

# Oxytetracycline and penicillin-G residues in cattle slaughtered in south-western Nigeria: Implications for livestock disease management and public health

**Authors:**

Hezekiah K. Adesokan<sup>1</sup>  
Charity A. Agada<sup>1</sup>  
Victoria O. Adetunji<sup>1</sup>  
Ibikunle M. Akanbi<sup>2</sup>

**Affiliations:**

<sup>1</sup>Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria

<sup>2</sup>Ministry of Agriculture and Natural Resources, Oyo State, Nigeria

**Correspondence to:**

Hezekiah Adesokan

**Email:**

latterglory70@yahoo.co.in

**Postal address:**

PMB 001, Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria

**Dates:**

Received: 15 Oct. 2012

Accepted: 26 Mar. 2013

Published: 11 July 2013

**How to cite this article:**

Adesokan, H.K., Agada, C.A., Adetunji, V.O. & Akanbi, I.M., 2013, 'Oxytetracycline and penicillin-G residues in cattle slaughtered in south-western Nigeria: Implications for livestock disease management and public health', *Journal of the South African Veterinary Association* 84(1), Art. #945, 5 pages. <http://dx.doi.org/10.4102/jsava.v84i1.945>

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After the discovery of indiscriminate antibiotic use in ready-for-slaughter cattle in south-western Nigeria, 90 tissue samples from randomly selected slaughtered cattle were evaluated for oxytetracycline and penicillin-G residues using high performance liquid chromatography and the data analysed by one-way Analysis of variance (ANOVA). The findings revealed residues of oxytetracycline (kidney:  $9.47 \mu\text{g/kg} \pm 3.24 \mu\text{g/kg}$ ; liver:  $12.73 \mu\text{g/kg} \pm 4.39 \mu\text{g/kg}$ ; muscle:  $16.17 \mu\text{g/kg} \pm 5.52 \mu\text{g/kg}$ ) and penicillin-G (kidney:  $6.27 \mu\text{g/kg} \pm 2.46 \mu\text{g/kg}$ ; liver:  $8.5 \mu\text{g/kg} \pm 2.80 \mu\text{g/kg}$ ; muscle:  $11.67 \mu\text{g/kg} \pm 2.94 \mu\text{g/kg}$ ) in all tissues screened. Significantly high levels (oxytetracycline:  $F = 16.77$ ; penicillin-G:  $F = 29.38$ ) were, however, found in muscles, followed by liver and then kidney – findings confirming recent antibiotic administration to the animals before slaughter. The dietary intakes through the tissues screened were 0.024% (oxytetracycline) and 0.017% (penicillin-G) of the acceptable daily intake (ADI). Although the concentrations in the tissues screened were below the maximum residue limits despite recent administration of these antibiotics before slaughter, the lower concentrations are suggestive of the probable low dosages often administered by those involved in indiscriminate use of antibiotics. This therefore raises serious concerns for the livestock industry as well as human health, given the resultant emergence and spread of resistant strains of bacterial pathogens that could ensue from prolonged use of low dosages of antibiotics. Additionally, the lower concentrations of the daily intakes notwithstanding, the plausible exposure to these antibiotics from other food sources is a cause for concern. Since antimicrobial misuse and its consequent effects are not just a problem limited to Nigeria but also a concern in sub-Saharan Africa, the need for national and international stakeholder intervention is emphasised.

## Introduction

Antimicrobial resistance (AMR) and the resulting failure of antimicrobial therapies in humans is a mounting public health problem of global significance and presents a major and growing threat to effective treatment of bacterial infections (Mather *et al.* 2012). This phenomenon is driven by many factors including the use of antimicrobial drugs in both humans and animals. The use of these antimicrobials in agriculture as a major driver of AMR in pathogenic bacteria of significance to humans is an issue of concern to public health sectors (Mølbak 2004; Wassenaar 2005). The prophylactic and metaphylactic use in animal populations has been a particular concern (Aarestrup *et al.* 2001; Klare *et al.* 1999), especially when the drug classes are the same as, or related to, the pharmaceuticals used in the control of human infections. For example, veterinary use of the non-steroidal anti-inflammatory drug (NSAID) diclofenac has been reported as the main, and perhaps the only, cause of population declines of Asian Gyps vultures (Prakash *et al.* 2012). In addition, avian exposure to fluoroquinolones was reported to be associated with isolation of fluoroquinolone-resistant *Escherichia coli* from broilers, which could affect the drug therapeutic potential in humans and veterinary medicine (Moniri & Dastehgoli 2005). Misuse and overuse of antimicrobial drugs creates selective evolutionary pressure that enables antimicrobial-resistant bacteria to increase in numbers more rapidly than antimicrobial-susceptible bacteria and thus increases the opportunity for individuals to become infected by resistant bacteria (USDHHS/FDACVM 2012), particularly through the food chain.

