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Quality Evaluation of Oxytetracycline Injection and Water Soluble Powder Veterinary Products Marketed in Nairobi City, Kenya

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The antibiotic oxytetracycline is extensively applied in veterinary medicine in diverse formulations including injectable solution, water soluble powder and wound sprays. The drug is liable to degradation during processing and shelf life, which may compromise its quality attributes. A survey on the quality of oxytetracycline injection and water soluble powder products available in agroveterinary (agrovet) and pharmacy stores in Nairobi city, Kenya was carried out. Thirteen injectable and 11 water soluble powder samples were subjected to British Pharmacopoeia and United States Pharmacopeia quality specifications. Whereas all the injections complied with assay specifications, only three complied with colour of solution and none complied with pH specification. Furthermore, all water soluble powder samples failed the pH test, while two did not comply with assay specifications. The results obtained demonstrate the existence of substandard oxytetracycline products in the study area. Proper adherence to good manufacturing and distribution practices as well as post market surveillance studies are required to ensure optimal quality of veterinary drugs in the market.

Key words: Quality control, veterinary antibiotic, assay, pH, substandard and falsified medicine, degradation

INTRODUCTION

Oxytetracycline (OTC) is a natural tetracycline antibiotic commercially obtained through fermentation of *Streptomyces rimosus* since its discovery in 1948. The drug is widely used as a routine antibacterial agent in veterinary medicine in diverse formulations such as injection, water soluble powder (WSP) and wound sprays. Oxytetracycline is taken up by susceptible bacteria through active transport thus leading to intracellular accumulation. For antibacterial action, OTC binds to the 30S ribosomal subunit thereby arresting the initiation stage of protein synthesis.[1]

Commercial OTC injection exists in various strengths (5-30% w/v), while the WSP is formulated with excipients either alone or in combination with vitamins and minerals. These formulations may undergo deterioration during processing and shelf-life due to pH, light, temperature and microbiological effects. The respective degradation products include, 4-epi-oxytetracycline, apo-oxytetracycline and anhydro-oxytetracycline (Figure 1), all of which are inactive.[2]

Antimicrobials including OTC are applied in veterinary practice for prophylaxis, metaphylaxis and therapeutic purposes. It is therefore imperative that these products be of good quality with regulated distribution, marketing and utility. Nonetheless, there have been reports of treatment failures in Kenya from agroveterinary (agrovet) staff and farmers wherein OTC tops the list. This may be attributed to poor quality products or antibiotic misuse which may in turn predispose the drug to antimicrobial resistance with disastrous consequences. Evidently, there are no restrictions on self-medication despite its contravention of regulations and veterinary practice ethics.[3] These practices greatly undermine the One Health paradigm for management of antimicrobial agents in human, veterinary and environmental ecosystems for improved planetary health.[4]

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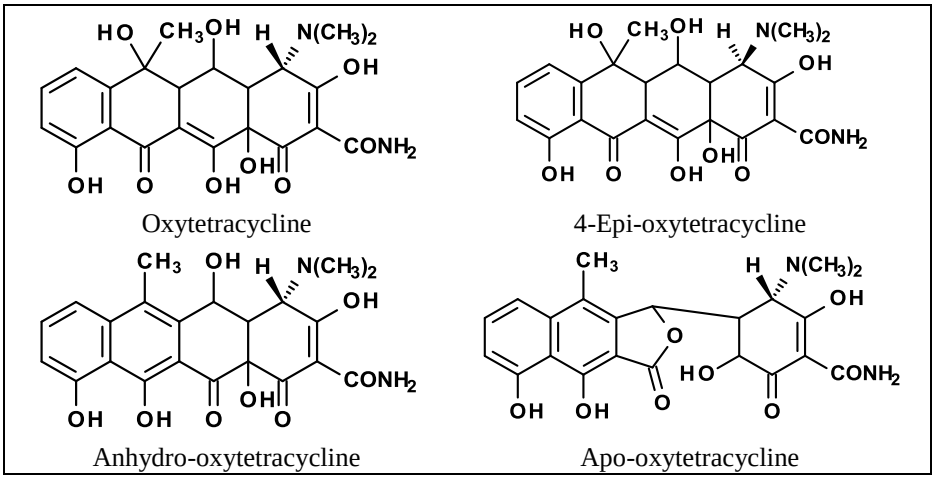

