

Evaluation of Tetracycline Residues in Pork Organs and Tissues Sold for Human Consumption in Lagos and Ogun States of Nigeria

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Abstract

A study was conducted to evaluate the concentrations of tetracycline residues in organs and tissues of pork sold for human consumption in selected markets in Lagos and Ogun States of Nigeria. Thirty-six (36) fresh pork samples (kidney, liver and muscles) were collected from markets in twelve local government areas in each of the two states, making a total of 72 samples. The controls were growing pigs raised without the use of any antibiotics in the University Pigsty. They were slaughtered after nine weeks of feeding, samples were collected and analysed. The sample analysis was done using the microbiological assay method whereby tetracycline residues were detected after incubation on the Antibiotic Agar 2 medium seeded with *Bacillus subtilis* (ATCC 6633). Results showed that the concentration of the antibiotic residues in the muscle samples ranged from 0.099 µg/g in Odeda L.G (Ogun State) to a significantly higher ($p < 0.05$) 0.447 µg/g in Agege L. G (Lagos State). In the liver samples, the highest value of 0.455 µg/g was also recorded in Agege L. G compared to 0.154 µg/g in Abeokuta North L. G (Ogun). The kidney samples ranged from 0.241 µg/g in Owode L. G (Ogun) to 0.574 µg/g in Lagos State. The mean concentration value of 0.209 µg/g of the tetracycline residues was recorded in the Lagos State was significantly higher ($P < 0.001$) than that of Ogun State (0.070 µg/g). This result emphasize that tetracycline antibiotics were used by farmers without adhering to the withdrawal time period by drug manufacturers, posing a threat to human health which might lead to antibiotics resistance problems amongst others. It is concluded that the samples from Lagos state have more of the tetracycline residues than that of Ogun, therefore antibiotics should be judiciously used (if at all) in the animal production practices.

Keywords: Tetracycline; Residues; Antibiotic resistance; Agar; *Bacillus*; Kidney; Liver

Introduction

Antibiotics are widely used as feed additives in farm animals for the purpose of improving the health status and conditions of the farm animals. In animal production, farmers used different production systems and methods to rear the farm animals, and different health conditions are presented at different environments and times (Olomu and Oboh, 1995).

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There are different systems of pig production ranging from extensive, semi-intensive and intensive systems, depending on farmer's preference and availability of capital. (Adebambo, 1988; Olomu and Oboh, 1995 and Tewe, 1997). In animal production, different feed additives are used and antibiotic forms a major part. Antibiotic as a chemotherapeutic agents are chemical substance produced wholly or partially by a micro-organism (usually a bacterium or fungi), which has the capacity to inhibit the growth (bacteriostatic) or to destroy (bactericidal) bacteria and other organisms (Stewart and Beswick, 1977; Gracey, 1986 and Bedford, 2000). Pena., *et al.* (2004) reported that of all the antibiotics, tetracyclines have been one of the most widely used.

In food-producing animals, the use of antibiotic agents has become a very important public health issue; this is due to the fact that these agents are being increasingly used in animal farm production. Antibiotic residues can be defined as existence of small amount of an antibiotic or its breakdown product(s) which remains in or on the animal tissues or products following treatment with antibiotics (Strasie., *et al.* 1984; Walker, 1993). It is also the portion of the antibiotic drug or its metabolite retained in the animal tissue meant for human consumption (Collison., *et al.* 1992; Mellor, 2000).

There is need to prevent the case of abuse of antibiotic usage, which can lead to resistance syndrome in bacterial organism. Also, public opinion had demanded that food of animal origin for human consumption must have low limits of tolerance or maximum residue limits (MRLs) and must be free of any residue of substances like antibiotics, pesticides etc. (Mudambi and Rajagopal, 1985; Lamorde, 1988; Hall., *et al.* 2003).