According to a World Health Organization report (WHO 2011), there is an urgent need for action in countering antibiotic resistance through a holistic, intersectoral, and multifaceted approach that should focus on all efforts to reduce unnecessary use of antibiotics, including those used in food production. Such specific regulatory strategies include eliminating the use of antibiotics as growth promoters in food animals as well as requiring that antibiotics be administered to animals only when prescribed by a veterinarian. This notwithstanding, livestock traders in Ibadan, south-western Nigeria engage in indiscriminate administration of antibiotics to ready-for-slaughter beef cattle (Adesokan *et al.*, unpublished data, 2012) without any consideration of the fate of

these antibiotics in the slaughtered animals. Whilst these traders supply most of the cattle consumed in this region, to our knowledge the fate of the antibiotics often administered to these ready-for-slaughter cattle and the resultant daily dietary intakes by humans in this region have not been investigated. The aim of this study was therefore to evaluate tissues from these slaughtered animals for the presence of oxytetracycline and penicillin-G residues and to estimate the intake of each of these antibiotics from the tissues, with a view to determining the implications for livestock disease management and the possible health risks associated with the consumption of beef in south-western Nigeria.

## Materials and methods

### Study site

This study was carried out at Bodija Municipal Abattoir, Ibadan, south-western Nigeria and Ibadan, which lies at 7°32'N and 3°54'E. Ibadan has a population of over 1.3 million (NPC 2006) and is the largest city in West Africa and the third largest in Africa after Cairo and Johannesburg. It has a major abattoir, which is the main meat processing facility in the city, where an average of 250 cattle are slaughtered daily, and supplies meat to most parts of the city and its surrounding areas. A previous study conducted by the authors (Adesokan *et al.*, unpublished data, 2012) at the Tropical Cattle Market, which supplies most of the cattle slaughtered at this abattoir, discovered an indiscriminate use of antibiotics, mainly oxytetracycline and penicillin, on the trade cattle meant for immediate or eventual slaughter.

### Animal sampling and tissue collection

Using a simple random sampling technique, an average of one in every ten cattle slaughtered was sampled with a total of ten cattle sampled per day. This was done over a three-day visit to the abattoir giving a total of 30 animals screened. Three different specimens, including kidney, liver and muscles of approximately 50 g each, were obtained from the 30 animals resulting in 90 specimens collected in all. These specimens were wrapped in polythene bags and transported in an insulated flask packed with ice to the laboratory for drug residue analysis.

### Reagents

All reagents used were of analytical grades from Sigma Chemical Co, St Louis, MO, USA, including acetonitrile (HPLC grade), methanol (HPLC grade), oxalic acid, hydrochloric acid, methylene chloride, petroleum ether, and distilled water.

### Antibiotic (oxytetracycline and penicillin-G) residue analysis of samples

High performance liquid chromatography standard methods were used for antibiotic residue analysis. Oxytetracycline and penicillin-G were analysed according to a previously described technique (Olatoye & Ehinmowo 2010; Shaikh & Moats 1993). The only variation was that each sample was run twice whilst the mean values were regarded as the

residue levels in the respective tissues. The limits of detection (LOD) and quantification (LOQ) for TC residues were 0.01 µg/g and 0.10 µg/g, respectively. The limits of detection and quantification for penicillin were 0.005 µg/g and 0.010 µg/g respectively.

### Statistical analysis

The one-way Analysis of variance (ANOVA) test statistic was used to determine any significant differences in the mean residue levels of oxytetracycline and penicillin-G in the three types of animal tissue sampled (Figure 1).