Figure 1: Chemical structures of oxytetracycline and degradation products

Literature on sub-Saharan African studies has demonstrated the existence of substandard and falsified veterinary medicines in circulation.[5] Similarly, market surveillance reports have exposed quality problems with OTC injection and WSP. A study carried out in Addis Ababa, Ethiopia to determine the quality of OTC injections in distribution within the legal market revealed that all products sampled complied with the USP specifications.[6] Conversely, a survey in Ibadan city, Nigeria by Saba *et al.* on OTC injectable solutions (5% and 20% w/v) revealed that all samples did not comply with assay specifications.[7] The analytical method utilized absorbance measurement in the visible region (540 nm) but yielded less than optimal linearity parameters (e.g. r^2 of 0.9421). In another study, Tshibamba *et al.* developed and validated a spectrophotometric method with acceptable validation parameters for the identification and assay of OTC.[5] The method was used in the analysis of 47 samples collected in the Democratic Republic of Congo (DRC), comprising forty-five 20% w/v and two 5% w/v injections. Whereas all samples complied with identification, 19 (40.4%) of them failed in assay. In a Tanzanian post market surveillance (PMS) of veterinary products, 71 samples of OTC injectable and WSP products were subjected to assay using pharmacopoeial specifications whereof 15.6% of injections and 5.1% WSP were non-compliant. The authors postulated that the poor quality medicines encountered could be due to poor manufacturing processes, poor formulation, or intentional actions aimed at reducing production costs. Furthermore, degradation caused by high temperature and humidity during storage and distribution processes could lead to reduced potency.[8]

There is a dearth of studies on the quality of OTC injectable and WSP products in Kenya. Quality control reports from the Drug Analysis and Research Unit (DARU) and the Mission for Essential Medicines and Supplies (MEDS) laboratory have indicated that the poor quality of veterinary products encountered therein is of concern.[9-11] In this context, the current market survey was designed to evaluate the quality of oxytetracycline injections and WSP marketed in Nairobi City, Kenya.

MATERIALS AND METHODS

Samples

A total of 13 oxytetracycline injectable and five WSP brands were purchased from retail agrovet and pharmacy outlets in Nairobi city, using convenient sampling. For this purpose, one injection and up to three WSP batches of each brand were collected from the Central Business District (CBD), Uthiru shopping centre and Dagoretti market. The brands collected were: Oxylife®, Oxycycline®, Oxymet®, Adamycin®, Tetranor®, Pantoxy®, Centre-oxyte®, Egocin®, Abamycin®, Alamycin®, Adacycline®, Kenoxy-LA® and Oxy-T® injections; Egocin®, Ashoxy®, Vetoxy®, Vetaoxy® and Oxyvet® WSP. All samples were stored under ambient conditions until analysis.

Materials, reagents and solvents

Analytical grade HCl (Loba Chemie, Mumbai, India), K_2HPO4 (Rankem, New Delhi, India)

and ortho-phosphoric acid 85% w/w (Uni-Chem, Belgrade, Serbia) were used to prepare buffer solutions. HPLC grade acetonitrile and methanol were from Scharlab (Barcelona, Spain). Freshly distilled water was prepared in the laboratory. Sabouraud Dextrose Agar and Tryptone Soya Agar (Titan Biotech Limited, Rajasthan, India) were used for the microbial load test. Oxytetracycline.HCl USP reference standard (USP, Rockville, MD, USA) was a kind donation from the National Quality Control Laboratory (Nairobi, Kenya).

