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Oxytetracycline Residues in Edible Tissues of Cattle Slaughtered in Akure, Nigeria

Olatoye Isaac Olufemi[1]*, Ehinmowo Adebayo Agboola[2]

[1] *Department of Veterinary Public Health and Preventive medicine, University of Ibadan, Ibadan, Nigeria.*
[2]*. Veterinary Services Department Ministry of Agric., Fisheries, and Forest Resources, Akure, Ondo State, Nigeria.*

Abstract : This work is the first reported confirmatory and quantitative analysis of antibiotic residue in animal products in Nigeria. Meat and other edible portions of slaughtered cattle from Akure metropolitan abattoir from January to June 2008 were analyzed with high performance liquid chromatography (HPLC) for oxytetracycline residue. The extraction was done using hydrochloric acid and acetonitrile for deproteinisation, while clean up was by liquid- liquid partitioning using dichloromethane and petroleum ether. Elution, detection and quantification were done on Lichrosorb RP–18 in HPLC machine coupled with UV – detector. Out of a total of 180 beef samples analyzed during this study, 98 (54.44%) of the total samples had detectable levels of oxytetracycline residues from which 62(34.44%) had oxytetracycline residues at violative levels above the WHO/FAO maximum residue limits (MRLs). The mean residue for positive samples for muscle is 51.8µg/kg, kidney is 372.7µg/kg and liver is 1197.7µg/kg. While the standard deviation (SD) of residue in samples tested positive are 718.9µg/kg, 366.8µg/kg, and 90.53µg/kg in liver, kidney muscle respectively. These high level oxytetracycline residues in greater proportion of meat destined for human consumption at violative levels could be as a result of the indiscriminate use and misuse of veterinary drugs as commonly practiced among livestock producers and marketers without observing withdrawal period prior to slaughter. This result indicates that consumers are predisposed to health hazards and hinders international meat trade from Nigeria. Regulatory authority should therefore ensure compliance with good Agricultural practices including withdrawal period of drugs used for treatment of food animals and routine drug residues surveillance program should be established in the country to ensure food safety.

Key words: Oxtetracycline residue, meat safety, high performance liquid chromatography

* Corresponding author,
Department of Veterinary Public Health and Preventive medicine, University of Ibadan, Ibadan, Nigeria, Tel: +234-805-7852-655, E-mail: io.olatoye@mail.ui.edu.ng

Introduction

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 food of animal origin particularly meat, milk and eggs. Farm animals treated with antibiotics and their edible products are required to be held 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). In Nigeria like most developing countries antibiotics are used in animals indiscriminately for the prevention and treatment of bacterial infection (Dina and Arowolo 1991; Kabir et al. 2004). A greater proportion of cattle in Nigeria are reared by the nomadic herdsmen who administer chemotherapeutic agents without veterinary prescription (Alhaji 1976). When such laymen use these drugs, correct dosage are unlikely to be administered and the withdrawal periods are usually not observed. Tetracyclines are among various antibiotics widely used in livestock (Karimuribo *et. al.*, 2005; Nonga et al. 2009). Improper dosage of oxtetracycline especially at sub-therapeutic levels can result in acute or chronic public health problems, which could be toxicological, microbiological or immunological (FAO 1999). Human health problem that could arise from the consumption of unacceptable levels of oxtetracycline residues in meat include gastrointestinal disturbances, hypersensitivity, bone and teeth problems in children and development of bacterial resistance (Woodward 1991; Czeizel et. al. 1998; Larkin et al. 2004). Enormous amounts of antimicrobials are also used in

agriculture, subjecting microorganisms to the same forces of evolution as occur when antimicrobials are used in humans. Humans may encounter bacteria from animals through the food supply, through direct contact with animals, or through water. Because many of the antimicrobials used in animals are also used in human medicine, the use of antimicrobials in animals is part of the global problem of antimicrobial resistance.

