Terrestrial and Aquatic Environmental Toxicology ©2009 Global Science Books

Oxytetracycline and Procaine Penicillin Residues in Tissues of Slaughtered Cattle in Maiduguri, Borno State, Nigeria

Yaqub Ahmed Geidam[1*] • Hamidu Usman[2] • Hassan Ismail Musa[3] • Franca Anosike[1] • Yemisi Adeyemi[1]

[1] Department of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria
[2] Department of Chemistry, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria
[3] Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria

Corresponding author: * **ygeidam@yahoo.com**

ABSTRACT

A study was conducted in Maiduguri to detect the presence of antibiotics (oxytetracycline and procaine penicillin) residues in some tissues of cattle slaughtered for human consumption. Thin layer chromatography was utilized to qualitatively screen tissue samples by running in parallel with reference standards of both antibiotics. Out of the total 285 tissues sampled, muscle produced the highest incidence rate (32.6%) of oxytetracycline residue followed by liver (5.0%) and then kidney (3.1%). Similarly, muscle tissue produced the highest incidence rate (15.7%) of procaine penicillin residues followed by liver (13.0%) and then kidney (8.3%). The presence of residues of these commonly used antibiotics in tissues is a pointer to a serious public health risk as these antibiotics are also used in humans and may result in the development of bacterial strains resistant to these antibiotics. This underscores the need for a national residue monitoring program in Nigeria in accordance with international regulations.

Keywords: antibacterial, antibiotics, kidney, liver, muscle
Abbreviations: rpm, revolutions per minute; **TLC**, thin layer chromatography

INTRODUCTION

Antibiotics are naturally occurring, semi-synthetic and synthetic compounds with antimicrobial activity that can be applied parentally, orally or topically (Kemper 2008). Presently in Nigeria, many antibiotics are widely used for preventing and treating several diseases, as well as for promoting growth in food-producing animals (Kabir *et al*. 2002, 2004). In Nigeria, cattle are considered to be an important source of protein to the ever-increasing human population. The population of cattle in Nigeria was estimated to be about 13.9 million (Bourn *et al*. 1994) and believed largely to be owned by nomadic rearers (Schafer *et al.* 1997). The present situation where both trained and untrained persons are involved in offering animal health services and coupled with an increased uncontrolled variety of veterinary drugs marketed freely to herdsmen requires that the levels of these substances in animal tissues be monitored. These drugs given to animals orally or parentally may be found in tissues, particularly when the cattle are slaughtered without observing the withdrawal periods (Kabir *et al*. 2004). Long-acting drug preparations such as oxyteracycline, streptomycin and penicillin are particularly favoured for use. Under field conditions, compliance with drug use guidelines, including observance of withdrawal period is difficult (Kabir *et al.* 2002). Moribund animals as well as those with poor prognosis are sometimes slaughtered on the spot or at a nearby slaughter facility and consumed by humans. Such animals are at risk of harbouring antibiotics residues in their edible tissues. The occurrence of veterinary drug residues in edible animal tissues is a global problem as it is considered to be the basis for the development of antibiotics resistance (WHO 1990). Residues of antibacterial drugs have been reported in different categories of cattle such as beef cattle by Van Dresser and Wilcke (1989), dairy cows (*Bos indicus*) by Fajo *et al.* (1995) and bob veal calves by Wilson *et al*. (1991) as cited by Kabir *et al*. (2002). A lot of literature on standard regulatory protocols prohibiting slaughtering animals harbouring residues exists in many countries (EEC Council 1986; Fitzpatrick 1990; WHO 1990; Norcross and Brown 1991; Corrigan 1992). This situation is not totally different in recent times at both WHO and EU (WHO 1997, 1998; EC 1999; Lee *et al.* 2007) levels. Factors such as management, drug pharmacokinetics and demographic variables like age, sex, and species have been reported to influence distribution of drugs in tissues of animals (Black and Claxton 1982; Bevill 1984; Nouws 1992). Information obtained from these studies may be used to provide the basis for establishing routine screening procedures and tolerance levels in tissues of slaughtered animals for commonly used veterinary drugs (WHO 1990). The problem of antibiotics residues in animal tissues has continued to attract attention resulting in many countries and governmental authorities to establish monitoring programmes to determine antibiotic levels in foods, as well as the highest allowable residue levels (Caprioli *et al*. 2000; Ramírez *et al*. 2003).

