# Assessment of antimicrobial drug residues in beef in Abuja, the Federal Capital Territory, Nigeria

Gabriel K. Omeiza<sup>(1)</sup>, Itopa E. Ajayi<sup>(2)</sup> & Okwoche J. Ode<sup>(3)</sup>

# **Summary**

Drugs administered food-producing animals close to the time of slaughter often result in prohibited antimicrobial residues in the animal tissues at slaughter. Evidence based on the Premi® test confirmed the occurrence of antimicrobial drug residues in 89.3% of kidney and urine samples from cattle slaughtered within Abuja town where the residents rely heavily on beef as a source of protein. The administration of antibiotics close to the time of slaughter by marketers/herd owners and transporters was found to be significantly (*p*<0.05) higher when compared with butchers and abattoir workers. The practice of administering antibiotics to animals close to the time of slaughter was believed to be profitmotivated. The research suggests awareness campaigns amongst the stakeholders, the enactment of appropriate laws for the control of antibiotic use and the empowerment of veterinary public health practitioners in food regulatory agencies as some of the strategies which may positively reduce the risk of antimicrobial drug residues in food animals in Nigeria.

#### Keywords

Abattoir, Abuja, Antimicrobial, Beef, Drug, Nigeria, Residue, Risk, Slaughter.

# La valutazione dei residui di farmaci antimicrobici in carni bovine ad Abuja, capitale del Territorio Federale, Nigeria

#### Riassunto

I farmaci somministrati ad animali da reddito in tempi ravvicinati alla macellazione spesso inducono residui vietati di antimicrobici nei tessuti degli animali al momento dell'abbattimento. Prove effettuate con ®Premi test hanno confermato la presenza di residui di farmaci antimicrobici nell' 89,3% dei campioni di rene e di urina in bovini macellati nel territorio di Abuja dove i residenti utilizzano in maniera rilevante la carne bovina come fonte di proteine. La somministrazione di antibiotici in tempi ravvicinati alla macellazione è risultata significativamente più alta da parte dei venditori / proprietari dell'allevamento trasportatori (p<0.05) rispetto a quella effettuata dai macellai e dai lavoratori del mattatoio. Si ritiene che la prassi di somministrare antibiotici prima della macellazione sia motivata da ragioni economiche. La ricerca suggerisce che le campagne di sensibilizzazione presso i portatori di interesse, la promulgazione di leggi adeguate per il controllo dell'uso di antibiotici, la responsabilizzazione dei veterinari di sanità pubblica che operano nelle agenzie di controllo degli alimenti e l'attivazione di specifiche strategie possono positivamente ridurre il rischio di residui di farmaci antimicrobici negli animali da reddito in Nigeria.

<sup>(1)</sup> Department of Veterinary Public Health and Preventive Medicine, University of Abuja, P.M.B. 117, Abuja, Nigeria gabriel.omeiza90@gmail.com

<sup>(2)</sup> Department of Veterinary Anatomy, University of Abuja, P.M.B. 117, Abuja, Nigeria mailitopa@yahoo.com

<sup>(3)</sup> Department of Veterinary Pharmacology and Toxicology, University of Abuja, P.M.B. 117, Abuja, Nigeria

#### Parole chiave

Abuja, Antimicrobico, Bovino, Farmaco, Macello, Macellazione, Nigeria, Residuo, Rischio.

## Introduction

Beef is becoming the cheapest available source of animal protein in Nigeria. In most developing countries of the world with low capital income and uncontrolled population growth, beef is increasingly becoming a vital component of a balanced diet (28). The contribution of the beef sector to both the well-being of consumers and the economy of the country is important. It is therefore imperative that the sector be well managed by experts who ensure that animals for human consumption are healthy. Skilled manpower is also needed to meet the challenges of relative protein deficiencies which are commonly experienced in most developing nations of the world, including Nigeria. Unfortunately, in this part of the world, serious attention is not given to the sector but rather, it is left in the hands of individuals and businessmen whose interest is to maximise profits even when consumers' health may be compromised.