Oxytetracycline levels above the WHO/FAO recommended Maximum residues limits have been reported in edible tissues of slaughtered animal in Nairobi slaughterhouses in Kenya, (Muriuki et al. 2001). However, in Nigeria various studies have been conducted on drug administration and residues deposition in meat and animal products in Nigeria Oboegbulem and Fidelis, 1996; Dipeolu and Alonge 2002 and Kabir *et. al.,(*2004) have demonstrated the presence of antibiotic residues in meat and animal products. Most of these studies were based on microbiological screening techniques that does not specifically classify and quantify the antibiotics. Hence the degree of risks to the consumers could not been ascertained due to low of specificity and sensitivity. High Performance Liquid Chromatography procedures are widely used to quantify various antibiotic residues in food products with good sensitivity and specificity (Oka et al., 1985; Moats, 1986; Dipeolu and Kanamaru 1996; Senyuva et al.*;* 2001; Muriuki et al. 2001). There has not been any documented report of oxytetracycline residue study using HPLC in beef from Nigeria. This study was therefore to determine the oxytetracycline residue levels in edible tissues of cattle slaughtered in Akure municipal abattoir as major source of meat within Southwest Nigeria, where cattle are usually sourced from nomadic herdsmen ad cattle markets from different northern part of the country.

Materials and Methods

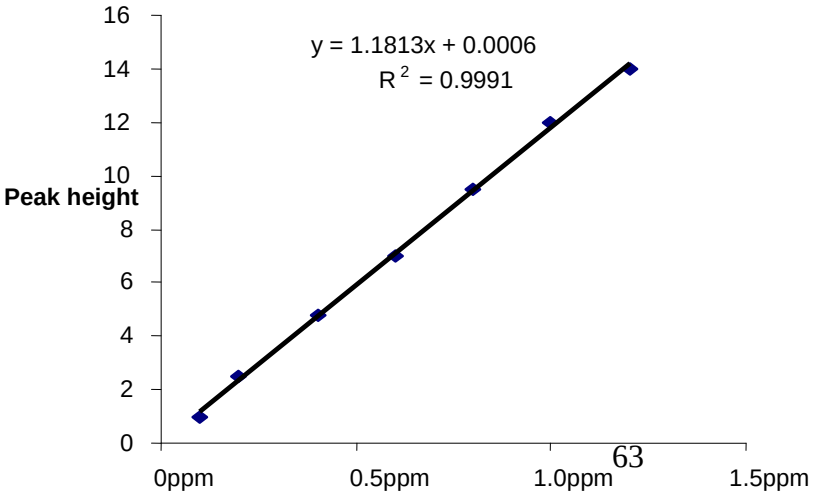
Equipment. High Performance Liquid chromatography (1000 series, CECIL, HPLC) equipped with a constant flow pump, variable wavelength U.V detector. Lichrosorb RP –18 (10µm, 250 x 4.6mm I.D) columns and a recorder operated at 10mV and chart speed of 5mm/min.

Chemicals. Analytical grade oxytetracycline standard from Sigma chemical Co, St Louis, USA., acetonitrile (HPLC grade), methanol (HPLC grade), oxalic acid, hydrochloric acid, methylene chloride, petroleum ether and distilled water. All chemicals were analysis grade and were properly degassed

Samples collection. Approximately 50gms of 60 liver samples, 60 kidney samples and 60 muscles (beef) samples were randomly obtained from slaughtered cattle at Akure municipal abattoir between January and June 2008. An average number of 40 to 55 heads of cattle are slaughtered daily according to the record of meat inspection from the State Ministry of Agriculture and Natural Resources. The samples were wrapped in polythene bags, transported in a cool box packed with ice packs to the laboratory in the department of Veterinary public health and preventive medicine stored at -20^{0}c until the time of analysis.

Preparation of Standard Curve. Oxytetracycline standard powder was accurately weighed and dissolved in methanol to make the stock solution and several serial dilution of the stock solution was made and injected to the HPLC to plot the standard curve of linear R^2 value = 0.9991(Figure 1).The detection limit for oxytetracycline was 0.01ppm while the retention time was 4minutes.

Figure 1. Calibration curve of oxtetracycline standard



Sample preparation. The extraction and clean up procedures developed by Moats in 1986 was employed. This involves Liquid - Liquid partitioning extraction procedures to obtain the analyte. 25g of each sample was homogenized with 3 volumes of 1N hydrochloric acid. 8mls of the homogenate was thoroughly swirled with 32mls acetonitrile and allowed to stand for 5minutes after which the supernatant was decanted though a glass wool on the stem of a glass funnel. 20mls of the filtrate was mixed with 20mls petroleum ether and 20mls methylene chloride in a separatory funnel and vigorously shaken resulting in separation to two layers. About 4mls of the water layer containing the analyte was collected for HPLC analysis.