In Nigeria, legislation regarding drug use and veterinary drug residue control is lacking; basic facilities for determination of residues are also unavailable at the level of abattoirs, farms and markets (Kabir *et al*. 2004). Similarly, there is no specific residue monitoring program. However, there are scanty reports on veterinary drug residues and on the extent of the residue problem in Nigeria in which a limited survey in eastern Nigeria by Oboegbulem and Fidelis (1996) reported finding residues in two out of 24 cattle slaughtered in the region. Another study by Kabir *et al*. (2002) reported 7.4% in the north western region. A study was therefore carried out to screen animals slaughtered in Maiduguri, north-eastern Nigeria to determine the incidence of oxytetracycline and procaine penicillin residues in some tissues of cattle slaughtered as a preliminary means of detecting animals harbouring antibacterial drug residues. Antibiotics are not completely eliminated in animal organisms, as they are bioactive substances, acting highly effec-

Received: 29 May, 2009. Accepted: 3 August, 2009.

Research Note

tively at low doses and excreted after a short time of residence. Antibiotics are optimised with regard to their pharmacokinetics in the organisms: organic accumulation is, as in other pharmaceutics, objectionable and thus, they are excreted as parent compounds or metabolites (Kümmerer *et al*. 2000; Thiele-Bruhn 2003). The levels of antibiotics excretion depends on the active components, routes and periods of administration and the host animal involved; but these levels varies between 40-90% for tetracyclines and sulphonamides (Berger *et al*. 1986; Haller *et al*. 2001; Halling-Sørensen 2001).

MATERIALS AND METHODS

Study area

The study was carried out in Maiduguri, the Borno State capital, which is located at Latitude 1l° 51′ N and Longitude 13° l0′ E in North-eastern Nigeria. The arid zone has rather austere climatic conditions with a hot dry season from late January to late June during which average daily peak temperatures, especially in April and May, are 34.4 to 37.8°C. The rainy season lasts from late June to mid September and provides an annual average of 46.3 cm rainfall. The cold north-easterly trade wind blowing across the Sahara desert in October to January brings with it cold and desiccant effects on the environment. Thus, nomadic herdsmen who own most of the cattle in the State are constantly on the move in most parts of the year in search of lush grazing ground and water. Fatigue imposed on already malnourished animals as a result of movement over long distances, and excessive heat load which is not easily shed, subject animals to severe stress, which in turn makes them succumb easily to infection by various agents and diseases that may necessitate treatment with antibiotics (Brisibe *et al*. 1996).

Sample collection

A total of 285 tissues comprising of muscle (89), liver (100) and kidney (96) of cattle routinely slaughtered at Maiduguri abattoir were screened for the presence of some antibiotics at slaughter. Fresh tissue samples, which included kidney, liver and muscle tissues of cattle were collected from Maiduguri Monday market and Maiduguri abattoir and transferred into sterile vials and transported to the laboratory on ice and processed immediately. The samples were analyzed for the residues of oxytetracycline and procaine penicillin.

