

Detection of antimicrobial drug residues in edible tissues from Bunaji cattle slaughtered in Minna modern abattoir

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Abstract

The occurrence of antimicrobial drug residues in edible tissues of slaughtered animals in Nigeria due to indiscriminate drug administration by livestock farmers necessitated this study in Bunaji cattle slaughtered in Minna modern abattoir. A total of 168 edible tissues comprising of meat, liver and kidney were collected from 56 Bunaji cattle slaughtered every two weeks for a period of four months. Microbial inhibition test was employed to screen samples for evidence of antimicrobial drug residues as well as quantitative test using ELISA test kit. 29 (51.78%) samples tested positive with zone of inhibition at various levels of residue (12, 13, 15, 17, 19 and 20mm), which signified high levels of residue. Samples from female cattle contained highest residue value of 20 (68.90%) and values at 20mm. Ages between 6-10 years had 17 (58.62%) while the lowest residue was recorded at older animals above 10 years 5 (17.24%). The quantitative test using ELISA test kit showed a total of 89 (52.78%) of the samples confirmed positive at various inhibition levels. Oxytetracycline residues from the positive results were determined for meat, liver and kidney. The results obtained indicated that, 100 % of the samples contained oxytetracycline residues at various optical density levels. 41.78 %, were above set residue limits of 0.2 mg/kg, 0.6 mg/kg and 1.2 mg /kg for meat, liver and kidney, respectively. From the results it is authenticated that consumers in Minna metropolitan are predisposed to health hazards due to high levels of antimicrobials residues in meat slaughtered in the abattoir. This emphasises the need for effective prevention of infectious diseases, enforcement of ante-mortem inspection, general enlightenment and possible establishment of antimicrobial residues monitoring unit in the study area.

Keywords: Antimicrobial, drug residues, meat, liver, kidney, Bunaji cattle

La Détection de résidus d'antimicrobiens dans les tissus consommables de bovins Bunaji abattus dans l'abattoir moderne de Minna

Résumé

La présence de résidus de médicaments antimicrobiens dans les tissus consommables d'animaux abattus au Nigéria en raison de l'administration de médicaments sans discernement par les éleveurs a nécessité cette étude sur des bovins Bunaji abattus dans l'abattoir moderne de Minna. Un total de 168 tissus consommables comprenant de la viande, du foie et des reins ont été prélevés sur 56 bovins Bunaji abattus toutes les deux semaines pendant une période de quatre mois. Microbial inhibition test was employed to screen samples for evidence of antimicrobial drug residues as well as quantitative test using ELISA test kit. 29 (51.78%) samples tested positive with zone of inhibition at various levels of residue (12, 13, 15, 17, 19 and 20mm), which signified high levels of residue. 29 échantillons (51,78%) ont été testés positifs avec une zone d'inhibition à divers niveaux de résidus (12, 13, 15, 17, 19 et 20 mm), ce qui signifie des niveaux élevés de résidus. Les échantillons de bovins femelles contenaient la valeur de résidu la plus élevée de 20 (68,90%) et les valeurs à 20 mm. Les âges entre 6 et 10 ans en comptaient 17 (58,62%) tandis que le résidu le plus bas était enregistré chez les animaux plus âgés de plus de 10 ans 5 (17,24%). Le test quantitatif

utilisant le test 'ELISA kid' a montré un total de 89 (52,78%) des échantillons qui se sont confirmés positifs à divers niveaux d'inhibition. Les résidus d'oxytétracycline des résultats positifs ont été déterminés pour la viande, le foie et les reins. Les résultats obtenus ont indiqué que 100% des échantillons contenaient des résidus d'oxytétracycline à divers niveaux de densité optique. 41,78%, étaient au-dessus des limites de résidus fixées de 0,2 mg/kg, 0,6 mg/kg et 1,2 mg/kg pour la viande, le foie et les reins, respectivement. À partir des résultats, il est authentifié que les consommateurs de la métropole de Minna sont prédisposés à des risques pour la santé en raison des niveaux élevés de résidus d'antimicrobiens dans la viande abattue dans l'abattoir. Cela souligne la nécessité d'une prévention efficace des maladies infectieuses, de l'application de l'inspection ante mortem, de la prise de conscience générale et de la création éventuelle d'une unité de surveillance des résidus d'antimicrobiens dans la zone d'étude.

