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DETECTION AND QUANTITATION OF TETRACYCLINE RESIDUES IN COMMERCIAL BROILERS FOR HUMAN CONSUMPTION IN SOUTH-EAST NIGERIA

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Abstract
Common and frequent misuse of tetracyclines (TCs) in poultry production results in the presence of their residues in edible tissues intended for human consumption, posing a serious public health threat. The presence and concentration of tetracyclines (oxytetracycline, chlortetracycline, doxycycline and tetracycline) in different poultry organs was determined in this study using immunoassay. The liver, kidney, gizzard and muscle of 50 birds were used in this experiment for the presence of tetracyclines and 45(90%) of them had detectable TCs residues, while 43(86%) were positive for tetracycline residues. Thirty six (72%) of the muscle samples were positive and all were above the maximum residue limit (MRL) of TCs in chicken muscle; 42 of the 43(86%) positive liver samples were above the MRL, 42 (84%) of the kidney samples were positive and all were below its MRL; 34 (68%) of the gizzard samples were positive and all were above the MRL of TCs. The mean TC concentration of each organ differed significantly (P < 0 .05) from the organ specific MRLs. The results indicate high levels of TCs in poultry meat meant for human consumption. Strict monitoring of the use of tetracyclines in poultry production and observance of prescribed withdrawal periods before birds are slaughtered and processed for human consumption is highly recommended.

Introduction

The tetracyclines (TCs) are a group of antibiotics commonly used as veterinary medicines and as growth promoters in food producing animals, and rank among the most frequently used in animal food production (Schmidt & Rodrick, 2003). A lot of antibiotics were used in veterinary medicine in the EU countries and TCs were among the most frequently used (Kools *et al*, 2008; Moulin *et al*, 2008). In the US, the large quantities of antibiotics were sold in 2007 and 2009 for use on animals and the commonest group is the TCs (Salah *et al*, 2013). Chlortetracycline, a member of this group was used by 87% of the farms sampled in Trinidad and Tobago as a feed additive given for either prophylaxis or for therapeutic purposes (Adesuyin *et al*, 2004). Chlortetracycline was also the most common residue detected in chicken in a work done in Barbados (Hall *et al*, 2004). In Nigeria, the tetracycline group of drugs is one of the most widely used antimicrobial drugs in poultry farming, In Kaduna state, Kabir *et al.* (2003) reported that oxytetracycline was the most widely used antimicrobial in poultry. Similar findings were reported by Ezenduka *et al.* (2011), in poultry farms in Enugu State. The common use of OTC as prophylaxis in poultry production is for dual purposes: as an anticoccidial and as an antibiotic (Harold *et al*, 1998). Due to the wide application (rapid or frequent use), concerns about their residues in foods of animal origin are growing. One of the most concerning problems associated with TCs residues is idiosyncratic reactions especially in hypersensitive consumers (Locksuwan, 2002). Tetracycline residues in meat potentially may stain teeth of young children (Mesgari *et al*, 2009). Development of resistant strains of bacteria following the ingestion of sub-therapeutic doses of antimicrobials and distortion of the metabolic activity of the microflora are also some of the hazardous effects associated with tetracycline residues (Wilson & Otsuki, 2003; Dayan, 1993; Mateu & Martin, 2001). To prevent any harmful health effects on consumers, Food and Agricultural Organization, World Health Organization and European Union (EU) have established the maximum residual limits (MRL) for veterinary drugs. The maximum residual limit set by the codex alimentarius and World Health Organisation(WHO) for Food and Agricultural Organisation(FAO)/WHO legislation for tetracycline (TTC) in chicken is as follows: muscle (0.2 ng/kg), kidney (1.2 ng/kg), liver (0.6 ng/kg), and egg (0.4 ng/kg). There are basically two methods that can be used to quantify the concentration of antimicrobial in foods: chromatographic and immunochemical methods. Both methods are quantitative and specific. They are used to confirm screened samples that are positive, and also determine the concentrations of the incriminated antimicrobials (Plum, 2008). Immunochemical methods like Enzyme Linked Immunosorbent Assay (ELISA) can be used both qualitatively and quantitatively. Compared to HPLC, it offers the advantage of having low cost, simpler to perform and tests more samples at a time (Amir, 2011). We aimed to determine the quantities of tetracycline residues in poultry meat

in South-East Nigeria and to compare the values against the Minimal Residual Limits using ELISA method.

Materials and methods

Sample collection

A total of 50 birds was randomly selected and purchased from the three major poultry markets in Enugu metropolis, South East Nigeria by visiting each market twice a week for 6 weeks and purchasing four birds per visit. A total of 200 samples constituting 50 of each organ: muscle, liver, kidney and gizzard as sample matrixes was harvested from the birds after slaughter.

Sample preparation

Each organ sample was prepared following the manufacturers instructions for extraction. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant decanted and stored frozen at -20°C for analysis.