Sample processing

Five grams each of muscle, kidney and liver tissues were measures and mixed with 5 ml of ethanol (May and Baker Laboratory Reagents Ltd, England). The samples were crushed and squeezed into fine particles in a Chinese mortar. The solvent was later transferred to 15 ml Falcon centrifuge tubes and centrifuged at 7000 rpm for 10 min. The clear supernatant was transferred into fresh glass test tubes and evaporated to dryness. After drying, the deposits were dissolved in 0.2 ml ethanol and made ready for TLC examination as described by Tajick *et al*. (2002). For comparison of extracted residues with standard antibiotics [oxytetracycline, obtained from Sigma (St. Louis, MO, USA) and procaine penicillin (Sishui Xierkang, Shandong, China)], working concentrations of standard antibiotics were prepared by dissolving 0.1 g of each powder in 4 ml methanol (Thangadu *et al*. 2002). The rationale for the choice of these antibiotics for the study is that they are the most commonly used antibiotics in the study area.

Preparation of silica plate

Glass plates of 20 × 20 cm dimensions were washed in acetone bath. For each plate, 2 g of silica gel F256 (Marik, Germany) was mixed in 5 ml of distilled water and shaken thoroughly to produce fine paste. Clean glass plates were coated with silica pastes by thin layer chromatography (TLC) gel spreader system (Shandon, UK) of 0.25 mm thickness. The plates were then activated at 120°C for 2 h prior to use as described by Boyer (1993).

Pointing, running and digestion

About 0.5 g of the samples was dissolved in 20 ml of methanol, an aliquot of which was spotted on the TLC plates. Treated plates were transferred to already equilibrated TLC tank containing acetone-methanol (1: 1) as the solvent system. The plates were removed from the tank after reaching the solvent front, chromatograms were then observed on UV light at 256 nm (Thangadu *et al*. 2002).

Evaluation of the thin layer chromatogram

The thin layer chromatogram was evaluated qualitatively based on a multistage distribution process as described by Tajick and Shoshreh (2006). This was achieved by parallel running with the reference antibiotics standards. Later, photometric evaluation was comparatively performed directly on the layers.

RESULTS AND DISCUSSION

Table 1 shows the distribution of oxytetracycline in the tissues of slaughtered cattle by organ. Of all the organs sampled, muscle tissue produced the highest incidence rate of 32.6% followed by liver (5.0%) and then kidney (3.1%). Out of the 285 samples screened, 37 (13.0%) of them were positive for the presence of oxytetracycline in the sampled tissues. The high positive samples of oxytetracycline could be as a result of indiscriminate use of these drugs in animals as buttressed by Kabir *et al*. (2002).

Similarly, **Table 2** shows the distribution of procaine penicillin in the tissues of slaughtered cattle by organ. Of all the organs sampled, muscle tissue produced the highest incidence rate of 15.7% followed by liver (13.0%) and then kidney (8.3%). The presence of these veterinary drug residues in animal tissues is considered a public health hazard. Penicillin in animal tissues was reported to have caused severe anaphylactic reaction in a consumer (Teh and Rigg 1992) apart from the known fact of causing the development of drug resistance. The high positive samples of oxytetracycline could be as a result of indiscriminate use of this drug as both prophylaxis and therapeutic means of disease control in animals (Kabir *et al*. 2002, 2004). Furthermore, Kemper (2008) enumerated the three risks derived from immoderate appliance of antibiotics as environmental contamination with original substances or derivatives, the indirect impact on health via resistant micro-organisms and the direct organic damage. Additionally, the influences on the biotic environment are a matter of concern (Kemper 2008). The main interest regarding the use of antibiotics in human and animal treatment is the development of resistant bacterial strains representing a health risk to humans and animals. Especially, the application of veterinary antibiotics to food animals is supposed to enhance the selection for

Table 1 Distribution of oxytetracycline in the tissues of slaughtered cattle by organ.

