A cluster sampling technique was employed. Fish farms in Sokoto State were divided into three according to their location within three senatorial zones namely. Sokoto South, Sokoto North and Sokoto East Senatorial zones. Fifty one (51) fish farms were identified by this study, but only 18 fish farms (13 from north, 3 from South and 2 from east) were active during this study and were all selected for sample collection. Ten (10) table size fish were randomly selected from each of the selected farms at the time of harvest. However, only eight (8) samples could be obtained from farm 3 in the Sokoto South Zone due to unavailability of the fish at the time of sample collection, while two (2) farms each from north and east had eleven samples. Random selection of samples from each farm was done at the time of harvest.

### Sample Population

The sample population includes some of the table size catfish cultured in fish farms across Sokoto State which were collected at the time of harvest from each of the selected farms.

### Sample Size

The sample size was determined using the formulae;

$$n = Z^2 P_{\text{exp}} (1 - P_{\text{exp}}) / d^2$$

Where  $n$  is required sample size,  $P$  is prevalence from previous research work,  $Z$  is level of confidence (95%) and  $d$  is the desired precision (Thrusfield, 2007). Using a prevalence rate of 11.1% detected in a study by Mensah *et al.* (2019).

$$n = (1.96)^2 \cdot 0.111 (1 - 0.111) / (0.05)^2 = 152$$

### Sample Collection

Fifty grams of muscles from each of the 180 ready-for-market-catfish were sampled from the selected fish. The samples were collected aseptically in sample bags and transported in a cool ice pack to the Central Laboratory of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

### Detection of Antibiotic Residues

A total of 180 samples were analyzed. The samples were considered positive when the concentration of the Oxytetracycline exceeds the maximum recommended level (MRL) of 200ppb and considered negative when the concentration of Oxytetracycline is less than MRL of 200ppb.

### Sample Analysis

Oxytetracycline ELISA kit produced by Nanjing ParsBiochemCO., Ltd 188th Kaicheng Road Jiangning Nanjing city China was used. Each kit consists of a 96-well microtiter plate, buffer and standards for Oxytetracycline.

### Kit Sensitivity and Cross-Reaction Rate

Kit Sensitivity: 0.05ppb (ng/ml)  
 Kit Cross-Reaction Rate: Tetracyclines (100%); Oxytetracycline (107%); Chlortetracycline (16.7%) and Doxycycline (4.2%).

### Principle of the Test Procedure

Oxytetracycline (OTC) ELISA Test Kit is based on an indirect competitive enzyme immunoassay method. The Oxytetracycline (OTC) coupling antigen has been coated in the plate wells. During the detection, samples were added along with the primary antibody specific for the target drug (OCT). If the target is present in the sample, it would compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a Horseradish peroxidase enzyme, targets the primary antibody that is complexed to the drug-coated on the plate wells.

### Test Procedure

#### Sample Pre-Treatment and Drug Extraction

Five grams of the fish muscle was weighed; it was then

homogenized using sterile pestle and mortar. About 2.0g of the homogenized sample was collected into 50ml centrifuge tube, 6ml 1% solution of trichloroacetic acid was added and oscillated for 2min before centrifugation at 4000 r/min at room temperature for 10 min. After centrifugation, 1ML supernatant was transferred to another centrifuge tube and then 1ML of methanol was added, oscillated for 1minute then centrifuged again at 4000 r/min at room temperature for 10 min. 1ML supernatant was then collected into another centrifuge tube and blow-dry at 50--60°C. This is followed by an addition 1ML redissolving solution to dissolve the dried residue. Finally, 1ML of the solute was added to another centrifuge tube before 1ML Methanol was added and oscillated for 1min and centrifuged at 4000 r/min at room temperature for 10 min. About 50µl of the final supernatant was used for the assay.

### Enzyme-Linked Immune Assay Steps

Prior to the commencement of the assay, the microtiter plate and the reagents required were taken from 4°C cold storage environment and placed at room temperature for over 30 min. This is followed by addition of 50µl of the sample into marked well and then another 50µl of antibody working solution into each well. The plates were covered with the adhesive membrane oscillated gently for 5 seconds and then incubated for 30 min at 37°C. After the incubation, the adhesive membrane was removed and discarded. 350µl washing buffer was pipette into every well, kept for 30 seconds and then drained. 100µl of antibody working solution was then added into each well and incubated for 30 min at 37°C. The same washing procedure was repeated. 50ul Substrate A solution was pipette into each well, this is then followed by another 50ul Substrate B solution to each well, oscillated gently for 5seconds, then incubated for 15 min at 37°C. After incubation 50µl of stop solution was added to each well, oscillated gently to stop the reaction. Addition of the stop solution changed the blue colour of the reaction to yellow. This is then preceded by reading of absorbance 450nm with a microplate reader. These procedures were carried out within 10min.

