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ARTICLE

Spectrophotometric Analysis of Oxytetracycline Brands Available Over-the-Counter for Veterinary Use in South Western Nigeria

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Abstract

Chemotherapy with oxytetracycline in livestock is reportedly faced with increasing therapeutic failure and resistant bacteria strains circulating in humans, animals and the environment. This study determined the actual concentrations in some dosage forms of oxytetracycline available in Southwest Nigeria labelled for veterinary use by spectrophotometric method of analysis.

Eight 5% (50mg/ml) and four 20% (200mg/ml) preparations of injectable oxytetracycline were purchased from veterinary pharmacies in Ibadan.

The mean concentration of oxytetracycline obtained from formulations ranged between 0.484mg/ml and 0.757 mg/ml. All the commercial formulations contained less than the labelled concentration when 1mg/ml of these samples was compared with 1mg/ml of the analytical grade of oxytetracycline. Therapy failures and development of bacterial resistance to oxytetracycline could therefore result from proliferation of fake or sub-standard drugs and indiscriminate use or abuse.

There is need for strict regulation and quality control of importation, marketing and use of veterinary drugs in Nigeria. Also, there is need for pharmaco-vigilance and pharmaco-epidemiological investigation of chemotherapeutic agents, which is recommended to ensure their efficacy for animal health and food safety.

Introduction

Oxytetracycline is obtained from *Streptomyces rimosus*, a soil actinomycete and is one of the first two members of the tetracycline group of antibiotics ever discovered (Kim *et al.*, 1994). Oxytetracycline hydrochloride (Syn: 5-Hydroxytetracycline hydrochloride) is chemically recognized as [4S-(4alpha,4aalpha,5alpha,5aalpha,6beta,12aalpha)]-4-(Dimethylamino) 1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2 naphthacenecarboxamide monohydrochloride (Fig 1). Tetracyclines act by inhibition of bacterial protein synthesis through prevention of the association of the bacterial ribosome with aminoacyl tRNA (Chopra *et al.*, 1992; Schnappinger and Hillen, 1996).

Tetracyclines are widely administered for prophylactic and therapeutic purposes, as well as at sub-therapeutic doses for growth promotion in livestock (Gustafson and Kiser, 1985; Karimuribo *et al.*, 2005). Up till the 1950s, about 98% of commensals and pathogenic bacteria were reported sensitive to tetracycline (Levy, 1992). Resistance to tetracycline has since been on the increase and recent studies showed that about 85 to 91% of food-borne

pathogens were resistant to tetracycline (Ojo *et al.,* 2009; Olatoye, 2010). Resistance to tetracycline have occurred due to their indiscriminate use and abuse in medical, veterinary and agricultural practices (Dina and Arowolo, 1991; Chopra and Roberts, 2001; Dipeolu, 2002).

In Nigeria, like in most developing countries, antibiotics are widely available over-the-counter and mostly used without prescriptions (Dina and Arowolo, 1991; Ezenduka *et al*., 2011). Withdrawal periods are not usually observed and residues have been reported in tissues of food animals and their products (Kabir *et al*., 2004; Olatoye and Ehinmowo, 2010). Several brands of tetracycline imported and sold in veterinary shops, livestock markets and by drug peddlers are routinely used by farmers without veterinary diagnosis or prescriptions (Dina and Arowolo, 1991; Fagbamila *et al*., 2010). Most of these drug formulations have been reported ineffective against tetracycline susceptible organisms (Ojo *et al.,* 2009). WHO (2006) estimated that about 30% of medicines sold in Africa are counterfeit of which antimicrobial agents being the most popular target. Substandard products with lower-than-stated doses promote resistance, and those containing no antimicrobial drug at all promote microbial dissemination (Okeke *et al.,* 2007). The proliferation of fake or sub-standard drugs could contribute to widespread resistance to numerous antibiotics with tetracycline resistance being one of the most common (Shakoor, *et al*., 1997). There is a large market for drugs in Nigeria with about 130 existing pharmaceutical manufacturers. Only 60 of these are actively manufacturing, while up to 70% of drugs are imported into the country (Okoli, 2000). The suspected sharp practices by some drug manufacturers and importers in the production and packaging of drugs, and obvious therapy failures on the field necessitated this study. The study was designed to evaluate the concentrations of oxytetracycline in some formulations (brands) available in Nigeria for veterinary application using the spectrophotometric analytical method.