In Nigeria, there is no strict legislation that governs antibiotic residues in animal tissues (14). The National Agency for Food, Drug Administration and Control (NAFDAC) is charged with the responsibilities of regulating drug use but the emphasis is limited to human drug preparations only and there is no fully established veterinary department that regulates veterinary drug use based on human and animal health safety, as well as the effectiveness of antibacterial drugs (16, 29).

Proper abattoir practices are essential to ensure an uncontaminated meat supply. However, in Nigeria, abattoir systems are substandard. There is no monitoring by official veterinarians, as occurs in other jurisdictions, and there are no proper ante-mortem and postmortem inspections by veterinary officers.

Detection of antibiotic residues in food poses no risk to consumers' health if the level falls below the maximum residue limit stipulated by the world regulatory bodies (29). However, risks to human health may arise when antibiotic residues with no safe limits are found in foods of animal origin destined for human consumption. Mercer (18) and Santos and Ramos (21) reported that under no circumstance should chloramphenicol be found as residues in food. In addition, NAFDAC banned the use of nitrofurans in food animals in Nigeria (19).

Antibiotics are essential in the control of bacterial infections and improved performance in herds of cattle (13, 17) but should not be used as a substitute for good husbandry practices. The problem arises when treated animals are slaughtered without recourse to withdrawal periods for the specific drug (9). This situation is more likely to occur with prolonged administration and over dosage of antimicrobial agents (26). Toxic levels of antimicrobial residues in food animals are known to cause diverse health hazards to susceptible human consumers (24). For this reason, a 'zero tolerance' was specified by the Federal Drug Agency in the United States, for antibiotic residues in meat and meat products intended for human consumption (12).

Abuja town is currently faced with the problem of a population explosion, with an increasing percentage of the population consuming beef daily as a delicacy. The occurrence of antimicrobial residues has previously been reported in some other locations within Nigeria (10, 14). This study sought to assess the magnitude antimicrobial residue contamination of beef in abattoirs within the town, with a view to providing evidence to support proactive measures to protect consumer health.

# Materials and methods

# Investigation of antimicrobial use in beef animals prior to slaughter

A questionnaire was designed to collect information from four slaughterhouses in different areas of the Abuja metropolis. A total of 210 respondents among abattoir workers, butchers, transporters and marketers/herd owners of livestock were required to provide

information on their respective profiles, types of drugs, frequency of use, purpose, the time of administration of drugs to animals prior to slaughter and their respect of recommended withdrawal periods. The replies were collated and the data subjected to Chi-square statistical analysis. Mean differences at p < 0.05 were considered significant.

# Determination of the presence of antimicrobial drug residues in urine, kidney, liver and muscle samples at slaughter

The study was performed using the Premi® test kit obtained from DSM Nutritional products: version 0505, Geleen, The Netherlands. The kit consists of agar ampoules spores the thermophilic containing of Bacillus stearothermophilus. bacterium, spores germinate when heated and produce carbonic acid. This acid causes the bromocresol purple indicator in the ampoule to change from purple to yellow. The presence of antimicrobials in test samples inhibits the growth of the bacteria and the ampoule remains purple, indicating a positive result (7). A 5 ml sample of urine was aseptically collected directly from the bladders of four randomly selected slaughtered animals in each of the four different abattoirs (a total of 16 animals) in Abuja. Tissue samples (50 g) of kidney, liver and muscle were also collected from the same slaughtered animals. This exercise was repeated twice a week for 14 weeks between October 2008 and January 2009. All samples were placed in separate polythene bags and transported on ice packs to the laboratory for immediate analysis. Prior to

the test, the kit was pre-heated at 80°C for 10 min and 64°C for 2 h 45 min, consecutively. The high temperature was adequate to eliminate other bacterial contaminants in the test medium. Tissue extracts were obtained from the samples with an extractor incorporated in the kit.