HPLC analysis for oxytetracycline. The analysis and quantification of the oxytetracycline residues in the analyte was done using a high-performance liquid chromatography machine equipped with a constant flow pump and a variation wavelength U.V detector set at 280nm and flow rate of 2mls/min. Elution of oxytetracycline from the analyte was done on a Lichrosorb RP- 18 (10µ, 250 x 4.0mm 1D) Column with Methanol-Acetonitriel-0.01m aqueous Oxalic acid solution, PH 2.0 (1: 1.5: 2.5) as the mobile phase as described by Oka et al.1985 and adopted by Muriuki et al. 2001. 20µl of the analytes from each sample were injected in duplicate to obtain average peak height of positive samples corresponding to the retention time of 4 to 4.5 minutes as the reference standard. Quantification of oxytetracycline residues in the samples were obtained and calculated from the peak heights extrapolated from the calibration curves of the standard.

Results

Out of the 180 beef samples analyzed during this study, 98(54.4%) comprising of 48 (80%) liver, 18 (30%) kidney and seven (1.17%) muscle samples had detectable levels of oxytetracycline residues while remaining 82 samples (45.6%) had no detectable residues. Out of the positive samples, 62 i.e. 63.2% had oxytetracycline residue at violative levels while 36(36.8%) had residue below the WHO / FAO recommended MRLs for oxytetracycline in beef. The mean ($p>0.05$) residue of oxtetracycline are 1197.0±718.9µg/kg, 372.7±366.8µg/kg, 51.80±90.53µg/kg in liver, kidney muscle respectively. The ranges of oxtetracycline in the tissues are 424-2370µg/kg, 338-1016µg/kg and 0-220µg/kg in liver, kidney, muscle respectively. There were significant difference in the mean ($p>0.05$) oxytetracyline residues of the different tissues using one-way ANOVA (table 1).

TABLE 1; Monthly mean values of oxytetracycline residues (µg/kg) in carcasses from Akure municipal Abattoir (January – June 2008)

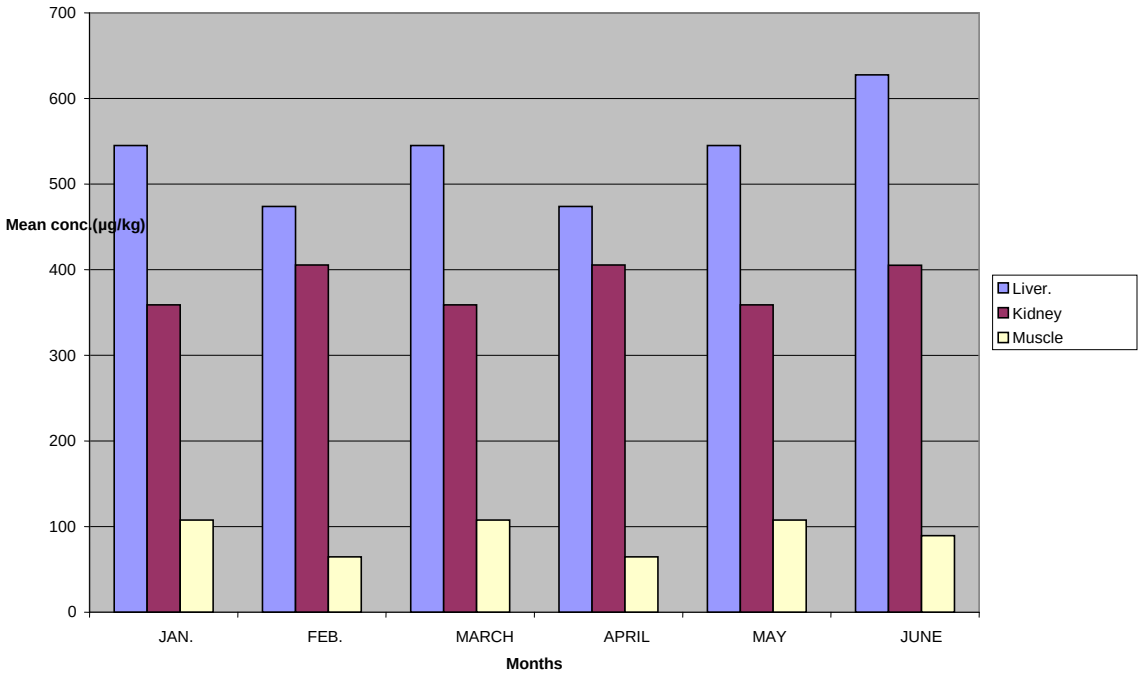
Month		Liver	Kidney	Muscle
January	Positive	10/10	6/10	4/10
	Mean	1720	355.8	83.20
	Range	1016 to 2370	338 to 1016	220 to 240
February	Positive	8/10	6/10	1/10
	Mean	674.0	389.6	20.40
	Range	424 to 1354	1016	0 to 204
March	Positive	10/10	6/10	4/10
	Mean	1720	355.8	83.20
	Range	1016 to 2370	338 to 1016	204 to 220
April	Positive	8/10	6/10	1/10
	Mean	674.0	389.6	20.40
	Range	424 to 1354	424 to 1016	0 to 204
May	Positive	10/10	6/10	4/10
	Mean	1720	355.8	83.20
	Range	1016 to 2370	338 to 1016	204 to 220
June	Positive	8/10	6/10	2/10
	Mean	834.8	398.2	42.40
	Range	592 to 2032	424 to 1016	204 to 220