In Nigeria, data banks for these types of studies are very few as previous studies on antibiotics residues were reported by Oyekunle and Olubi (1992). Factors such as route of administration, the dosage and withdrawal period are important in the formation of residue of tetracycline antibiotics in the organs of animals. "Withdrawal period" is the interval from the time an animal is removed from medication until the permitted time of slaughter to prevent the occurrence of residues in slaughtered animals. It is the period between the administrations of the last dose of a drug and slaughtering of the animal as prescribed on the drug label by the manufacturer. When such tissues are consumed, it can lead to serious health implications and problems of which the major hazard is antibiotic drug resistance. This research therefore wants to evaluate the existence of any deposits of tetracycline residues in organs and tissues of pork sold for human consumption in selected markets in Lagos and Ogun states of Nigeria.

Material and Methods

Description of the Study Area: The study was conducted in two States of Nigeria-Lagos and Ogun States respectively. They are both located in the south-western region of the country with a humid climate (Figures 3 and 4). Ogun State was created alongside six other States in 1976, out of the old western State of Nigeria. It is neighbored in the East by Ondo State, in the West by Republic of Benin, in the North by Oyo State and in the South by Lagos State and the Atlantic Ocean. The State has a tropical climate with rain-forest vegetation on its southern part and a derived savannah on its Northern end. It covers a total land mass of 16,409.26 sq. km. The average annual rainfall is about 1200 mm, with annual mean temperature of 34.5°C. It has a population of 2.4 million. It has Twenty local government and the following were used for the study:-*Ifo, Ewekoro, Sagamu, Ijebu-Ode, Abeokuta South, Abeokuta North, Egbado South, Egbado North, Obafemi-Owode, Odeda, Ado-Odo/Ota and Ikene.*

Lagos State has an annual temperature ranging from 21°C-34°C while annual rainfall is between 2000 mm- 3000 mm with high relative humidity. It has twenty local governments and the following were used:-*Oshodi/I sola, Mushin, Alimosho, Epe, Ifako/Ijaye, Somolu, Kosofe, Agege, Ikorodu, Apapa, Ibeju/Lekki and Lagos-Island.*

Pigs, Housing Diet and Management: The samples used for the control experiment were collected from Six (6) grower pigs (four Large White and two Hampshire breeds) raised at the Teaching and Research Farm of the University of Agriculture, Abeokuta. The floors of the pig house were all concreted, while roofing was made of asbestos material. Grower ration (Table 1) was given to the pigs and were allowed access to water *ad libitum*. They were intensively managed for nine (9) weeks and throughout this period; no antibiotic was administered to the pigs.

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The growth performance of the pigs was measured and determined on weekly basis. The weight gain, feed intake and feed to gain ratio were measured and calculated. This was used as control. After the ninth week, the pigs were all slaughtered and 25 gm samples of *kidney, liver and muscles*, each were aseptically collected for analyses.

Ingredients	%
Maize	380.00
Maize offal	400.00
Brewer's Dry Grain (BDG)	105.00
Full-Fat Soybean (FFSB)	50.00
Fish Meal	50.00
Bone Meal	13.00
Oyster Shell	1.00
Salt	0.50
Premix	0.50
Total	1000

Calculated Analysis

Energy (kcal/kg)	2635.46
Crude protein (%)	16.38

Table 1: Composition of experimental diet (%).

Premix supplied per kg DM of complete diet: 100 mg Fe as FeSO_4 ; 100 mg Zn as ZnSO_4 ; 20 mg Mn as MnO ; 10 mg Cu as CuSO_4 ; 0.30 mg I as Cal; 0.30 mg Se as Na_2SeO_3 ; 5.506 IU vitamin A; 551 IU vitamin D3; 33 IU vitamin E; 3.6 mg vitamin K; 5.5 mg riboflavin; 25 mg D-pantothenic acid; 33 mg niacin; 27 μg vitamin B12; 1.7 mg folic acid; 220 μg biotin; 120 mg choline.

Data Collection: Three samples each including kidney, liver and muscle of pigs were randomly collected from the open markets of each of the twelve locations (local governments) in each state, and were collected in triplicates, making a total of 36 samples from a state. A total of 72 samples from the two states were used for the study.