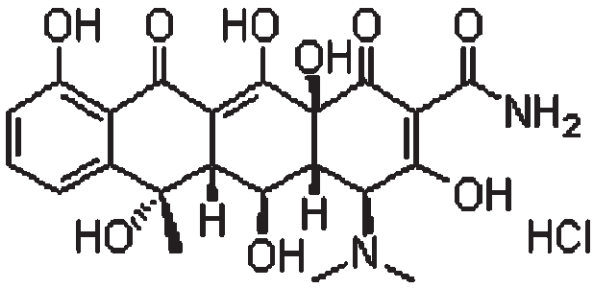

Fig 1: Molecular structure of oxytetracycline hydrochloride
Molecular Formula $C_{22}H_{24}N_2O_9 \cdot HCl$
Molecular Weight 496.90

MATERIALS and METHODS

Chemicals and reagents

Analytical grade of oxytetracycline hydrochloride was purchased from Sigma-Aldrich St. Louis, MO, USA. Random samples of commercially available injectable oxytetracycline brands for veterinary use [5% (50mg/ml) (n = 8) and 20% (200mg/ml) (n = 4)] were purchased from veterinary pharmacies in Ibadan, Nigeria and these were used at least six months before their expiry dates. The drug samples included in the study were all imported brands with National Administration of Food and Drug Administration (NAFDAC) registration identifications and designated as A, B, C, D, E, F, G and H for 5% formulations, while the 20% formulations were designated as I, J, K, and L. HPLC-grade of methanol was also purchased from Sigma-Aldrich (USA).

Preparation of Standard curve

Laboratory grade analytical standard

oxytetracycline were accurately weighed and dissolved in methanol to produce different concentrations of 1, 0.75, 0.50, 0.25 and 0.1mg/ml. The absorbances of these concentrations were measured in triplicate at 540nm on a spectrophotometer (SM22PC Surgienfield Instrument, England) after obtaining zero absorbance with methanol. The calibration curve used in this study (Fig. 2) was prepared by obtaining linear relationship between these known concentrations of the standard and their absorbance.

Sample preparation and test protocol

Dosage forms (commercial preparations) of injectable oxytetracycline (1mg/ml each) were dissolved in methanol and the absorbances of these solutions on the spectrophotometer at 540nm were recorded. The concentrations of these solutions were determined using the linear equation obtained from the known concentrations and corresponding absorbance of the standards curve of oxytetracycline. The procedure was repeated four times for each brand, and the mean values and standard error of mean were calculated.

The method was validated using relative standard deviation (RSD) obtained from the standard oxytetracycline and the correlation coefficient (R^2) of the standard curve.

Statistical analysis

The results obtained were expressed as mean values ± standard deviation (SD). Data were analysed using one way analysis of variance (ANOVA) on GraphPad Prism 4.0 version. The statistical significant difference between the mean values were determined at $p<0.05$.

RESULTS

The 5% formulations of oxytetracycline were presented as brands A, B, C, D, E, F, G or H, while the 20% formulations were presented as brands I, J, K or L. The mean concentrations of oxytetracycline obtained for the 5% (50mg/ml) formulations ranged between 36.2mg/ml (3.6%) and 37.9mg/ml (3.8%) which is between 24.3 - 27.5% less than the standard oxytetracycline as shown in Table I, while the mean concentration of the 20% (200mg/ml) oxytetracycline samples ranged between 96.8mg/ml (9.7%) and 101.2mg/ml (10.1%) (Table II).

All the formulations contained less than the labelled oxytetracycline concentration indicated values. Significantly lower concentrations were obtained in drugs A, E, G, I, J and K. Most of the 20% oxytetracycline brand samples contained less than 50% of the concentrations indicated by their manufacturers, while the 5% oxytetracycline brands had about 30%

less than the expected concentrations (Table 1).

