

Full Length Research Paper

Oxytetracycline residue levels in beef in Dodoma region, Tanzania

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Antibiotic residues in food of animal origin pose a threat to both human and animal's health due to an increasing level of resistant strains of pathogenic bacteria to a wide range of antibiotic drugs. A cross-sectional study was conducted to assess the levels of oxytetracycline (OTC) residues in raw beef in Dodoma region, Tanzania. The OTC levels were determined by using liquid chromatography-mass spectrometry (LC-MS). A total of 60 beef samples were collected from various slaughterhouses and butcheries. Twenty-one out of 60 samples (35%) had OTC residues and no samples had OTC levels above the maximum allowed residues limits (200 µg/kg). The highest OTC concentration was 4.95 ng/g, while the mean concentration was 0.69 ± 0.09 ng/g. The obtained levels were not expected to induce adverse effects and the beef is safe for consumers. Though the findings indicates the meat in the market is safe for consumers, it calls for a proper management of antimicrobial drugs use for animal production as an additional advantage to consumers.

Key words: Liquid chromatography-mass spectrometry (LC-MS), residues levels, raw beef, oxytetracycline.

INTRODUCTION

To obtain sound animal products from milk and meat, animals have to be kept healthy. The care includes feeding, management and control of animal diseases. Some of the drugs used for treatment of animal diseases in Tanzania include tetracyclines (TCs) and beta-lactams like penicillins and cephalosporin (Katakweba et al., 2013). The TCs which are among the first antibiotics, have bacteriostatic activity against both Gram-positive and negative bacteria and are widely used for the

treatment of livestock (Nonga et al., 2009). The commonly used antibiotics in livestock production is the oxytetracycline. The presence of OTC, residues in raw beef may cause health problems to consumers, such as bone and teeth problems in children, gastrointestinal disturbance and hypersensitivity reactions (Larkin et al., 2004). The OTC is named [4S-4a,4a,5a,5a,6b,12a]-4-(dimethylamino)-,4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 5, 6, 10, 12, 12a-exahydroxy-6-methyl-1, 11-dioxo-2-

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naphtacenecarboxamide with Molecular weight of 496.5. When antibiotics are not used correctly, there is a possibility of losing the efficiency of these drugs in the management of ailment in human and animals (Bilatu 2012). The Food and the Agriculture Organization (FAO) and the World Health Organization (WHO); recommend the maximum OTC residue of 200, 600 and 1200 µg/kg in muscles, livers and kidneys, respectively (Food and Agriculture Organization/World Health Organization, 2014). Tetracycline residues levels in animal products depend on the initial dosage and the duration between the drug administration and animal product collection (Uekane et al., 2011; Abbasi et al., 2012). The antibiotic residues can remain in the animal's body after slaughtering if withdrawal period is insufficient (Hemmat et al., 2014). Hence, the aim of this study was to analyze the levels of OTC residues in raw beef samples collected from Dodoma region, Tanzania.

MATERIALS AND METHODS

A total of 60 raw beef samples were randomly collected from different districts (Bahi, Mpwapwa, Kongwa, Dodoma Urban and Rural and Kondoa) in Dodoma region, Tanzania. A Purposive sampling was used to select these districts, then random sampling was used to pick the beef samples from slaughterhouse and butchers, 30 samples from slaughterhouse and 30 samples from butchers. A total of 10 beef samples were collected from each district. A standard sample size calculation was used to calculate the sample size. Antibiotic-free meat samples (blank matrix) were collected from the Central Veterinary Research Institute of Zambia.

Sample pretreatment and extraction

The samples were kept at -20°C until analysis and were allowed to defrost at room temperature. A representative portion of the defrosted sample (10 g) was weighed and mixed with 25 mg of EDTA per gram sample. The sample and the EDTA were homogenized for 1 min using a blender. The blended sample was further ground using a mortar and pestle. One gram of homogenized sample was accurately weighed into 15 mL polypropylene centrifuge tube. To the sample, 10 µL of 10 µg/mL carbamazepine D10 internal standard solution equivalent to 100 ng/g concentration was added.