The Premi® test was performed in accordance with the manufacturer's instructions. volume (100 µl) of each test sample was pipetted onto the surface of the test ampoule. The ampoule was kept at room temperature for 20 min for a pre-diffusion stage before covering with aluminium foil and incubating at 64°C. Distilled water was used as a negative control and 100 µg/ml<sup>-1</sup> amoxicillin as a positive control. During the incubation period (2 h 45 min), the ampoules were examined and then re-examined at intervals until negative control had turned from purple to yellow. When this occurred, the ampoules were incubated for a further 10 min to negate variation between ampoules and the results were then read from the lower two-thirds of the agar. A purple colour indicated a positive result, whereas a change to yellow indicated a negative result.

## **Results**

# Investigation of antimicrobial use in beef animals prior to slaughter

The results from the investigation of antimicrobial use in local beef production in Abuja are presented in Table I. A total of 210 respondents were interviewed. Transporters and marketers/herd owners were those

Table I
Use, amongst the stakeholders, of antimicrobial drugs in beef production in Abuja

Purpose of drug administration								
Stakeholders	Routine drug use	Prevent zoonoses	Prevent losses due to deaths	Prevent weight loss due to transportation stress	Total			
Abattoir workers	8	13	5	0	26 (12.4%)			
Butchers	13	0	8	10	31 (14.8 %)			
Transporters	31	0	16	25	72 (34.3%)			
Marketers/herd owners	38	0	28	15	81 (38.6%)			
Total	90 (42.9 %)	13 (6.2%)	57 (27.1 %)	50 (23.8 %)	210(100.0%)			

who used antimicrobials most in the preslaughter treatment of animals with 34.3% and 38.6%, respectively. There were a variety of reasons reported for administering antimicrobials with those who reported the administration of drugs to animals 'as a routine', accounted for 42.9%, followed by those who administered drugs 'to prevent losses due to death' 27.1%, those 'to prevent weight loss due to transportation stress' (23.8%) and then those 'treating animals to prevent zoonoses' at 6.2%. The activities of marketers/herd owners and transporters with respect to pre-slaughter administration of drugs were significantly (p<0.05) higher compared to those of butchers and abattoir workers.

# Antimicrobial drug and time interval of administration prior to slaughter

Results from this study showed that a large proportion stakeholders (53.2%)administered drugs to cattle designated for meat at 24 h prior to slaughter and many others (26.4%) at 48 h prior to slaughter (Table II). Long-acting oxytetracycline and a penicillin/streptomycin combination were the drugs used most frequently. penicillin and oxytetracycline/penicillin antibiotics were also popular amongst the drugs administered to cattle within less than 48 h prior to slaughter.

# Antimicrobial drug residues in urine, kidney, liver and muscle samples of cattle at slaughter

An insignificant number of animals studied were found to have antimicrobial drug

residues in both liver and muscle tissues. There was, however, a statistically significant difference (p<0.05) between the occurrence of residues in the kidney and urine on one hand, and liver and muscle tissues on the other. In the kidney and urine samples tested, 50 out of 56 (representing 89.3%) in each case were positive for antimicrobial drug residues (Table III).

## Discussion

The study revealed that antibiotics were being inappropriately administered to cattle prior to slaughter in Abuja. The withdrawal period for oxytetracycline injection is 28 days for beef cattle (3) yet this was the most common antibiotic administered in the pre-slaughter period.

In our study, there were variations in the time of administration of the drugs, most of the treatments were given within 48 h prior to slaughter. This suggests the increased likelihood of antimicrobial drug residues in the subsequently offered for consumption. Situations of antimicrobial drug residues in food of animal origin are well documented when drugs are given for prolonged periods, at extra label doses and/or outside prescription (26). Similar studies have revealed the presence of antimicrobial drug residues in laying birds and eggs 6-12 days post treatment (1, 15).