## Results

### Residues of oxytetracycline and penicillin-G in the animal tissues screened

A total of 90 tissues (kidney = 30; liver = 30; muscle = 30) from 30 slaughtered cattle were screened for the presence of oxytetracycline and penicillin-G residues. Each of these specimens contained detectable levels of oxytetracycline and penicillin-G (Table 1). The highest levels of the two antibiotics were found in the muscles, followed by the livers, the lowest being found in the kidneys (Figure 1). The mean residue levels of oxytetracycline were 9.47 µg/g ± 3.24 µ/kg, 12.73 µg/g ± 4.39 µ/kg and 16.17 µg/g ± 5.52 µ/kg in the kidneys, livers and muscles respectively, and 6.27 µg/g ±

**TABLE 1:** Residue levels of oxytetracycline and penicillin-G in tissues of slaughtered cattle in south-western Nigeria.

Sample Number	Oxytetracycline (µg/kg)			Penicillin-G (µg/kg)		
	Kidney	Liver	Muscles	Kidney	Liver	Muscles
1	9.5	11.5	14.5	6.0	8.0	10.0
2	12.5	15.5	17.5	8.0	9.0	12.0
3	5.5	7.5	11.5	4.0	6.0	9.0
4	10.5	14.5	21.5	6.0	8.0	12.0
5	8.5	13.5	17.5	4.0	6.0	9.0
6	9.5	11.5	13.5	6.0	1.0	14.0
7	4.5	7.5	9.5	10.0	12.0	18.0
8	7.5	11.5	14.5	5.0	8.0	12.0
9	16.5	18.5	22.5	5.0	6.0	10.0
10	14.5	22.5	26.5	11.0	12.0	15.0
11	10.5	18.5	22.5	5.0	9.0	12.0
12	10.5	12.5	17.5	9.0	14.0	10.0
13	14.5	18.5	23.5	11.0	14.0	17.0
14	8.5	7.5	12.5	4.0	6.0	9.0
15	3.5	5.5	9.5	9.0	11.0	13.0
16	7.5	10.5	15.5	6.0	8.0	11.0
17	9.5	10.5	2.5	3.0	6.0	10.0
18	11.5	18.5	22.5	8.0	8.0	13.0
19	8.5	16.5	22.5	8.0	10.0	14.0
20	5.5	8.5	11.5	3.0	6.0	9.0
21	10.5	12.5	14.5	5.0	9.0	12.0
22	12.5	14.5	18.5	9.0	11.0	15.0
23	7.5	9.5	12.5	4.0	6.0	9.0
24	5.5	8.5	11.5	4.0	10.0	4.0
25	8.5	9.5	14.5	5.0	7.0	10.0
26	4.5	6.5	10.5	9.0	11.0	14.0
27	9.5	12.5	14.5	4.0	7.0	9.0
28	14.5	18.5	22.5	9.0	11.0	15.0
29	10.5	11.5	13.5	4.0	6.0	9.0
30	11.5	17.5	23.5	4.0	9.0	14.0
Mean ± s.d.	9.47 ± 3.24	12.73 ± 4.39	16.17 ± 5.52	6.27 ± 2.46	8.5 ± 2.80	11.67 ± 2.94

**TABLE 2:** Result of data analysis using one-way Analysis of variance.

Variable	Sum of Squares	df*	Mean Square	F**	Sig.***
<b>Oxytetracycline</b>					
Between groups	673.489	2	336.744	16.770	.000
Within groups	1747.000	87	20.080	-	-
<b>Total</b>	<b>2420.489</b>	<b>89</b>	-	-	-
<b>Penicillin-G</b>					
Between groups	441.756	2	220.880	29.381	-
Within groups	654.033	87	7.518	-	-
<b>Total</b>	<b>1095.789</b>	<b>89</b>	-	-	-