### Calculations

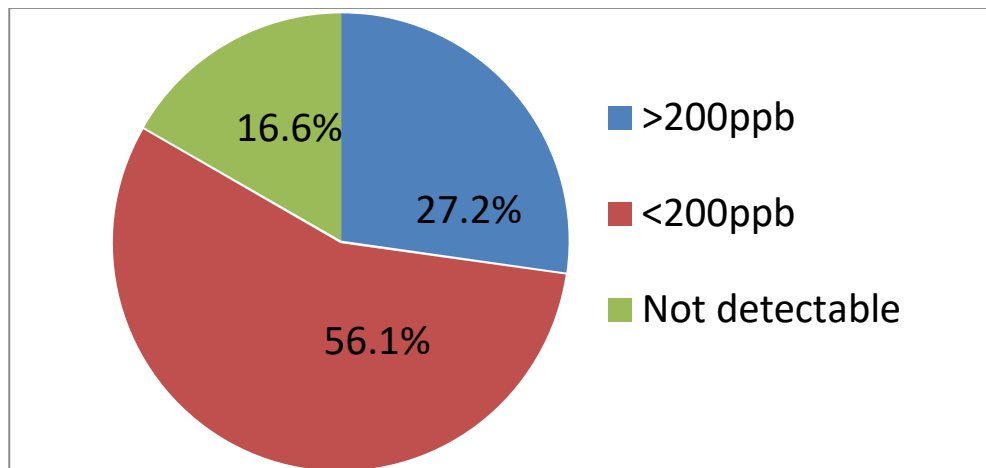
Software provided by the kit manufacturer was used to calculate % absorbance and concentration of Oxytetracycline residue from each sample. The results were tabulated and analyzed.

### Statistical Analysis

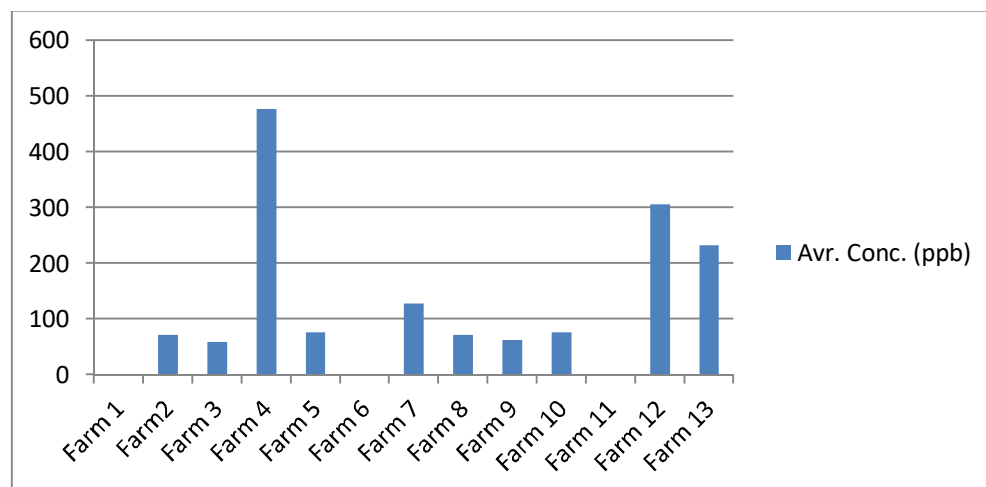
Results of analysed catfish samples were tabulated and also represented in percentages and graphs. The associations between the variables were determined using both Chi-square and Analysis of variance (ANOVA).

## RESULTS

Forty-nine (27.22%) samples out of 180 catfish samples



**Figure 3:** Graphical Representation of the Overall Results of the Analyzed Samples



**Figure 4:** Average Residue Concentration from Each Farm in Sokoto North Senatorial Zone

were observed to contain Oxytetracycline residue concentration above the recommended maximum residue limit (MRL) of 200ppb. Whereas 101 (56.11%) of the sampled catfish were negative with only traceable Oxytetracycline concentration less than the recommend MRL of 200ppb (Figure 3). Thirty (30) samples representing 16.67% were found to be negative with no traceable Oxytetracycline concentration.