Tetracycline residues have caused excessive flatulence in humans (4). Degraded tetracycline has been incriminated in Fanconi syndrome in predisposed humans (11).

Table II
Drug and time of administration before slaughter

Frequently administered	Time of drug administration prior to slaughter					
drugs	<24 h	24 h	48 h	72 h	>72 h	Total
Procaine penicillin	1	29	14	6	0	50
Penicillin/streptomycin	2	27	12	7	2	50
Oxytetracycline/penicillin	0	24	16	8	2	50
Oxytetracycline long acting	0	41	3	2	4	50
Streptomycin	14	12	21	3	0	50
Total	17 (6.8%)	133 (53.2%)	66 (26.4%)	26 (10.4%)	8 (3.2%)	250

Table III
Results of samples tested in the four abattoirs in the study

Organ	Abatto	Abattoir A		Abattoir B		Abattoir C		oir D	Tests		
	+	-	+	-	+	-	+	_	+	-	Total
Kidney	11	2	10	1	15	0	14	3	50 (89.3%)	6	56
Liver	2	12	0	15	1	14	0	12	3 (2.9%)	53	56
Muscle	1	17	0	10	0	16	0	12	1 (1%)	55	56
Urine	13	1	11	2	14	3	12	0	50 (89.3%)	6	56
Total	27	32	21	28	30	33	26	27	104 (100%)	120	224

<sup>+</sup> positive

Penicillin can cause hypersensitivity reactions and even death in highly susceptible human subjects (24); its degradation products are known to combine with host proteins and become antigenic (20). Risks of cancer, bacterial resistance and continuous changes in susceptibility patterns to anti-microbial agents (8) are some of the realities that cannot be avoided with consumption of antimicrobial drug residues in animal tissues. Inappropriate use of antibiotics in both humans and animals can lead to the emergence of multidrugresistant (MDR) bacteria, methicillin-resistant Staphylococcus aureus (MRSA) and extended spectrum beta-lactamases-producing enteric bacteria (5, 29) and multiple resistant Escherichia coli (6). The presence of MDR pathogens reduces the therapeutic options available to treat those human cases requiring antibiotic therapy. In the context of developing countries, where laboratory capacity for their identification and the range of available antibiotics may be limited, these multi-drug resistant pathogens are problematic.

A disease outbreak caused by pathogenic bacteria which are resistant to most antibiotics is a serious public health concern in a community (15).

The tolerance limits established for the sum of residues of tetracyclines including chlortetracycline, oxytetracycline in tissues of cattle, dairy calves, swine, sheep, chickens, turkeys, catfish, lobsters and salmonids are 2 parts per million (ppm) in muscle, 6 ppm in liver and 12 ppm in fat and kidney (7). In our study, high proportions (89.3%) of kidney and urine samples tested positive for antimicrobial

residues but muscle and liver tissues revealed insignificant numbers. The different profiles of antimicrobial residues observed in the various animal tissues could, amongst other factors, be due to the time of drug administration prior to slaughter, route of administration, nature of drug relative to its protein\*-binding ability, the route of excretion, degree of tissue perfusion and even the age of the animal (23, 25).

The high occurrences of antimicrobial residues in both kidney and urine were supported by earlier reports (30). Some antibiotics, for instance, aminoglycosides, are known to be sequestered in the kidneys even long after detectable concentrations have disappeared from other organs or tissues (2, 27). Nonspecific inhibitory substances against the growth of the test micro-organisms have occasionally been detected in kidney and urine of slaughtered animals (22). The Premi® test used is an automated screening method that enables qualitative the detection antimicrobial residues in tissues of meat, fish and eggs (7). The sensitivity of the test procedure for aqueous solutions of various antimicrobials was determined; penicillin G was detected at 0.0025 ppm, streptomycin at 2.0 ppm, oxytetracycline and tetracycline were both detected at 0.3 ppm (7).