The control sample was taken from the pigs reared and fed without using any antibiotic in the sty unit of the Federal University of Agriculture, Abeokuta Ogun State (FUNAAB). The meat samples were analyzed for residues of tetracycline using a modification of the microbiological assay method as described by Katz and Fassbender, (1972). This involved the use of a standard test organism *Bacillus subtilis* ATCC 6633 to detect the residues of tetracycline in the pork samples.

Preparation of sample: Twenty-five (25) gm each sample of kidney, liver and muscle of pork (in triplicates) was aseptically weighed into stomacher bag and 100 ml of phosphate buffer pH 4.5 added. This was homogenized in a machine for 3 min.

Aliquots of this were then decanted into centrifuge tubes and centrifuged (4000 rpm for 10 min). One (1) ml supernatant was inoculated into the wells bored on the seeded agar and incubated overnight at 37°C. Each sample was analysed in replicates. The zones of bacteria growth inhibitions produced were measured and the concentrations of the tetracycline residues in the meat samples were determined by extrapolations from the prepared standard curve for the antibiotic.

Statistical Analysis: All data collected were subjected to analysis of variance (ANOVA) using a 2 x 3 and a 3 x 12 factorial arrangement with the following models:-

Model 1:

$$Y_{ijk} = U + ST_i + OR_j + STOR_{ij} + E_{ijk}$$

Where

Y_{ijk} = Dependent variable

U = Overall mean

ST_i = Effect of the i -th state

OR_j = Effect of the j -th organ

$STOR_{ij}$ = Effect of the ij -th interaction of state and organ

E_{ijk} = Random residual error term

Model 2:

$$Y_{ijk} = U + LG_i + ORG_j + LGORG_{ij} + E_{ijk}$$

Where

Y_{ijk} = Dependent variable

U = Overall mean

LG_i = Effect of i -th local govt

ORG_j = Effect of j -th organ

$LGORG_{ij}$ = Effect of ij -th interaction of local govt and organ

E_{ijk} = Random residual error

The factorial in a randomized complete block design was used. Means were separated using Duncan's multiple range tests.

Results and Discussion

In Lagos State the tetracycline residues obtained in the pork sold in the markets at the local governments identified showed that twenty-four (24) of the samples in Lagos State had positive antibiotic residues in them representing 66.67% compared to twelve (12) samples representing 33.3% indicating a negative tetracycline residues in the samples. In Ogun State, tetracycline residues were obtained from seventeen (17) samples representing 47.22% compared to nineteen (19) representing 52.78% which were negative for antibiotic residues. In all, the residues of Tetracycline occurred in 41 samples which is 56.94% out of the total of 72 samples while the remaining 31 (43.06%) were negative for the antibiotic residues (Table 4).

In Lagos state, residues of tetracycline were recorded in samples obtained from the markets in eight (8) Local Government Areas of the state, while the samples from the markets in the remaining four (4) Local Government Areas showed no evidence of tetracycline residue deposition. Tetracycline residues were also not detected in the control samples.

The location of sample collection had a significant effect ($P < 0.05$) on tetracycline residues in the organs. Kidney, liver and muscle samples collected in Apapa, Epe Somolu and Kosofe LGA's had no tetracycline residues in them while the remaining LGA's recorded antibiotic residues depositions. The concentration of the tetracycline residues in the kidney sampled ranged between 0.202 $\mu\text{g/g}$ (Oshodi/ Isolo L.G) to 0.574 $\mu\text{g/g}$ (Agege L.G) and in liver, it ranged between 0.195 $\mu\text{g/g}$ to 0.455 $\mu\text{g/g}$ while muscle recorded between 0.194 $\mu\text{g/g}$ to 0.447 $\mu\text{g/g}$ from the same location (Table 3 and Figure 1).

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Antibiotic Residues	Lagos State	%	Ogun State	%	Total	%
Tetracycline +ve	24	66.67	17	47.22	41	56.94
Tetracycline -ve	12	33.33	19	52.78	31	43.06
Total	36	100	36	100	72	100

Table 2: Occurrence of the Tetracycline Residues in Pork samples in Lagos and Ogun States.