Five milliliters acetonitrile were added to the sample and vortexed for 1 min. Each sample was centrifuged for 10 min at 7000 rpm and the supernatant was collected into a separate 15 mL centrifuge tube by decantation. 5 mL acetonitrile were again added to the residue and vortexed for 1 min. The samples were then centrifuged for 10 min at 7000 rpm. Both supernatants were combined in a 15 mL centrifuge tube bringing the total volume to 10 mL. All samples were briefly mixed using a vortex and dried under a stream of nitrogen gas to 2 mL, then sample clean up was done by Supelclean ENVI-carb active coal (Mgonja et al., 2016).

Sample analysis by LC-MS method

The HPLC was equipped with DAD detector and mass spectroscopy (Model Agilent Technologies 6130 Quadrupole LC/MS) to target the flowing parent ions using Single Ion Monitoring

(SIM) mode 461 mass per charge ratio (m/z) for OTC. The analytical column was reversed-phase Eclipse XDB C-18. 4.6 x 150 mm set at a flow rate of 0.5 ml/min. The column temperature was 25°C. Mobile phase A was HPLC water with 0.1% formic acid and solvent C was acetonitrile with 0.1% formic acid. The starting mobile phase composition at 0 min was 85% water: 15% acetonitrile at 0.5 ml/min, which are other mobile phase composition. The wavelength of the DAD detector was set at 275 and 355 nm, respectively. Internal calibration curves were prepared by spiking the blank matrix with pure chromatographic standard solutions in the range between 200 and 2500 ng/g injected for each compound and estimates of the amount of the analyte in samples were interpolated from these graphs.

Validation

To test the analytical method trueness, 14 samples were prepared. Each contained 1 g of homogenized muscle tissue of the negative control sample (blank matrix). Seven samples were spiked with 20 µL of 10 ng/mL solutions, equivalent to 200 ng/g of analyte. The other seven samples were spiked with 250 µL equivalent to 2500 ng/g of the analyte. All samples were processed using the described LC-MS method.

Recovery experiment

Samples recovery was determined with blank bovine muscle spiked at 200 ng/g. To test the recovery, 10 samples that contained 1 g of homogenized muscle tissue of the negative control were prepared. They were spiked with 20 µL of 10 µg/mL spiking solution equivalent to 200 ng/g of the analyte. Four samples were used to calculate the recovery mean and six samples were used to calculate the recovery-corrected content.

Ethical issues

Permission for this study was granted by the Ethical Committee of the Sokoine University of Agriculture.

Data analysis

The data were analysed using SPSS version 20. Descriptive statistics were used to compute means, standard deviations and range, a p-value of less than 0.5 was considered statistically significant.

RESULTS AND DISCUSSION

A validated method was capable of detecting OTC residues in raw beef samples. In this method, the most complicated step was during the meat clean up, due to the fact that meat is a complex matrix. Therefore, clean up by Supelclean ENVI-carb active coal could be enough to remove the interfering substances. In addition, carbamazepine D10 was used as internal standard to correct internal and external error.

The concentrations of residue levels in each sample were calculated in ng/g. The obtained mean concentration was then compared with that of WHO