Equipment

A high-performance liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) was used for identification and assay of the samples. It consisted of a LC-20AD Prominence solvent delivery mechanism, a SIL-20AC Prominence autosampler and supported by a CBM-20A prominence communication bus module. Eluents were monitored by means of a SPD-M20A Prominence UV/Visible detector set at 254 nm. Separation was achieved using a HyperClone BDS C18 (Phenomenex Inc, Torrance, CA, USA) of dimensions 250 mm × 4.6 mm ID, particle size 5 μm maintained at 40 °C. The mobile phase was delivered isocratically at a flow rate of 1.0 ml/min. Neat samples were subjected to pH determination on a 3510 Jenway pH meter (Bibby Scientific Ltd, Staffordshire, UK) equipped with an A1131B refillable pH electrode and an A7662 stainless steel temperature probe. The equipment was calibrated in the 4.0 - 10.0 pH range using standard buffer solutions.

Specifications

Oxytetracycline injections were tested for colour and clarity of solution, pH, identification, sterility and assay in accordance to the BP (2017) veterinary specifications.[12] On the other hand, WSP samples were subjected to USP (2020) tests for pH, microbial load and assay.[13] However, identification and assay were carried out using the HPLC method described by Giugiu.[14] The mobile phase consisted of acetonitrile-methanol-80 mM potassium phosphate, pH 7.5 (17.5: 17.5: 65 v/v/v). The mixture was degassed by means of ultrasonication for 15 min before use.

Physical tests

The injectable solutions were visually observed for colour and clarity. The pH was determined on neat injections, and WSP solutions prepared according to label instructions.

Standard preparation

A stock solution of OTC.HCl (0.2 mg/ml) was prepared by dissolving 20 mg of the reference standard in 0.01M HCl. Twenty-five millilitres of the stock solution were diluted to 100 ml using 0.01M HCl to obtain a final concentration of 0.05 mg/ml.

Sample preparation

Oxytetracycline injections were sonicated for 10 min and serially diluted to a final concentration of 0.05 mg/mL using 0.01M HCl. The WSP samples were dispensed into a beaker and weight equivalent to 25 mg OTC.HCl dissolved in 0.01M HCl to 100.0 ml solution. The solution was filtered, and 5.0 ml diluted to 25.0 mL (0.05 mg/ml) with 0.01M HCl. All samples were freshly prepared prior to analysis.

RESULTS AND DISCUSSION

All injectable solutions were packaged in amber-coloured glass vials. However, samples SD and SM neither had secondary packaging nor package inserts. Oxytetracycline readily undergoes photodegradation due to structural conjugation and phenolic functions hence the need to package them in amber-coloured containers. Secondary packaging offers additional protection against environmental conditions that may trigger deterioration.[15] Current good manufacturing practices (cGMP) stipulate that pharmaceuticals be properly labelled and bear package inserts to provide additional product information on indications, dosing, storage and safety. Thus, lack of package inserts may lead to inappropriate use of drugs, leading to under-dosing, over-dosing, adverse effects and drug resistance.[16]

The results of the quality tests carried out on the samples under study are presented in Table 1. According to the BP (2017) specifications, OTC.HCl solution for injection is clear and

yellow with pH 2.0 - 3.0. All the injection samples were clear solutions but 10 (77%) were brown-dark brown in colour. The colour of tetracyclines intensifies on degradation implying that the brown-coloured samples may have undergone chemical degradation under suboptimal storage conditions or improper packaging material thus causing exposure to actinic light. Besides, all samples had a basic pH hence not compliant with specifications. The pH of a liquid formulation impacts on the solubility, stability, biological tolerability, and pharmacological activity of the active pharmaceutical ingredient. Tetracyclines are unstable at alkaline pH which favours degradation to terranoic acid and iso-oxytetracycline.[17] The BP (2017) specifications provides for an assay tolerance range of 90.0 - 110.0% of the label claim. All injection samples complied with the assay specifications within the range 92.6-104.7% percent of the label claim. The assay results obtained are similar to those achieved in an Ethiopian study but at variance with the outcome of Nigerian (Ibadan City) and DRC studies.[5,7]

Eleven WSP samples representing five brands were analysed. Sample PE4 lacked a batch number which may be attributed to a manufacturing defect. This batch was encountered during sampling and taken as an extra (fourth) batch for the brand on this account. Sample PV1 did not indicate the withdrawal period for meat and dairy products from treated animals. All samples did not comply with pH specifications while all 10 samples subjected to the test for microbial load conformed. One sample (PE4) was not tested due to insufficient sample. Nine samples complied with the USP (2020) specifications for assay except PO1 and PP1 that yielded values below the lower limit (90% label claim). Sample PO displayed a secondary peak which could be due to the OTC degradation products. These samples were the closest to expiry with three months of shelf-life left at the time of analysis. Notably, brands PE and PP demonstrated batch to batch variations in the assay values depicting high coefficients of variation (7.5 and 8.3 respectively). This calls for stringency in good manufacturing practices (GMP) to ensure process control and batch consistency.