Tetracycline residues detection

The tetracyclines were detected by using tetracycline ELISA kit (ABRAXIS USA). In preparation, the microtitre plates and the reagents were stabilized to room temperature before use. The lypholysed conjugate was reconstituted first with 1ml of conjugate diluent, vortexed and diluted with the same conjugate diluents at 1:10 ratio. The standards and control were reconstituted, with 1ml of deionized water. The wash buffer (5X) concentrate was diluted at a ratio of 1:5. For each plate, a worksheet was prepared, the standards and samples were run in duplicates.

Data from the study was analyzed in GraphPad Prism Statistical software version 5.02 (www.graphpad.com). Gaussian distribution of data sets was tested, using D'agostino Omnibus Normality test before choosing Wilcoxon Signed Rank Test. The test was used to analyse the difference in the median value of each organ and it's MRL. Probability was accepted at $p<0.05$.

Results

Organ distribution of tetracycline residues

Out of 200 organs sampled from 50 commercial birds, 21 were undetected (had no residue), 25 were within the detection limit of the kit while 178(89%) were above the detection limit and are positive for tetracycline residues. Approximately, 72% of muscle, 86% of liver, 82% kidney and 68% of gizzard tested positive (Table 1). Tetracycline residues were detected in 45 of the 50 birds and 43(86%) were positive since they were above the detection/control limit of the kit.

Comparison of mean values of the organs with their MRL's

The concentrations of all the 36(72%) muscle samples were above the MRL. The median value of TC residues at 0.79ng/kg is higher than its MRL at 0.2ng/kg. The residue concentrations of 42 out of 43(86%) positive liver samples were above the MRL. The median value of the TC residues at 0.87ng/kg is higher than its MRL at 0.60ng/kg. The concentrations of all the 41 positive kidney samples were below the MRL. The median value of the detected TC residues in kidney at 0.89 is lower than its MRL at 1.20. The concentrations of all the 34 positive gizzard samples were above the MRL of muscle tissue. The median value of the detected TC residues in gizzard at 0.67ng/kg is higher than its MRL at 0.20ng/kg. The differences in the median values of the TC residues in the different organs are all statistically significant with their P values <0.001 (Table 2)\

Discussion

The tetracycline ELISA kit detects the presence of drugs in the tetracycline class of drugs including oxytetracycline, chlortetracycline, tetracycline, doxycycline etc in foods in varying degrees; hence the residues detected in this study could be any of the tetracyclines. The 90% prevalence of tetracycline residues in chicken in this study is very suggestive of constant use of tetracycline group of drugs either added as feed additives for prophylaxis or used therapeutically for treatment. This conforms to the findings of Kabir *et al.* (2003) and some other studies in Nigeria, that tetracyclines are the most widely used antibiotic in poultry production in the country, owing to its wide application in treating many disease conditions, they are also inexpensive and readily available. A similar study in Iran also reported a high prevalence (100%) of TC residues in chicken tissues and also indicated that TC's are the most widely used antibiotics in poultry production (Farahmand *et al*, 2006).

All the organ types with the exception of the kidney records tetracycline levels higher than the WHO recommended MRL. There is the possibility that consumers are exposed to violative levels of tetracyclines, which could lead to public health problems including antibiotic resistance and contamination of food chain. It is generally known that antibiotic resistance has become a challenging clinical problem in South-East Nigeria. More studies would be required to explore the link between high tissue residues of tetracyclines in poultry meat and antibiotic resistance in human. Although the urinary system is the main excretory route of tetracyclines and

almost all the detected kidney samples were above the control limit of 0.5ng/kg, the mean concentration was below the WHO recommended MRL. This may be associated with the fact that OTC is excreted by both bile and kidney. Bile excretes 40% while kidney excretes 60% (Brander *et al*, 1993) hence, the residue concentration in the kidney may not be as high as 1.2ng/kg. In particular, orally administered tetracyclines must have been bio-transformed and metabolized in the liver (first pass effect) and the concentration reduced before excretion.

Conclusion

Commercial broilers sold in Enugu metropolis contain violative levels of tetracycline residues and consumers in the area may be exposed to deleterious levels of these residues. Despite their low toxicity, residues of TCs in meat should be avoided because they may pose potential health threats to consumers. The importance of antibiotics in livestock production cannot be overemphasized as they represent a key component in the strategy used in disease control in humans and animals. Therefore, their prudent use in livestock production is of utmost importance to avoid the occurrence of violative level of antimicrobial residues in human food chain and subsequent development of resistant strains of microorganisms.

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Table 1: Tetracycline residues detection according to concentration

Organ	Frequency [Concentration (ng/kg)]			Proportion detected (%)
	< 0.1(undetected)		> 0.5 (% positive)	
Muscle	2	12	36(72)	48 (96)
Liver	2	5	43(86)	47 (94)
Kidney	7	2	41(82)	43 (86)
Gizzard	10	6	34(68)	40 (80)
Total	**21**	**25**	**154(77)**	**178 (89)**

Table 2: Statistical comparison of the organs with their maximum Residue Limits (MRL)

Organ	Median value(ng/kg)	MRL(ng/kg)	P value
Muscle	0.79	0.2	< 0.0001
Liver	0.87	0.6	< 0.0001
Kidney	0.89	1.2	< 0.0001
Gizzard	0.67	0.2	< 0.0001