Location	Kidney	Liver	Muscles	Mean
Agege L.G	0.574 ^a	0.455 ^a	0.447 ^a	0.492 + 0.033
Alimosho L.G	0.436 ^b	0.402 ^b	0.402 ^b	0.413 + 0.011
Mushin L.G	0.345 ^c	0.345 ^c	0.320 ^c	0.337 + 0.014
Ibeju/Lekki	0.344 ^c	0.311 ^c	0.233 ^d	0.296 + 0.017
Ifako/Ijaye L.G	0.308 ^c	0.311 ^c	0.305 ^c	0.308 + 0.003
Ikorodu L.G	0.303 ^c	0.258 ^d	0.217 ^c	0.259 + 0.013
Lagos Island L.G	0.217 ^d	0.217 ^d	0.195 ^e	0.209 + 0.004
Oshodi/Isolo L.G	0.202 ^d	0.195 ^e	0.194 ^e	0.197 ^b + 0.004
Apapa L.G	0.000 ^e	0.000 ^f	0.000 ^f	0.000 + 0.000
Somolu	0.000 ^e	0.000 ^f	0.000 ^f	0.000 + 0.000
Epe L.G	0.000 ^e	0.000 ^f	0.000 ^f	0.000 + 0.000
Kosofe	0.000 ^e	0.000 ^f	0.000 ^f	0.000 + 0.000
Control	0.000 ^e	0.000 ^f	0.000 ^f	0.000 + 0.000
Mean	0.227	0.208	0.193	0.209
SEM	0.032	0.027	0.026	0.016

^{af}means with different superscript within the same column are significantly different ($P < 0.05$).

Table 3: Concentration of tetracycline residues in different pig organs across LGA's in Lagos State ($\mu\text{g/g}$).

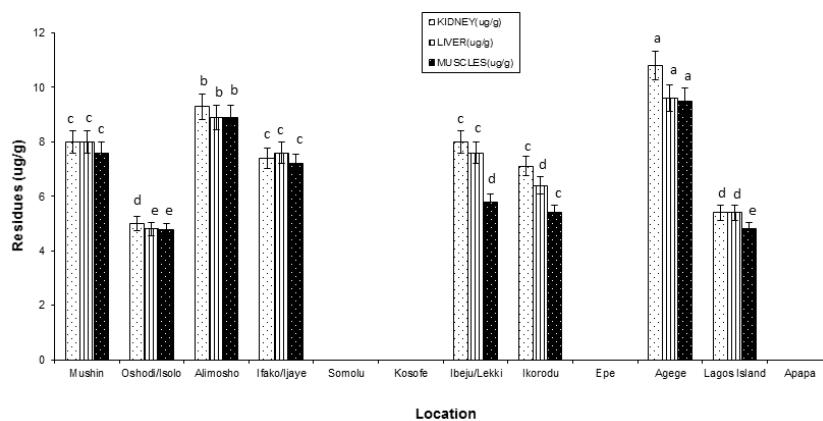


Figure 1: Concentration of tetracycline residues showing error bars in different pig organs across LGA's in Lagos State.

In Ogun state, the residues of tetracycline were recorded in kidney, liver and muscle samples obtained from the markets in some of the local Government areas of the state (Table 4 and Figure 2). The residues of Tetracycline in kidney sample was significantly highest ($p < 0.05$) in Obafemi Owode local government with a value of 0.241 $\mu\text{g/g}$ compared to 0.199 $\mu\text{g/g}$, 0.185 $\mu\text{g/g}$, 0.154 $\mu\text{g/g}$ and 0.118 $\mu\text{g/g}$ in Sagamu, Ikenne, Ifo and Odeda local government areas in Ogun State respectively. Other kidney samples in the other local government areas were negative for any residue and this includes Abeokuta North, Ado-Odo Ota, Ewekoro, Egbado South, Egbado North and Ijebu-Ode local government areas. The residues of antibiotic residues in liver sample showed only five, out of the twelve local government had antibiotic residues with Abeokuta North local government which recorded a significantly highest ($p < 0.05$) value of 0.154 $\mu\text{g/g}$ compared to a significantly lowest value of 0.118 $\mu\text{g/g}$ in Ifo local government area. Other local government areas including Ado-Odo/Ota, Ewekoro and Ijebu-Ode among others had not tetracycline residues in their liver samples. The muscle samples had residues of tetracycline antibiotics in six samples collected where Obafemi-Owode local government had a significantly highest ($p < 0.05$) value of 0.169 $\mu\text{g/g}$. The lowest value of 0.099 $\mu\text{g/g}$ was recorded in Ikenne local government area. Overall, the highest residue of tetracycline residue was recorded in the kidney sample with a value of 0.241 $\mu\text{g/g}$ while the lowest overall value of 0.108 $\mu\text{g/g}$ was recorded in the muscle sample; this however, corroborated the findings of Oyekunle and Olubi, (1992); Pena., et al. (2004); and Salehzadeh., et al. (2006) where they reported that kidney and liver sample(s) recorded the highest residue of any antibiotics, this is so because they are the primary organs of detoxification and metabolism before the muscle and other tissues of the body can receive the metabolite.