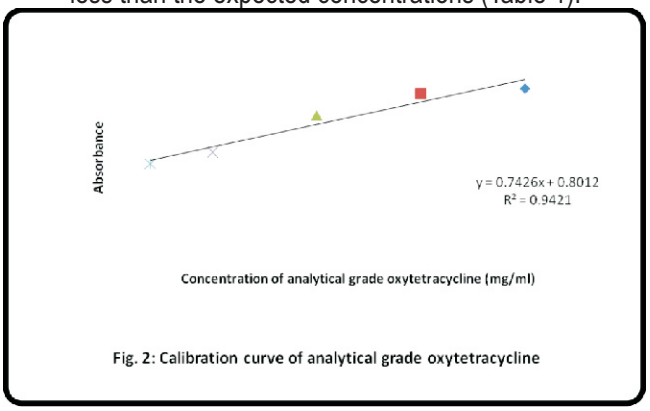

Fig. 2: Calibration curve of analytical grade oxytetracycline

TABLE I: Mean absorbance of 5% oxytetracycline brands (1mg/ml) with their corresponding concentrations.

Oxytetracycline Brands	Mean absorbance	Concentration (mg/ml)	% concentration present	Concentration (mg/ml) of oxytetracycline relative to 5% labelled
A	1.446±0.006	0.7471	74.71	37.4 (3.7%)
B	1.440±0.004	0.7386	73.86	36.9 (3.7%)
C	1.446±0.005	0.7476	74.76	37.4 (3.7%)
D	1.446±0.003	0.7471	74.71	37.4 (3.7%)
E	1.429±0.003	0.7247	72.47	36.2 (3.6%)
F	1.451±0.003	0.7538	75.38	37.7 (3.8%)
G	1.436±0.012	0.7332	73.32	36.7 (3.7%)
H	1.454±0.003	0.7574	75.74	37.9 (3.8%)

TABLE II: Mean absorbance of 20% oxytetracycline brands (1mg/ml) with their corresponding concentrations.

Oxytetracycline Brands	Mean absorbance	Concentration (mg/ml)	% concentration present	Concentration (mg/ml) of oxytetracycline relative to 20% labelled
I	1.258±0.005	0.4944	49.44	98.9 (9.9%)
J	1.260±0.013	0.4962	49.62	99.2 (9.9%)
K	1.251±0.006	0.4841	48.41	96.8 (9.7%)
L	1.267±0.003	0.5061	50.61	101.2 (10.1%)

DISCUSSION

The values of oxytetracycline concentration obtained for all drug formulations evaluated in this study were found lower than what is indicated on their labels. The method used was found to be linear (R^2> 0.94), the linear relationship in the calibration curve of the standard oxytetracycline, while accuracy and precision of the method was shown by RSD <4.21% obtained from standard oxytetracycline. The result of this study therefore implies that most of the commercially available oxytetracycline brands for veterinary use in the studied area were sub-standard as they contained lower concentrations of the active ingredients below the manufacturer's claims. The sub-standard antibiotic formulations imply that there is an

ongoing widespread administration of sub-therapeutic doses of antibiotics to sick animals which has its attendant problems including but not limited to; therapy failure, development and spread of resistant strains of micro-organisms in human and animal populations (Okonko *et al.*, 2008; Nkang *et al.*, 2010).

Several years of medical exploitation of antimicrobial chemotherapy, particularly in treatment of human infections, has imposed an enormous selection pressure on formerly sensitive bacteria to acquire genetic elements that code for resistance to antibiotics (Prescott *et al.*, 2000; Nkang *et al.*, 2010). Administration of sub-therapeutic doses to sick animals will no doubt serve to increase incidences of diseases and elevate the virulence of the microorganisms that evolve after such exposure. Severe economic losses are usually the fall out of uncurtailed disease outbreaks due to unmitigated increased morbidity and mortality (Okeke *et al.,* 2007). Aside this, development of resistance by microorganisms to antibiotics lead to heavy loss of revenue by genuine drug manufacturers due to evolvement of resistance in what was formerly sensitive organisms (Okeke and Sosa, 2011).

The reduction in the concentrations of the antibiotic formulations could also have been as a result of degradation of the active compounds in the storage process as appropriately manufactured drugs could also become substandard due to improper storage. The storage methods adopted by manufacturers and retailers of these drugs should be evaluated for possible contributions to degradation of drugs on the shelf. Okeke *et al.*, (2007) stated that antibiotics are unstable at ambient tropical conditions, but shelf lives and packaging are not adapted to preserve drug potency or mark their degradation in countries where these drugs are most needed. Proper storage in the tropics requires expensive electrical equipment, a constant storage temperature, pharmaceutical handling expertise, and an efficient supply chain, which do not exist in many parts of Africa. Storage methods should be standardized to ensure drug quality is maintained and prevent future re-occurrence of diminishing quality. The environmental temperature of the tropical region should also be considered for drugs exported from temperate regions.