Mots clés : antimicrobien, résidus de médicaments, viande, foie, rein, bovins Bunaji

Introduction

The Drug administration to domestic animals requires not only consideration of its effect on the animals but also the effect on humans who consume the product from such animals. Antibiotics are natural product of microorganisms or identical synthetic products or similar semi-synthetic products that inhibit the growth of or destroy microorganisms (Karbis, 2007). In animal health practice, antibiotics are widely used as therapeutic prophylactic or as growth promoting agents (Donoghue, 2003). Antibiotics are of different types, with various pharmacokinetic activities which are often neglected during slaughter, and hence become potential hazard to humans. The presence of antibiotics in meat and other food of animal origin is one of the most important indices for their safety to human consumers (Kozanova *et al.*, 2001). Many livestock farmers administer antibiotics indiscriminately for purposes of treating or preventing an ailment or as growth promoters without consulting experts for necessary advice, such animals are often slaughtered without recourse to the withdrawal period. The Indiscriminate use of antibiotics in livestock has raised serious public health concerns globally, especially in sub-Saharan Africa and particularly Nigeria (Shehu, 2018). Drug residue arises when treated animals are

slaughtered without recourse to pharmacokinetics of the specific drug administered, this situation usually occurs with prolonged administration and over dosage of antimicrobial agent, especially if these animals are slaughtered immediately (vinedresser, 1989) as cited by (Gabriel *et al.*, 2012). Toxic level of antimicrobial residues in food animals are known to cause divers health hazards to susceptible unsuspecting human consumers (Gabriel *et al.*, 2012).

Materials and methods

Experimental site

The samples were collected from Minna Modern abattoir located along Tayi village in Minna Niger state, Nigeria. Minna is situated on a latitude 9°31'05" and longitude 7°42'1" north, with annual rainfall of 1,200–1,300mm and mean temperature of 21–36 °C, the area has an altitude of 1,46m above sea level in the southern-guinea savannah vegetation zone of Nigeria. It is characterised by two seasons, the wet season (April – October) and dry season (November –March) (NSFCO, Minna, 2015).

Sample collection, duration and preservation

One hundred and sixty-eight (168) samples from 3 edible parts of cattle (liver, Muscles and kidney) weighing 50g each were

randomly collected from slaughtered Bunaji cattle in Minna modern abattoir for a period of 4 months at 2 week intervals, with special emphasis on age, sex and time intervals. The samples were properly packed and labelled in a poly bag and store under a refrigerator at a temperature of -20°C until the time for the analysis.

Location of the analysis

The analysis was carried out in the Department of Veterinary Public Health and preventive medicine, Faculty of veterinary medicine, Ahmadu Bello University Zaria, Nigeria.

Sample analysis

The following laboratory techniques were used for sample analysis:

Microbial inhibition test and Elisa kit test (confirmatory)

Laboratory procedure

An adapted microbial inhibition test was employed to screen samples for evidence of antimicrobial drug residues as described by Huber *et al.* (1969). Thereafter, based on the positive results obtained from the screening test, quantitative measurement of Oxytetracycline residues was done using Oxytetracycline ELISA test kits which was purchased from BIOTUVA Life sciences, 4283 Express Lane, suite 728504 Sanasota, FL34249.

Statistics analysis

The data obtained were expressed in percentages and presented as tables for both antimicrobial screening and Oxytetracycline residues. Statistical package for social sciences (IBM USA Version 16.0 2010) was used to analyse the data.

Results

A total of 168 samples of meat (Beef), liver and kidney samples from 56 Bunaji cattle of different sex and age were screened for antimicrobial drug residues through growth inhibition method using nutrient Agar seeded with *Bacillus subtilis*. The positive samples were later subjected to ELISA test kit for confirmation, and quantification for presence of oxytetracycline residues.

Screening of antimicrobial residues obtained from the tissue samples of Bunaji cattle slaughtered in the study area

The results for the screening to test for antimicrobial residues in the samples is shown in Table 1. Out of 168 samples analyzed for antimicrobial residues during this study; 89(52.97%) comprising of 29 (17.26%) meat, 37(22.02%) liver and 23(13.69%) kidney samples were positive at different inhibition zones ranging—from 13 to 20mm while the remaining 79(47.03%) were negative for antimicrobial residues.