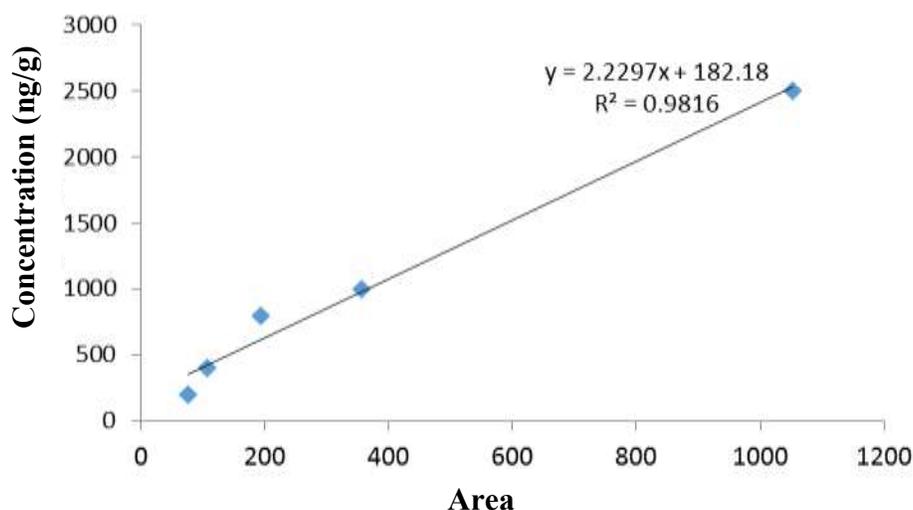


Figure 1. Linear calibration curve of the LC-MS method for OTC residues in beef.

(200 µg/kg). Of the 60 beef samples, 21 (35%) tested positive to OTC residues and 39 (65%) had no OTC residues. However, none of them had residue concentrations above the acceptable levels for muscle (Food and Agriculture Organization/World Health Organization, 2014).

The mean concentration of OTC residues was 0.69 ± 0.09 ng/kg. The detection and quantification limit were 18.2 and 54.6 ng/g, respectively. The correlation coefficients associated with the linear regression for the analytical OTC standard was 0.9816 (Figure 1). The retention time of the standard was 3.624 min.

TCs are important class of antibiotics in food, animal health and production. These antibiotics have been used for many decades in the treatment of diseases, promote growth and to maintain animals health (Olatoye and Ehinmowo, 2010; Bedada and Zewde, 2012). Katakweba et al. (2013) reported that OTC is one of the most commonly used antibiotics in livestock production in Tanzania. The easy access to these antibiotics and lack of awareness may lead to improper management of these drugs.

The results of this study indicate the presence of OTC residues in 35% of the samples and no samples had OTC residues concentration above the acceptable maximum residue levels recommended by the WHO and FAO. The OTC levels in this study were lower than that reported in other studies (Olufemi and Agboola, 2009; Bedada and Zewde, 2012); even though, Donkor et al. (2011) reported a comparable proportion of 21% OTC levels in beef samples from cattle in Ghana. On the other hand, a study conducted on beef from Morogoro and Dodoma municipalities, Tanzania showed 41.2% of the samples tested positive to OTC residues (Mmbando 2004) which is comparable to this study. The reasons for

these differences might be due to the method used, in this study, a simple and sensitive method (Mgonja et al. 2016) was used. In addition, samples collection season and type of TCs used might contribute to the differences. This is due to the fact that during the rain season, the animals are prone to diseases and where by more antibiotics are used during this time which can contribute to misuse of antibiotics. Another reason for these differences may be cold storage as Pavlov et al. (2005) reported decreased level of tobramycin in the poultry products stored at -18°C .

OTC residues in beef samples were also reported in 71.3 and 76.4% in studies conducted in Ethiopia (Addisalem and Bayleyegn 2012) and in Tanzania (Nonga et al. 2013), respectively which were both relatively higher as compared to the percentages observed in the current study. The presence of high levels of antibiotic residues in meat, may be the results of misuse and overuse of antibiotics which may cause microbial resistance (Nisha, 2008). Biswas et al. (2007) also revealed the presence of OTC residues up to 13.3% of the samples, but no sample had OTC residues concentration above MRLs.

Conclusions

OTC residues were detected in 35% raw beef samples from Dodoma, Tanzania by using LC-MS. The results show that beef samples had OTC level below the FAO/WHO MRLs (200 µg/kg), a mean concentration of 0.69 ± 0.09 ng/g. The obtained levels were not expected to induce adverse effects and the beef is safe for consumers. Though, the finding indicates the meat in the market is safe for consumers, it calls for a proper

management of antimicrobial drugs use for animal production, as an additional advantage to consumers.

Conflicts of Interests

The authors have not declared any conflict of interests.

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