Organ	n	Positive (%)	Negative (%)	Total (%)	Incidence rate (%)
Muscle	89	29 (32.6)	60 (67.4)	89 (31.3)	32.6
Liver	100	5 (5)	95 (95)	100 (35.0)	5.0
Kidney	96	3 (3.1)	93 (96.9)	96 (33.7)	3.1
Total	285	37 (13.0)	248 (87.0)	285 (100)	13.0

n= number of samples tested

Table 2 Distribution of Procaine penicillin in the tissues of slaughtered cattle by organ

Organ	n	Positive (%)	Negative (%)	Total (%)	Incidence rate (%)
Muscle	89	14(15.7)	75 (84.3)	89 (31.3)	15.7
Liver	100	13 (13)	87 (87)	100 (35.0)	13.0
Kidney	96	8 (8.3)	88 (91.7)	96 (33.7)	8.3
Total	285	35 (12.3)	250 (87.7)	285 (100)	12.3

n= number of samples tested

strains resistant to antibiotics used in human medicine. Transmission of these strains might be performed via direct contact with animals or via the food-chain to the consumers. As antibiotic resistance protects antibiotic-producing organisms from their own products, and other originally susceptible organisms from competitive attack, it is as ancient as antibiotics. Studies have shown that excretion rates of tetracyclines are dependent on the substance, the mode of application, the excreting species and time after administration, but it has been shown to vary between 40 and 90% for tetracyclines and sulphonamides (Berger *et al.* 1986; Haller *et al*. 2001; Halling-Sørensen 2001). Misuse of antibiotics by farmers and veterinarians alike, in addition to causing residues in edible tissues, is also contributing to the development of microbial drug resistance and the spread of resistant bacteria, including those with serious public health consequences within and across national borders (Levy *et al*. 1976; Lyons e*t al*. 1980; Wolfgang 1998; EU Scientific Steering Committee 1999).

REFERENCES

Bevill RF (1984) Factors influencing the occurrence of drug residues in animal tissues after the use of antimicrobial agents in animal feeds. *Journal of the American Veterinary Medical Association* **185**, 1124-1126

Black WD, Claxton MJ (1982) Distribution of neomycin in bull calves after intramuscular injection. *Canadian Veterinary Journal* **23**, 276-278

Berger K, Petersen B, Buning-Pfaue H (1986) Persistence of drugs occurring in liquid manure in the food chain. *Archive für Lebensmittelhygiene* **37**, 99-102

Bresibe F, Nawathe DR, Bot CJ (1996) Sheep and goat brucellosis in Borno and Yobe States of arid northeastern Nigeria. *Small Ruminant Research* **20**, 83-88

Bourn D, Wint W, Blench R, Woolley E (1994) Nigerian livestock resources. *World Animal Review* **78**, 49-58

Boyer RF (1993) *Modern Experimental Biochemistry* (2nd Edn), Bejamin Cummings Publishing Co., Redwood city, CA, 550 pp

Caprioli A, Busani L, Martel JL, Helmuth R (2000) Monitoring of antibiotics resistance in bacteria of animal origin: epidemiological and micribiological methodologies. *International Journal of Antimicrobial Agents* **14**, 295-301

Corrigan PJ (1992) Australia's National residue control program. In: *Proceedings of the 3rd World Congress on Food-borne Infections and Intoxications*, 16th-19th June, Berlin, pp 736-741

EEC Council (1986) Council directive concerning the examination of animals and fresh meat for the presence of residues (86/469/EEC). *Official Journal of the European Community* **L275**, 36-45

EU Scientific Steering Committee (1999) *Opinion of the European Union Scientific Steering Committee on Antimicrobial Resistance*. Available online: http://europa.eu.int/comm/food/fs/sc/ssc/out50_en.html

Fajo M, Norman BB, Farver TF, Lyons JF (1995) A comparison of the incidence of 1992 STOP test detected antibiotic residues between California and other Western states slaughtered cow populations. *California Veterinarian* **July**, 11-12

Fitzpatrick SC (1990) New food safety initiatives in the Food and Drug Administration. *Journal of Animal Science* **68**, 870-873

Haller MY, Muller SR, McArdell CS, Alder AC, Suter M (2001) Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography–mass spectrometry. *Journal of Chromatography A* **952**, 111-120