\* df: degree of freedom

\*\* F: Variance

\*\*\* Sig.: Significance

2.46  $\mu\text{g}/\text{kg}$ , 8.5  $\mu\text{g}/\text{g} \pm 2.80 \mu\text{g}/\text{kg}$  and 11.67  $\mu\text{g}/\text{g} \pm 2.94 \mu\text{g}/\text{kg}$  for penicillin-G in the kidneys, livers and muscles respectively (Table 1). These differences in the mean residue levels of oxytetracycline and penicillin-G in the kidneys, livers and muscles were respectively significantly different ( $F = 16.77$ ;  $df = 2.87$  and  $F = 29.381$ ;  $df = 2.87$ ) (Table 2). The residue levels ranged between 3.5  $\mu\text{g}/\text{kg}$ –16.5  $\mu\text{g}/\text{kg}$ , 5.5  $\mu\text{g}/\text{kg}$ –22.5  $\mu\text{g}/\text{kg}$  and 2.5  $\mu\text{g}/\text{kg}$ –26.5  $\mu\text{g}/\text{kg}$  in kidneys, livers and muscles for oxytetracycline and 3.0  $\mu\text{g}/\text{kg}$ –11.0  $\mu\text{g}/\text{kg}$ , 1.0  $\mu\text{g}/\text{kg}$ –14.0  $\mu\text{g}/\text{kg}$  and 4.0  $\mu\text{g}/\text{kg}$ –18.0  $\mu\text{g}/\text{kg}$  in kidneys, livers and muscles for penicillin respectively (Table 1). Concentrations detected in kidneys, livers and muscles were all below the WHO/FAO recommended maximum residue limits (MRLs) for oxytetracycline and penicillin-G.

### Estimation of oxytetracycline and penicillin-G intakes from animal tissues

The dietary intake of each antibiotic was calculated by multiplying the sum of the mean concentrations of a particular antibiotic in the different animal tissues screened by the weight of the tissues consumed in the daily diet of an average person (Urieta, Jalon & Eguileor 1996). The average consumption of beef was assessed on the basis of data obtained through the monitoring of the per capita consumption of beef tissues in a south-western state in Nigeria and reported to be about 38 g per day (Gomna & Rana 2007). Considering this amount and the mean concentrations of oxytetracycline (12.79  $\mu\text{g}/\text{kg}$ ) and penicillin-G (8.81  $\mu\text{g}/\text{kg}$ ) as analysed in these animal tissues screened, the dietary intakes of oxytetracycline and penicillin-G were calculated as 0.49  $\mu\text{g}/\text{day}$  and 0.34  $\mu\text{g}/\text{day}$  (3.43  $\mu\text{g}/\text{week}$  and 2.38  $\mu\text{g}/\text{week}$ ), respectively. To evaluate the health risk of the estimated dietary exposure, it was compared with the acceptable daily intake (ADI) recommended by the Joint FAO/WHO Expert Committee for Food Additives (JECFA 1998) for each of the antibiotics: 30  $\mu\text{g}$  oxytetracycline/kg body weight and 30  $\mu\text{g}$  penicillin/kg body weight, which is equivalent to 2040  $\mu\text{g}$  oxytetracycline/day and 2040  $\mu\text{g}$  penicillin/day for an adult weighing 68 kg. Expressing the dietary intake of oxytetracycline and penicillin-G as a percentage of ADI, their intake through the tissues screened in south-western Nigeria results in 0.024% and 0.017% of the ADI, respectively.

## Discussion

Because antimicrobial drug use contributes to the emergence of drug resistant organisms, these important drugs must be

used judiciously in both animal and human medicine to slow the development of resistance. The results of this study show the presence of residues of oxytetracycline and penicillin-G, which are some of the leading antimicrobials used in most African countries, in all the tissues screened, with the highest concentrations found in the muscles, followed by the liver and then the kidney, with the lowest levels. This finding further provides evidence that indiscriminate antimicrobial use, such as when used on trade animals meant for almost immediate slaughter, leads to residues in tissues obtained from them. Although the concentration levels obtained in this study were below the maximum residual limits recommended (FAO 1999), their presence in all the tissues sampled is a matter of concern. This is so in the light of possible bioaccumulation when these residues present in the animal tissues accumulate continuously over the lifespan of the individuals through prolonged consumption. This is particularly of potential concern in Nigeria where cattle are the most frequently consumed meat animals (Adetunji & Rauf 2012; Gambo, Raufu & Ambali 2010) and consumption of raw unpasteurised milk is common amongst livestock owners (Cadmus *et al.* 2006), which could have a potentially greater negative effect on young children, who consume substantial quantities of milk. Moreover, oxytetracycline and penicillin-G can enter the human body from other sources such as poultry meat, given the uncontrolled use of these antibiotics amongst food animals in Nigeria.