The low quality of these drugs could have encouraged the practices of indiscriminate use, abuse and overuse of antibiotics in food-producing animals to achieve growth promotion and therapeutic results from these drugs. This has become of enormous concern in recent times due to increase in resistant bacterial strains in humans (National Research Council, 1999; Okesola and Oni, 2009; Okonko *et al.*, 2009).
Policies regulating antibiotic use vary from one country to another. Many countries have highlighted the need for better control of licensing of antibiotics and codes for prudent use by veterinary practitioners and farmers. In most developed countries, antibiotics intended for animal use are assessed for their potential to compromise human health. In such countries there is control-of-use legislation that restricts antibiotics registered for therapeutic or prophylactic use to registered veterinary surgeons, but allows over-the-counter sales to farmers or stock-feed companies of products registered for use as growth promoters (Barton, 2000). In Nigeria there is a palpable preponderance of sub-quality pharmaceutical products and all veterinary antibiotics are sold over the counter. Osibo (1998) posited a strong and urgent need for stricter regulation and

quality control of importation, marketing and use of veterinary drugs in Nigeria. Regulatory organs of the government such as National Administration of Food and Drug Administration (NAFDAC) must strive to enforce the extant laws in order to evaluate and monitor the quality of veterinary drugs manufactured or imported into Nigeria. The establishment of functional veterinary directorate in NAFDAC as approved by the Federal Government of Nigeria could be a positive step towards addressing these problems. This may appear a daunting task considering the low level of requisite technology in the country, but it pales into insignificance when compared to likely adverse outcome on both human and animal populations.

In conclusion, since a higher percentage of drugs used in this Nigeria are either imported wholly or merely packaged in the country, routine pharmco-epidemiological studies are necessary. All the drugs sampled in this study are imported drugs. It does appear that uncontrolled importation of drugs in Nigeria has encouraged sharp practices which place commercial motive or gain over ethical considerations. There is therefore an urgent need for enforcement of appropriate drug regulations and pharmaco-epidemiological surveillance of veterinary drugs in Nigeria to ensure their consistent efficacy for effective animal health and human safety.

REFERENCES

BARTON, M. D. (2000). Antibiotic use in animal feed and its impact on human health. *Nutr. Res. Rev.* 13:279 – 299.

CHOPRA, I., HAWKEY, P. M., and HINTON, M. (1992). Tetracyclines, molecular and clinical aspects. *J. Antimicrob. Chemother.* 29: 245–277.

CHOPRA, I. and ROBERTS, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.*, 65(2): 232-260

DIPEOLU, M.A. (2002). Residue of tetracycline antibiotic in marked goat and pigs in Lagos and Ogun States Nigeria. *Trop. J. Anim. Sci.* 5(2): 47-51

EZENDUKA, E.V., OBOEGBULEM, S.I., NWANTA, J.A. and ONUNKWO, J.I. (2011). Prevalence of antimicrobial residues in raw table eggs from farms and retail outlets in Enugu State, Nigeria. *Trop. Anim. Health Prod.* 43 (3): 557-559.

FAGBAMILA, I., KABIR, J., ABDU, P., OMEIZA, G., ANKELI, P., NGULUKUN, S., MUHAMMAD, M. and UMOH, J. (2010). Antimicrobial screening of commercial eggs and determination of tetracycline residue using two microbiological methods. *Int. J. Poultry Sci* 9 (10): 959-962

GUSTAFSON, R. H., and KISER, J. S. (1985). Nonmedical uses of the tetracyclines. In J. J. Hlavka and J. H. Boothe (ed.), Handbook of experimental pharmacology, vol. 78. Springer-Verlag KG, Berlin, Germany, 405–446.

KABIR, J., UMOH, V.J., AUDU-OKOH, E., UMOH, J.U. and KWAGA, J.K.P. (2004). Veterinary drug use in poultry farms and determination of antimicrobial drug residue in commercial eggs and slaughtered chicken in Kaduna state, Nigeria. *Food Contr.* 15: 99-105.