Table 1: Screening of antimicrobials residues obtained from the tissue samples of Bunaji cattle slaughtered in the study area

SAMPLE	POSITIVE (%)	NEGATIVE (%)	TOTAL
MEAT	29 (51.79)	27 (48.21)	56
LIVER	37 (66.07)	19 (32.92)	56
KIDNEY	23 (41.07)	33 (56.92)	56
TOTAL	89 (52.97)	79 (47.03)	168

Distribution of antimicrobial residues from the positive samples based on sex and age

The results for distribution of Antimicrobial residues from the positive samples based on sex and age is shown in Table 2. The result on sex variation shows that 26 (29.2%) of the total positive samples were males with 9(31.03%), 8(25.80%) and 9 (39.13%)

obtained for meat, liver and kidney, respectively, while female animals had the highest value of 63 (70.78%), comprising, 20 (68.96%), 29 (78.37%) and 14 (60.36%) for meat, liver and kidney, respectively. On age variation of the positive samples, 6-10 years had the highest value of 56(62.92%), 0-5 years 21(23.59%) and above 10 years with 12 (13.48%).

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Table 2: Distribution of antimicrobial residues obtained from tissue samples of Bunaji slaughtered in the study area based on sex and age

Samples	Sex		Age			Total
	Males (%)	Females	0-5 (%)	6-10 (%)	>10 (%)	
MEAT	9(31.03)	20(68.96)	7(24.13)	17(58.62)	5(17.24)	29
LIVER	8(25.80)	29(78.37)	9(24.32)	24(64.86)	4(10.81)	37
KIDNEY	9(39.13)	14(60.86)	5(21.73)	15(65.21)	3(13.04)	23
TOTAL	26(29.21)	63(70.78)	21(23.59)	56(62.92)	12(13.48)	89

The results of ELISA test kit for confirmatory and quantifications of Oxytetracycline residues

The results of ELISA test kit for confirmatory and quantifications of Oxytetracycline residues is illustrated in Table 3. Out of the 89 tissue samples

positive for antimicrobial residues, Oxytetracycline residues was detected on all the samples (100%) by the Elisa kit. 37(41.57%) were above the set residue limit, with 25(86.20%), 10(32.25%) and 2(8.69%) for meat, liver and kidney, respectively.

Table 3: Quantification of OTC residues obtained from the tissue samples using ELISA test kit

Samples	MRL	Range of residues result (%)			Mean LS
		Below limit	Within limit	Above limit	
Meat	0.2mg/kg	1(3.44%)	3(10.34%)	25(86.20%)	0.4122 NS
Liver	0.6mg/kg	27(72.97%)	0	10(32.25%)	0.2830 NS
Kidney	1.2mg/kg	21(91.31%)	0	2(8.69%)	0.2248 NS
Total		49(55.05%)	3(3.37)	37(41.57%)	

OTC: -oxytetracycline; MRL:-maximum residue limit.

Mean value not significant ($p>0.05$)

Distribution of oxytetracycline residues based on sex of the animals

Results of oxytetracycline residues based on the sex of the animals is shown in Table 4. The result of oxytetracycline residues based on sex showed female animals had

the highest residue values of 29 (32.98%) for total samples and 20 (68.96), 8 (21.62%) and 1 (4.34%) for meat, liver and kidney respectively; Males had 8 (8.99%) of the total samples, which comprises, Meat 5 (17.24), liver 2 (5.40%) and kidney 1 (4.34%).

Table 4: Distribution of OTC residues obtained from tissue samples of Bunaji cattle based on sex of the animals

SAMPLE	MRL	BELOW MRL		ABOVE MRL		TOTAL
		Male	Female	Male	Female	
MEAT	0.2mg/kg	2(6.89%)	2(6.89%)	5(17.24)	20(68.96%)	29
LIVER	0.6mg/kg	5(13.52%)	22(59.45%)	2(5.40%)	8(21.62%)	37
KIDNEY	1.2mg/kg	4(17.39)	17(73.91)	1(4.34)	1(4.34%)	23
TOTAL		11(12.35%)	41(46.06%)	8(8.99%)	29(32.98%)	89

Distribution of oxytetracycline residue based on age of the animals

Results for distribution of oxytetracycline residue based on age of animals is shown in

table 5. Oxytetracycline residues above set limit confirmed within age groups showed, 11(12.36%); 22 (24.71%) and 4(4.41%) as values obtained for ages 0-5; 6-10 and above 10 years respectively.