Table 1: Analytical results of oxytetracycline samples obtained from Nairobi City

Serial No.	Sample code	Formulation, OTC content	pH	Clarity of solution	Colour of solution	Assay (% label claim)	Tests failed
1.	SA	10% w/v injection	8.1	Clear	Brown	96.6 (1.0)	pH, colour
2.	SB	10% w/v injection	8.4	Clear	Brown	102.2 (0.4)	pH, colour
3.	SC	10% w/v injection	8.4	Clear	Dark Brown	103.1 (0.5)	pH, colour
4.	SD	10% w/v injection	8.4	Clear	Yellow	99.0 (0.3)	pH
5.	SE	10% w/v injection	8.0	Clear	Dark Brown	99.3 (1.7)	pH, colour
6.	SF	10% w/v injection	8.1	Clear	Yellow	99.5 (0.8)	pH
7.	SG	10% w/v injection	8.4	Clear	Brown	95.0 (1.8)	pH, colour
8.	SH	10% w/v injection	8.2	Clear	Yellow	97.7 (0.7)	pH
9.	SI	20% w/v injection	8.3	Clear	Brown	100.9 (1.7)	pH, colour
10.	SJ	20% w/v Injection	8.3	Clear	Dark Brown	98.8 (0.9)	pH, colour
11.	SK	20% w/v injection	8.5	Clear	Dark Brown	92.6 (0.9)	pH, colour
12.	SL	20% w/v injection	8.1	Clear	Brown	97.6 (0.4)	pH, colour
13.	SM	30% w/v injection	8.4	Clear	Brown	104.7 (2.4)	pH, colour
14.	PE1	5% w/w WSP	4.3	-	-	108.7 (1.7)	pH
15.	PE2	5% w/w WSP	4.6	-	-	95.4 (0.5)	pH
16.	PE3	5% w/w WSP	4.5	-	-	93.0 (2.8)	pH
17.	PE4	5% w/w WSP	4.0	-	-	90.8 (1.8)	pH
18.	PO1	20% w/w WSP	4.4	-	-	86.1 (2.7)	pH, Assay
19.	PV1	20% w/w WSP	3.7	-	-	97.8 (1.6)	pH
20.	PP1	20% w/w WSP	3.7	-	-	88.8 (1.1)	pH, Assay
21.	PP2	20% w/w WSP	3.7	-	-	94.0 (1.5)	pH
22.	PP3	20% w/w WSP	3.6	-	-	103.1 (1.9)	pH
23.	PB1	20% w/w WSP	3.5	-	-	108.5 (1.8)	pH
24.	PB2	5% w/w WSP	3.6	-	-	102.9 (1.6)	pH

OTC – oxytetracycline, WSP – water soluble powder, - – not applicable, Figures in parentheses represent the coefficient of variation.

CONCLUSION

Twenty-four oxytetracycline samples consisting of 13 injections and 11 WSP were purchased from agroveterinary retail outlets in the Nairobi City. They were subjected to quality control procedures including colour, clarity, pH, identification and assay tests. All the samples complied with pharmacopoeial specifications for identification. However, two WSP did not comply with assay specifications while all samples failed the acidity test. Only three out of 13 injectables complied with the specifications for colour of solution. These results underscore the significance of stringent regulation and frequent quality evaluation surveys for assured quality, safety, and efficacy of veterinary pharmaceuticals. Post-market surveillance programmes on oxytetracycline preparations are required to ascertain the quality of drugs in circulation. Instructively, countrywide studies may elucidate a more comprehensive quality profile of oxytetracycline injections and WSP marketed in Kenya.

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