Discussion

Tetracyclines have served for decades as an important class of antibiotics in food animal health and production. As such, they have also been a source of concern for residue monitoring authorities around the world. In response to this concern the World Health Organization and the Food and Agriculture Organization (FAO 1999) joint committee on residues of some veterinary drugs in animals and foods recommend maximum residue limits for various drugs in edible tissue of food animals. The recommended maximum residue limit (MRL) for oxytetracycline in beef is muscle (200µg/kg), liver (600µg/kg) and kidney 1200µg/kg. As revealed by this study, 54.4% of the samples had detectable levels of oxytetracycline residues is a indication of widespread misuse of veterinary drugs by food animal producers

across Nigeria, since these animals were sourced from different parts of the country. About 63.2% (comprising 44 liver, 4 kidney and 14 muscle samples) contain residue levels above (WHO 1999) recommended standard. Liver and kidney samples yielded more positive result of the residue than muscle Figure 2. This agreed with findings of most workers (Moat 1986; Dipeolu and Alonge 2002; Muriuki et al. 2001). These organs of metabolism and excretion of the drugs are delicacies by some meat consumers portent greater risk of accumulation of residues in this group of consumers.

FIgure 2. Monthly mean OTC residue in the tissues (Jan.-June 2008)



The number of samples positive for oxtetracycline obtained in this study was higher the 45.6% reported from slaughtered cattle in Kenya by (Muriuki et *al.*2001). This may be due to the fact that greater proportion of cattle rearing in Nigeria are done by the nomadic herdsman who have access to veterinary drugs and always purchased drugs over the counter for administration to their animal without veterinary prescription and supervision. This is also an indication of lack of adequate veterinary ad public health regulatory control in the country. This portent great risks and hazards to human health that could result in allergy, cancer, embryo toxicity and antibiotic resistance effects on the consumers. (Aliu *et al.* 2001) portrayed economic implication of drug residues in carcasses inducing physical and chemical changes that can result in condemnation and severe economic losses to the stakeholders. This

is also a critical factor in international meat trade that can deprive the country earnings from meat and meat products in the international market. Oxytetracyline and other antibiotics routinely misused in Nigeria livestock are deposited within their tissues at significant levels rendering most of the meat unsafe and unwholesome. This work is the first reported quantitative and confirmatory analysis of antibiotic residues in food animal products in Nigeria, therefore regulatory authorities should ensure compliance with good agricultural practices including withdrawal period of drugs used for treatment of food animals and embark on routine national residues surveillance program to ensure food safety.

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