Table 5: Distribution of Oxytetracycline residues obtained from tissue samples of Bunaji cattle based on age

Samples	MRL	Residues						Total
		Below /-			Above residue			
		0-5	6-10	>10	0-5	6-10	>10	
MEAT	0.2mg/kg	1(3.44)	2(6.89)	1(3.44)	6(20.69)	16(55.17)	3(10.34)	29
LIVER	0.6mg/kg	6(16.21)	16(43.24)	5(13.51)	3(8.11)	6(16.21)	1(2.70)	37
KIDNEY	1.2mg/kg	3(13.04)	15(65.21)	3(13.04)	2(8.67)	0	0	23
TOTAL		10(11.23)	33(37.01)	9(10.11)	11(12.36)	22(24.71)	4(4.41)	89

Discussion

The overall results from this study when considered regardless of the tissue types, indicated high incidence of 89(52.97%) for antimicrobial drug residues and 37(41.5%) above set residue limit for oxytetracycline (Prajapast *et al.*, 2018). The high occurrences of antimicrobial residues found in this study on meat, liver and kidney have been reported by several authors; 89.3% from kidney and urine (Gabriel *et al.*, 2012); 69.74 % in edible tissues (Olaloye and Ogunidipe, 2009). However, the result is higher than 46% in beef in Oyo (Akinwumi, 2012); 16.11% (Dipeolu and alonge, 2002) and 25% in milk (Yusuf *et al.*, 2017). The outcome from this study found 41.57% of the samples to have oxytetracycline maximum residue value, the result however agreed with that reported by Omotoso and Omojola (2018) but lower than 71.1% in Cattle beef in Tanzania (kamiroso *et al.*, 2005); 71.3% from cattle in Ethiopia (Zeroda, 2012); 54.4% in muscle samples reported in studies from Kenya (Muruiiki *et al.*, 2001), respectively. The occurrence of antimicrobial residues and Oxytetracycline residues above set limit showed that, females had the highest residue of 70.78 % and 32.98 % for antimicrobial and Oxytetracycline residues respectively. This could be attributed to indiscriminate administration of drugs during pregnancy at a time that they probably have compromised immune system. 62.92 % of antimicrobial residues and 24.71%

Oxytetracycline residues were obtained above based on age variation between 6-10 years, the result is higher than 14.7 % reported by Zeenatudeen, (2015) on raw cow milk. High levels of antimicrobial and Oxytetracycline residues was found in liver and meat which could be attributed to their being responsible for absorption and metabolism of drugs. Marketers, herdsman and butchers all administered veterinary drugs indiscriminately, with Oxytetracycline long acting drugs which had slow absorption rate.

The high prevalence of 41.57 % of Oxytetracycline residues obtained from the study using Elisa test kit by quantification is an indication of wide spread misuse of Veterinary drugs for therapeutic, prophylactic and as growth promoters by food animal producers in the study area. Veterinary drugs are easily accessible over the counter and in rural cattle Markets in Nigeria without prescription by veterinary personnel. Most times these animals are treated in the butcher's house or lairage, where antimicrobial inspection is not fully employed before slaughter. The indiscriminate use of antimicrobial substances and non observance of withdrawal period has been reported as the major cause of violative residues in animal food products (Riviere and soundlof, 2001) which had also led to availability of unsafe meat to consumers. Olaloye and Ehinmowo (2014) reported drugs residues due to indiscriminate and wide spread misuse of veterinary drugs which could be attributed

to the fact that greater proportions of cattle rearing in Nigeria is mostly by nomadic herdsmen.

The presence of drug residues in animal products portends great risk and hazard to human health that could result in Allergy, kidney disease, toxicity and drug resistance (Alhendi, 2000).

Conclusion

The high percentage of meat with antimicrobial residues obtained from this study indicates that, consumers in Minna metropolitan are predisposed to health hazards due to the presence of antimicrobial residues in meat in slaughtered animals from the abattoir, this emphasises the need for the enforcement of antimortem inspection, effective prevention of infectious diseases, general enlightenment on drug withdrawal periods, and possible establishment of antimicrobial residues monitoring unit in Minna modern abattoir.

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