Halling-Sørensen B (2001) Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. *Archive of Environmental Contamination Toxicology* **40**, 451-460

Kabir J, Umoh JU, Umoh VJ (2002) Characterisation and screening for antimicrobial substances of slaughtered cattle in Zaria, Nigeria. *Meat Science* **61**, 435-439

Kabir J, Umoh VJ, Audu-okoh E, Umoh JU, Kwaga JKP (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

Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological Indicators* **8**, 1-13

Kümmerer K (2001) Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. *Chemosphere* **45**, 957-969

Lee J, Chung H, Chung Y, Lee K (2007) Development of an analytical protocol for detecting antibiotic residues in various foods. *Food Chemistry* **105**, 1726-1731

Levy SB, Fritzgerald GB, Macone AB (1976) Spread of antibiotic resistance plasmids from chicken to chicken and from chicken to man. *Nature* **260**, 40-42

Lyons RW, Samples CL, De Silva HN, Ress AK, Julian EM, Clark PJ (1980) An epidemic of resistant *Salmonella* in a nursery. Animal-to-human spread. *Journal of American Medical Association* **243**, 546-547

Norcross MA, Brown JL (1991) Food safety and inspection service initiatives for tissue residue reduction in meat and poultry. *Journal of the American Veterinary Medical Association* **198**, 819-824

Nouws JFM (1992) Pharmacokinetics in immature animals: A review. *Journal of Animal Science* **68**, 863-869

Oboegbulem SI, Fidelis AP (1996) Detection of antimicrobial residues in poultry meat and slaughter cattle in Nigeria. *Meat Science* **43**, 71-74

Ramirez A, Gutierrez R, Draz G, Gonzalez C, Perez N, Vega S (2003) High-performance thin-layer chromatography-bioautography for multiple antibiotic residues in cow's milk. *Journal of Chromatography B* **784**, 315-322

Schafer C, Hoffmann I, Steinbach J (1997) The role of traditional livestock husbandry in the supply of milk. *Meat and Draught Power in the Northwest of Nigeria Animal Research and Development* **46**, 14-29

Tajick MA, Shohreh B (2006) Detection of antibiotics residues in chicken meat using TLC. *International Journal of Poultry Science* **5**, 611-612

Tajick MA, Rahimian H, Alizadeh A, Rezacian V (2002) Analysis of isolated lipids from scherotia of *Rhizoctonial solani* AG-1-ZA using TLC. In: *Proceedings of 14th Iranian Plant Protection Congress*, Ramanshah, Iran, 291 pp

Teh WL, Rigg AS (1992) Possible penicillin allergy after eating chicken. *Lancet* **ii**, 1632

Thangadu SSK, Shukia SK, Anjanegulu Y (2002) Separation and detection of certain β-lactams and floroquinolone antibiotic drugs by thin layer chromatography. *Analytical Sciences* **18**, 97-100

Thiele-Bruhn S (2003) Pharmaceutical antibiotic compounds in soils – a review. *Journal of Plant Nutrition and Soil Science* **166**, 145-167

Van Dresser WR, Wilcke JR (1989) Drug residues in food animals. *Journal of the American Veterinary Medical Association* **194**, 1701-1710

WHO (1990) Evaluation of certain veterinary drug residues in food. In: *36th Report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Technical Report Series, No. 799, Geneva, Switzerland

World Health Organization (1997) The medical impact of the use of antimicrobials in food animals. Berlin, Germany, 13-17 October, 1997

World Health Organization (1998) Use of quinolones in food animals and potential impact on human health. Geneva, 2-5 June, 1998

Wolfgang W (1998) Medical consequences of antibiotic use in agriculture. *Science* **279**, 996-997

Wilson DJ, Franti CE, Norman BB (1991) Antibiotic and sulfonamide agents in 'bob' veal calf muscle, liver and kidney. *American Journal of Veterinary Research* **52**, 1383-1387