#### Sokoto North Senatorial Zone

A total of 131 catfish were sampled from the 13 farms located within Sokoto North Senatorial Zone. Twenty-eight (21.37%) of the samples have Oxytetracycline concentration above MRL (200ppb), while 73 (55.725%) have concentration lower than MRL (200 ppb) and 30 (22.9%) showed no traceable Oxytetracycline residue (Figure 4).

#### Sokoto South Senatorial Zone

A total of 28 catfish were sampled from the only 3 active fish farms in the zone for Oxytetracycline residue detection

and quantification (Figure 5). All the ten samples from Farm 1 were positive with an average residue concentration of 269 ppb, while all samples from Farm 2 of the zone were negative with no detectable Oxytetracycline residue. All the samples from Farm 3 of the zone were negative with an average Oxytetracycline residue concentration of 54.88 ppb which is below the maximum residue limit (MRL) of 200ppb.

#### Sokoto East Senatorial Zone

From the Sokoto east agricultural zone, a total of 21 catfish samples were obtained from only 2 active fish farms. The result showed that all samples in Farm 1 were positive with an average Oxytetracycline residue concentration of 325ppb, all samples in Farm 2 showed no detectable residue (Figure 6).

#### Overall Residue Level across all Agricultural Zones

The relationship between the three zones in terms of Oxytetracycline residue was statistically determined using ANOVA and was discovered that the statistical association

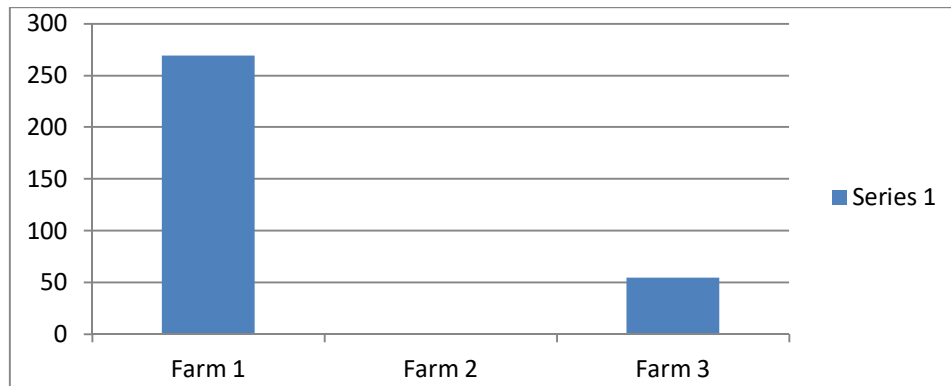


Figure 5: Average Residue Concentration from Each Farm in Sokoto South Senatorial Zone

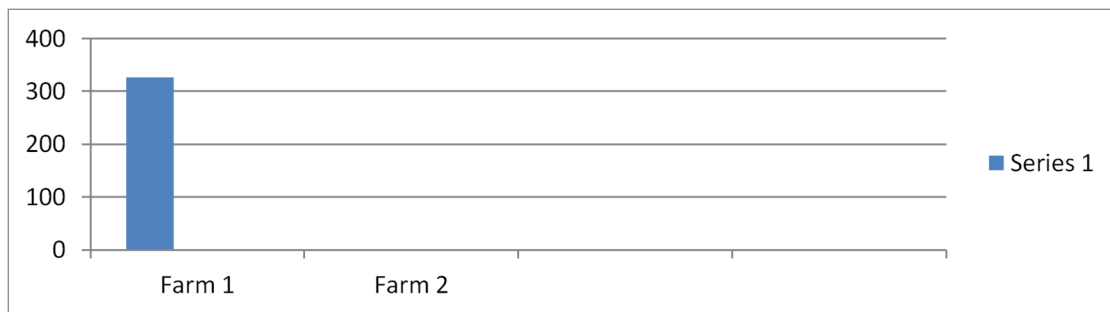


Figure 6: Average Residue Concentration from Each Farm in Sokoto East Senatorial Zone

Table 1: Relationship between Results from All Three Zones.