# **Conclusions**

This study highlights that the presence of antimicrobial drug residues in beef consumed in Abuja poses a potential public health risk that needs to be addressed. There should be massive public enlightenment of all

287

negative

stakeholders in the beef industry in regard to the potential public health risks associated with the inappropriate use of antimicrobial drugs in food-producing animals. The World Health Organization has advocated the need to promote awareness of the adverse health consequences associated with drug residues amongst the developing nations of the world (13). Proper legislation to restrict the inappropriate treatment of animals should be enacted. The veterinary profession should be strengthened and empowered to take on the responsibility of overseeing the safety of the beef supply in Nigeria. A total overhaul of drug dispensing systems in the country is advocated. Drugs should not be purchased without a prescription from a qualified veterinary doctor. NAFDAC should be encouraged to create a veterinary department to effectively regulate the use of all animal drugs. Proper regulation and policing of antibiotic use in live animals, together with effective surveillance in slaughtered animals to detect events of non-compliance, are essential components to ensure a safe beef supply.

Our research has provided evidence of the widespread occurrence of antimicrobial drug residues in beef consumed by most inhabitants of Abuja, the Federal Capital Territory of Nigeria. The suggestions put forward in this study to tackle the problem should be implemented to avert adverse human health effects associated with the inappropriate use of antibiotics in animals.

# Acknowledgments

The authors acknowledge Drs D. H. M. Du-Sai and Alex Otu of Ahmadu Bello University, Zaria, for their financial assistance. The valuable technical advice of Mr M.B. Odoba is also much appreciated.

#### References

- 1. Anadón A., Bringas P., Martínez-Larrañaga M.R. & Díaz M.J. 1994. Bioavailability, pharmacokinetics and residues of chloramphenicol in the chicken. *J Vet Pharmacol Ther*, **17**, 52-58.
- 2. Andreini G. & Pagnattelli P. 1972. Kenamycin blood levels and residues in domestic animals. *Veterinaria*. **21**, 51-72.
- Anon. 1999. A freedom of information summary. Abbreviated New Animal Drug Application (ANADA). Oyxtetracycline injection Geomycin 200. ANADA 200-232. The Federal Food, Drug, and Cosmetics Act). Pliva, d.d., Zagreb, 5 pp (www.fda.gov/downloads/AnimalVeterinary/Products/ ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM061645.pdf accessed on 10 September 2012).
- 4. Anthony H.M. 1977. Tetracycline sensitivity as a cause of excessive flatus. Br Med J, 2 (6103), 1632.
- 5. Aqil F., Khan M.S., Owais M. & Ahmad I. 2007. Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*. Methods Find. *J Basic Microbiol*, **45** (2), 106-114.
- 6. Belloc C., Lam D.N., Pellerin J.L., Beaudeau F. & Laval A. 2005. Effect of quinolone treatment on selection and persistence of quinolones resistant *Escherichia coli* in swine faecal flora. *J Appl Microbiol*, **99**, 954-959.
- 7. Cantwell H. & O'Keeffe M. 2006. Evaluation of the Premi® test and comparison with the one-plate test for the detection of antimicrobials in kidney. *Food Addit Contam*, **23**, 120-125.
- 8. Carter G.R. & Cole Jr J.R. 1990. Diagnostic procedures in veterinary bacteriology and mycology, 5th Ed. Academic Press Inc., San Diego, California, 479 pp.
- 9. Coles E.H. 1968. Determination of packed cell volume. *In* Veterinary clinical pathology (E.H. Coles, ed.). W.B. Saunders Co., Philadelphia, 17-19.
- 10. Fagbamila I., Kabir J., Abdu P., Omeiza G., Ankeli P., Ngulukun S., Muhammad M. & Umoh J. 2010. Antimicrobial screening of commercial eggs and determination of tetracycline residue using two microbiological methods. *Int J Poult Sci*, **9**, 959-962.