The low levels detected in all the tissues sampled could be attributed to the probably low doses of antimicrobials commonly administered by livestock traders in order to maximise the number of doses available, an assertion supported by an earlier report that nomadic herdsman administer chemotherapeutic agents without veterinary prescription and most likely at incorrect dosages (Olatoye & Ehinmowo 2010). Meanwhile, previous studies suggest that antimicrobial use in food animals at low dosages for prolonged periods is a significant risk factor for the development of antimicrobial resistance amongst bacterial pathogens (Blake *et al.* 2003; Varga *et al.* 2009). Previous investigators have isolated various bacterial organisms that were resistant to oxytetracycline as well as penicillin-G in the same study area. For instance, various organisms resistant to antimicrobials, including oxytetracycline in cooked food sold on a south-western university campus (Oluyeye *et al.* 2009) as well as oxytetracycline-resistant *E. coli* from faecal samples of ready-to-be-slaughtered cattle in an abattoir in south-western Nigeria (Ajayi *et al.* 2011) have been reported. In another study conducted in south-western Nigeria (Aibinu *et al.* 2007), strains of *E. coli* resistant to tetracycline and penicillin-G from both animal and human samples were reported. In general, reports from different parts of Nigeria have observed temporal trends in the prevalence of resistance amongst enteric organisms such as *E. coli* and *Shigella* (Okeke *et al.* 2005). This practice by livestock traders and herders therefore becomes an issue that needs urgent attention if efforts towards stamping out the menace of antimicrobial resistance in animals and humans globally are to produce the desired results.

Most often, concern for the presence of residues in food animals is associated with possible effects on consumer health, which could be in the form of immediate hypersensitivity reactions. This is often the case in people already sensitised from possible effects on gut flora of low levels of antimicrobials, which might select for resistance and have potentially toxic effects. As discussed earlier, prolonged continuous ingestion of antibiotics could promote the development of resistance to antimicrobials in an individual (Labio *et al.* 2007), resulting in resistance to treatment with the antibiotics when the need arises (Dipeolu 2010). This becomes worse when such people do not have access to alternative antibiotics, which in most cases are unaffordable.

Additionally, it is of interest to note that all the tissue samples analysed contained detectable residues of oxytetracycline and penicillin-G. This substantiates the observation by the authors (unpublished data, 2012) that antibiotics were often administered to the majority of the cattle meant for slaughter in this region within the last 24 h to 48 h prior to slaughter. In addition, it might be due to the fact that these two antimicrobials are amongst the drugs most commonly administered by livestock workers in Africa in general. This finding is in agreement with the recent report from a survey carried out in South Africa (Eagar, Swan & Van Vuuren 2012), where tetracyclines constituted the second largest group of antimicrobials and penicillin the majority of the parenteral dosage forms sold. Meanwhile, oxytetracycline has been shown to result in resistant genes in bacteria, following exposure of these bacteria to low doses of this drug over an extended period of time (Khachatryan *et al.* 2006). Furthermore, it has been shown that drug concentrations several hundredfold below the minimal inhibitory concentration for susceptible bacteria could select for resistant bacteria, even when present at a very low initial fraction (Gullberg *et al.* 2011). Thus, the surprisingly high frequencies of antibiotic-resistant bacteria found in animals from relatively pristine environments (Gilliver *et al.* 1999; Osterblad *et al.* 2001; Sjolund *et al.* 2008) could conceivably be partly explained by enrichment due to sub-minimum inhibitory concentrations (sub-MIC) selective effects.

This resistance can develop quickly and extend from a single individual to other members of its species as well as to people living and working in that environment, through direct contact. In addition, penicillin-G, as a beta-lactam, is known to select for an extended spectrum of beta-lactamase resistance that has resulted in Gram-negative bacteria such as *Escherichia coli* strains expressing extended-spectrum beta-lactamases (ESBLs) or plasmidic C beta-lactamases in food animals emerging globally, and has limited the treatment strategies available for bacterial infections (Commonwealth Department for Health & Aged Care 1999; DANMAP 2004). The concern, therefore, besides the effects on the livestock industry, is the fact that many analogues of these antibiotics are used in disease management in humans (Snyman 2006) and could consequently add to the development of cross-resistance to antimicrobials administered in human health.