MRL Location	Below MRL	Above MRL	Total
North	103	28	131
South	18	10	28
North	10	11	21
<b>Total</b>	<b>131</b>	<b>49</b>	<b>180</b>

P = 0.630

Kappa value=0.18p

was not significant ( $p > 0.05$ ). This is despite the fact that over 72% of the samples were obtained from the Sokoto North agricultural zone (Table 1).

## DISCUSSION

The existence of antibiotic residues in foods of animal origin such as meat and fish has attracted attention from local, national and international public health agencies (Ašperger *et al.*, 2009). Several studies have reported that the antibiotic resistance acquired by a large number of bacterial species may be due to exposure to these drugs and resistance genes may be transmitted to human and fish pathogens (Grigorakis and Rigos, 2011). Besides, human consumption of a large quantity of fish and fish products with antibiotic residues which may cause unfavourable changes in intestinal microbiota and create immunological response reactions in susceptible persons (Mottier *et al.*, 2003). The results of the present study are of serious concern since 27.22% of the samples revealed

detectable Oxytetracycline residue above maximum recommended limit (MRL). This high prevalence (27.22%) of positive residue level could be as a result of indiscriminate use of antibiotics in aquaculture or lack of observance of the withdrawal.

This study confirmed that catfish farmers in Sokoto State were also engaged in uncontrolled administration of antibiotics in aquaculture in Sokoto State. Oxytetracycline detected in catfish is very important to human health and medicine. Due to this importance, they are still found on the list of the 2016 revised World Health Organization (WHO) of critically important antimicrobials for human medicine (WHO CIA list), where they are classified as critically important and highly essential antimicrobials respectively (WHO, 2016). Based on the results of this study, consumers of catfish in Sokoto State have 27.22% chances of consuming unsafe and unwholesome cultured catfish. This could increase their chances of acquiring antimicrobial resistance in their vicinity. The results of this study are consistent with the findings of the previous study conducted in Ibadan, where an appreciable prevalence

(39.4% and 30.0% of liver and fillets respectively) of Oxytetracycline residue was observed thereby confirming the drug as the most frequently used antibiotic in catfish (Olatoye and Basiru, 2013). The result of this study is also consistent with the study conducted in Gaza, Palestine, which revealed the presence of antibiotic residues (86.7%) in most of the domestically farmed fish species such as Gilthead, Seabream, Red and Nile tilapia sold in Gaza strip (Asmaa, 2017). It is also supported by the Occurrence of antibiotic residues in raw fish *Clarias gariepinus* and *Oreochromis niloticus* from intensive rearing system in Benin republic by Mensa *et al.* (2019) in which tetracycline residues were found in 11.1% of the fish muscle samples regardless of species and age group.

Within the study areas, Sokoto central zone with the highest number of farms (72.5%) has the lowest proportion of positive samples (17.56%) compared to Sokoto central and Sokoto north whose proportions for the positive sample were 35.71% and 47.62% respectively. There seems to be a significant difference when the proportion of positive and negative samples between the three senatorial zones were analyzed ( $P > 0.05$ ). This might be as a result of the continuous veterinary enlightenment which is more available in the Sokoto Central and Sokoto South senatorial zones, compared to the Sokoto North senatorial zone.

## Conclusion

This study assessed the use of antibiotics in aquaculture and determined the presence of Oxytetracycline residues in cultured catfish intended for human consumption in Sokoto State. Findings revealed that more than 27% of the sampled catfish contained Oxytetracycline residues above the maximum recommended level of 200 ppb, rendering them unwholesome and unsafe for consumers. The indiscriminate use of antibiotics was linked to limited awareness of public health risks, inadequate veterinary services, and weak regulatory oversight on veterinary drug usage in aquaculture. Given these findings, urgent policy action is required. Government at all levels should intensify awareness campaigns on modern aquaculture practices, responsible antibiotic use, and strict observance of withdrawal periods. Through participatory extension services, aquaculturists should be encouraged to adopt innovative fish farming techniques that minimize reliance on antibiotics. Strengthening and enforcing regulations on veterinary drug use, alongside improved access to veterinary services, will help safeguard public health. Furthermore, farmers should be motivated to form cooperatives, enabling them to benefit from extension programs, shared resources, and collective advocacy. By implementing these measures, Sokoto State can promote safer aquaculture practices, protect consumers, and ensure the sustainability of fish farming as a vital source of food and livelihood.

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