- 11. Gross J.M. 1963. Fanconi syndrome (adult type) developing secondary to ingestion of outdated tetracycline. *Ann Intern Med*, **58**, 523-528.
- 12. International Dairy Federation (IDF) 1991. Detection and confirmation of inhibitors in milk and milk products. IDF, Brussels IDF Bulletin No. 258.
- 13. Joint Expert Committee on Food Additives (JECFA) 2004. Evaluation of certain veterinary drug residues without ADI/MRL. WHO Technical Report Series, 919, 175.
- 14. Kabir J., Umoh V.J., Audu-Okoha E., Umoh J.U. & Kwaga J.K.P. 2004. Veterinary drug use in poultry farms and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State, Nigeria. *Food Control*, **15**, 99-105.
- 15. Kozarova I., Mate D., Hussein K., Raschmanova K., Marcincak S. & Jevinova P. 2004. High-performance liquid chromatographic determination of sulphadimidine residues in eggs. *Acta Vet (Beograd)*, **54**, 427-435.
- 16. Lehmann R.P. 1972. Implications of the recommendations contained in the report of the Commissioner concerning the use of antibiotics in animal feed. *J Anim Sci*, **35**, 1340-1341.
- 17. McGrane P., Rowe M.T. & Anger S. 1996. Evaluation of Delvotest SP and Charm AlM-96 for the detection of a range of antibiotics in milk. *Milchwissenschaft*, **51**, 330-332.
- 18. Mercer H.D. 1975. Antimicrobial drugs in food-producing animals. Control mechanisms of governmental agencies. *Vet Clin North Am*, **5**, 3-34.
- 19. National Agency for Food and Drug Administration and Control (NAFDAC) 1996. Ban on the use of nitrofuran in livestock and poultry feeds. NAFDAC, Alert No. 10, Lagos.
- 20. Rang H.P., Dale M.M., Ritter J.M. & Moore P.K. 2003. Pharmacology, 5th Ed. Churchill Livingstone, London, 639-641.
- 21. Santos L. & Ramos F. 2006. Analytical methodologies for chloramphenical residues determination in food matrices: a brief review. *Curr Pharm Anal*, **2**, 53-57.
- 22. Schmidt U. & Cremmling K. 1975. Untersuchungen fiber unspezifische Reaktionen im Hemmstofftest beim Antibiotika-Nachweis in Schweinenieren. *Fleischwirtschaft*, **55**, 1736-1737.
- 23. Schwark W.S. 1992. Factors that affect drug disposition in food-producing animals during maturation. *J Anim Sci*, **70**, 3635-3645.
- 24. Seymour E.H., Jones G.M. & McGilliard M.L. 1988. Comparisons of on-farm screening tests for detection of antibiotic residues. *J Dairy Sci*, **71**, 2292-2296.
- 25. Shruti C. & Jonathan H. 2008. Factors affecting drug absorption and distribution. *Anaesth Intens Care Med*, **9**, 167-171.
- 26. Van Dresser W.R. & Wilcke J.R. 1989. Drug residues in food animals. *J Am Vet Med Assoc*, **194**, 1701-1710.
- 27. Van Schothorst M. & Peelen-Knol G. 1970. Detection and identification of some antibiotics in slaughter animals. *Neth J Vet Sci*, **3**, 85-93.
- 28. Wolfe R. 2006. The underappreciated role of muscle in health and disease. Am J Clin Nutr, 4, 475-482.
- 29. World Health Organization (WHO) 1969. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics. *In* 12th Report of the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 430, Geneva, 52 pp (whglibdoc.who.int/trs/WHO TRS 430.pdf accessed on 10 September 2012).
- 30. Yoshimura H., Itoh O. & Yonezawa S. 1982. Microbiological and thin-layer chromatographic identification of aminoglycoside antibiotics in animal body. *Nihon Juigaki Zasshi,* **44**, 233-239.