Furthermore, a cursory look at the different concentrations of these antibiotics across the three tissue types reveals consistently highest concentrations in muscles followed by livers and then the kidneys for most (97%) of the animals sampled (Figure 1). This is in contrast to what should be expected in cases where antimicrobials were administered long before slaughter, since organs of metabolism and excretion are expected to have higher concentrations of these residues than the muscles over time. This assertion is also corroborated by Landoni & Errecalde (1992), who indicated an ascending order of concentrations in the muscles, livers and kidneys respectively with increasing time. This is equally reflected by the increasing concentrations in the MRLs recommended (FAO 1999) as 200 µg/kg, 600 µg/kg and 1200 µg/kg in muscles, livers and kidneys respectively, particularly for oxytetracycline. The highest concentrations in the muscles when compared with the livers and kidneys might therefore be linked to administration of these antimicrobials to the animals shortly before slaughter. This finding therefore suggests that those whose preference is muscle consumption in the study region might be at higher health risk than those who eat liver and kidney.

Despite these findings, this study had some limitations. Firstly, the breed or sex of the animals sampled were not taken into consideration. This might have also possibly provided insights into patterns of residue distribution based on these factors. Secondly, the sample size was small, and a larger sample size could probably have given more information on the situation at this study site.

Notwithstanding the limitations, the findings confirming the presence of oxytetracycline and penicillin-G residues in all the tissues sampled are sufficient to provide the insights needed for public health intervention. Although the results obtained in this study show that daily intakes of oxytetracycline and penicillin-G through the tissue types analysed were below the acceptable daily intake, efforts should be made to still further reduce the levels by enforcement of regulations against indiscriminate use of antibiotics on ready-for-slaughter animals to avoid synergistic potentiation of their effects on consumers by antibiotics from other food sources. The application of appropriate drug withdrawal periods would also prove useful in combatting the menace of antibiotic resistance in both animals and humans.

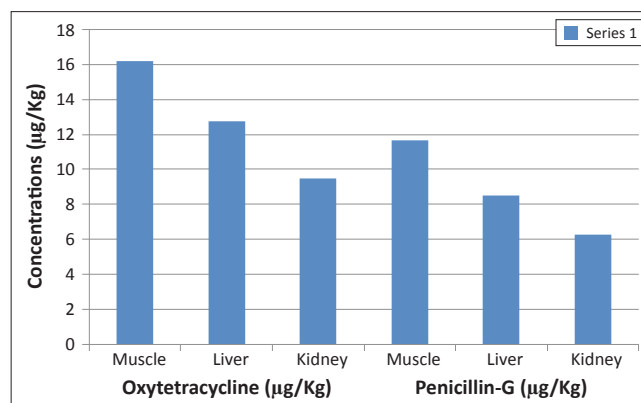


FIGURE 1: Mean distribution of oxytetracycline and penicillin-G residues in tissues of slaughtered cattle in south-western Nigeria.

## Conclusion

In conclusion, since misuse of antimicrobials, which in most cases leads to the presence of residues in foods of animal origin, is not only limited to Nigeria, but it's also a problem in most of sub-Saharan Africa, there is a need for both national and international stakeholders in the livestock industry and public health sector to intervene urgently to prevent the potentially grave impacts of antimicrobial resistance on both animal and human health globally.

## Acknowledgements

The authors are grateful to the head of the Bodija Municipal Abattoir, Ministry of Agriculture and Natural Resources, Oyo State, Nigeria, for her assistance.

## Competing interests

We declare that there is no financial or personal relationship which may have inappropriately influenced us in writing this article.

## Authors' contributions

H.K.A. (University of Ibadan) was responsible for the initiation of the concept and design of this study and the writing of the manuscript. C.A.A. (University of Ibadan) was involved in the laboratory work and statistical analysis of the findings. V.O.A. (University of Ibadan) made substantial contributions to the design of the work and writing of the manuscript. I.M.A. (University of Ibadan) was involved in the collection of samples as well as laboratory analysis.

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