KARIMURIBO, E.D., MDEGELA, R.H., KUSILUKA, L.J.M. and KAMBARAGE, D.M. (2005). Assessment of antimicrobial usage

and antimicrobial residues in milk on small holder farms in Morogoro, Tanzania. *Bull. Anim. Health Prod. Afri.* 53: 234-241.

KIM, E., BIBB, M.J., BUTLER, M.J., HOPWOOD, D.A. and SHERMAN, D.H. (1994). Sequences of the oxytetracycline polyketide synthase-encoding otc genes from *Streptomyces rimosus*. *Gene* 141 (1): 141-142.

LEVY, S. B. (1992). Active efflux mechanisms for antimicrobial resistance. *Antimicrob. Agents Chemother*. 36:695–703

NATIONAL RESEARCH COUNCIL-NATIONAL ACADEMY OF SCIENCES. (1999). The use of drugs in food animals: Benefits and risks, National Academy Press: Washington, DC pp 184.

NKANG, A. O., OKONKO, I. O., LENNOX, J. A., EYAREFE, O. D., ABUBAKAR, M. J., OJEZELE, M. O., BABALOLA, E. T., OGUNNUSI, T. A., ONAJOBI, B. I. And AMUSAN, T. A. (2010). Assessment of the efficacies, potencies and bacteriological qualities of some of the antibiotics sold in Calabar, Nigeria. *Afr. J. Biotech*. 9(41): 6987-7002,

OKEKE, I.N., ABODERIN, O.A., BYARUGABA, D.K. OJO, K.K. and OPINTAN J.A. (2007). Growing Problem of Multidrug-Resistant Enteric Pathogens in Africa. Emerging Infectious Diseases. 13(11): 1640-1646

OKEKE, I.N., and SOSA, A. (2011). Antibiotic Resistance in Africa – Discerning the enemy and plotting a defense. http://www.tufts.edu/med/apua/about_issue/africahealth.pdf

OKESOLA, A.O. and ONI, A.A. (2009). Antimicrobial resistance among common bacterial pathogens in south western Nigeria. *American-Eurasian J. Agric. Environ. Sci.* 5(3): 327-330.

OKOLI, S. (2000). Pharma Industry in Distress. *Pharmanews* 22(3):1.

OKONKO, I.O., FAJOBI, E.A., OGUNNUSI, T.A., OGUNJOBI, A.A. and OBIOGBOLU, C.H. (2008). Antimicrobial Chemotherapy and Sustainable Development: The Past, the Current Trend, and the future. *Afr. J. Biomed. Res.* 11(3): 235-250.

OKONKO, I.O., SOLEYE, F.A., AMUSAN, T.A., OGUN, A.A., OGUNNUSI, T.A. and EJEMBI, J. (2009). Incidence of Multi-Drug Resistance (MDR) Organisms in Abeokuta, Southwestern Nigeria. *Global J. Pharmacol.* 3(2): 69-80.

OLATOYE, I.O. and EHIMOWO AA, 2010. Oxytetracycline residues in edible tissues of cattle slaughtered in Akure, Nigeria. *Nig. Vet. J.* 31 (2): 93-102.

OSIBO, O.O. (1998). Faking and counterfeiting of drugs. *West Afr. J. Pharmacy*. 12(1):53 – 57.

PRESCOTT, J. F., BAGGOT, J. D. and WALKER, R. D. (2000). Antimicrobial Therapy in Veterinary Medicine, 3rd ed. Ames, Iowa, USA, 612.

SCHNAPPINGER, D. and HILLEN, W. (1996). Tetracyclines: antibiotic action, uptake, and resistance mechanisms. *Arch. Microbiol.* 165: 359–369.

SHAKOOR, O., TAYLOR, R.B. and BEHRENS, R.H. (1997). Assessment of the incidence of substandard drug in developing countries. *Trop. Med. Int. Health*. 2(9): 839-45.

WORLD HEALTH ORGANIZATION (WHO) (2006). Counterfeit

medicines: an update on estimates. Geneva: WHO International Medical Products Anti-Counterfeiting Task Force; 2006.