The higher tetracycline's residues obtained in Lagos State samples when compared to those in Ogun State could be as a result of increased awareness of antibiotic use, also in Lagos State being a cosmopolitan centre and meeting point of trade could be a reason why farmers tends to use antibiotics as feed additive for improving the growth performance of their animals to attain slaughter weight on time without adhering to the drug manufacturer label on withdrawal periods.

Location	Kidney	Liver	Muscle	Mean
Obafemi-Owode L.G	0.241 ^a	0.000 ^d	0.169 ^a	0.164 + 0.022
Sagamu L.G	0.199 ^b	0.142 ^b	0.114 ^b	0.151 + 0.013
Ikenne L.G	0.185 ^c	0.142 ^b	0.099 ^d	0.141 + 0.012
Ifo L.G	0.154 ^d	0.118 ^c	0.108 ^c	0.127 + 0.007
Abeokuta South L.G	0.154 ^d	0.142 ^b	0.000 ^a	0.098 + 0.024
Odeda L.G	0.118 ^e	0.000 ^d	0.099 ^d	0.073 + 0.018
Abeokuta North L.G	0.000 ^f	0.154 ^a	0.000 ^e	0.051 + 0.025
Ado Odo/Ota L.G	0.000 ^f	0.000 ^d	0.108 ^c	0.036 + 0.018
Ewekoro L.G	0.000 ^f	0.000 ^d	0.000 ^e	0.000 + 0.000
Egbado South L.G	0.000 ^f	0.000 ^d	0.000 ^e	0.000 + 0.000
Egbado North L.G	0.000 ^f	0.000 ^d	0.000 ^e	0.000 + 0.000
Ijebu-Ode L.G	0.000 ^f	0.000 ^d	0.000 ^e	0.000 + 0.000
MEAN	0.088	0.065	0.058	0.070
SEM	0.015	0.011	0.010	0.007

^{a-f}means with different superscript within the same column are significantly different ($P < 0.05$).

Table 4: Concentration ($\mu\text{g/g}$) of tetracycline residues in different pig organs across LGA's in Ogun State.

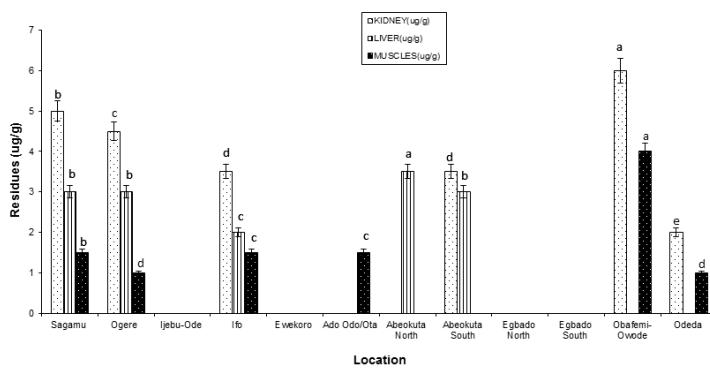


Figure 2: Concentration of tetracycline residues showing error bars in different pig organs across LGA's in Ogun State.

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