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High-Performance Liquid Chromatography (HPLC)-DAD Based Monitoring of Oxytetracycline Residues in Chicken Meat: A Case Study of Kamrup District, Assam, India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The experiment was conducted during Kharif (June–August, 2024) at Instrument Precision Laboratory, Department of Pharmacology and Toxicology, College of Veterinary Science, Khanapara, Guwahati-781022 to assess oxytetracycline residues in poultry meat samples.The samples were obtained from 5 different cities of Kamrup districts of Assam, India. A total of 125 poultry meat samples, consisting of thigh muscle tissues were collected from local road side chicken stalls of Azara, Kamakhya, Khanapara, Narangi and Noonmati areas. The samples were

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collected and stored in sterile sealed containers and transported to the laboratory under ice packs and kept at -20°C till use. Residue analysis was conducted using Ultra-High-Performance Liquid Chromatography coupled with quaternary pump system and diode array detector at 205 nm. The isocratic mobile phase consisting of 0.01 M oxalic acid, acetonitrile, and methanol in a 50:30:20 was used. Separation was achieved with C18 column. Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined to be 0.005 µg g^{-1} and 0.008 µg g^{-1}, respectively. The mean retention time for OTC was 3.2 min. Among the 125 samples tested for oxytetracycline residue, only 2 samples were found to be positive; however, none of the positive samples exceeded the Maximum Residue Limit (MRL). Therefore, the findings suggest that a high compliance rate with withdrawal periods, suggesting effective regulatory adherence and minimal public health risk from oxytetracycline residues in the sampled chicken.

Keywords: Oxytetracyline; residue; chicken; Kamrup; UHPLC; MRL; withdrawal period; public health.

1. INTRODUCTION

The use of antibiotic in both medical and agro-veterianary sector has taken a surge over the past decades. The indiscriminate use of antibiotic in India makes it one of the largest consumers of Antibiotic in the world (Van Boeckel et al., 2019). Antibiotic resistance is a global concern which is gradually causing detrimental effects on mankind. Either the dose of the antibiotic has to be raised or a complete change in the antibiotic regime results in longer hospital stays and unnecessary additional cost for the treatment. Above all, fatalities are also encountered in antibiotic resistance cases (Llor & Bjerrum, 2014). The growing concern of antimicrobial resistance the world facing today can be attributed to the overuse and misuse of antibiotics, particularly in treating viral infections. Availability of over-the-counter antibiotics without proper diagnosis is one of the reasons for bacterial resistance in India (Mongia et al., 2020). Antibiotics play a pivot role in combating various illness caused by bacterial infections, however, their indiscriminate use over the decades has led to the global challenge of antimicrobial resistance (AMR). The World Health Organization (WHO) has identified AMR as one of the top global health threats (WHO, 2020). Factors such as accessibility, healthcare policies and public awareness are the most influencing factors contributing to the consumption of antibiotics across various countries. Developed countries although often uses antibiotics massively, they stick to the guidelines set by regulatory authorities such as European Surveillance of Antimicrobial Consumption (ESAC) set by European Union that to a large extend helps and minimizes the irrational use of antibiotics and thereby slows down the pace of antibiotic resistance. On the contrary, over the counter availability of antibiotics without prescription in developing nation has contributed to higher rates of AMR. Moreover, antibiotics are commonly used in agricultural and veterinary sectors as feed additives to promote growth and Livestock which further exacerbate the antimicrobial resistance even more. The resistant microbes in the environment then reaches the human through contaminated food, water, air and direct (Laxminarayan et al., 2016). Many effective strategies were developed by the Indian Government such as National Action Plan on AMR to counteract the nuisance of AMR.

Oxytetracycline is extensively used in the veterinary sector in India for treating bacterial infections in livestock, poultry, and aquaculture on account of its low cost, broad spectrum and ease of availability (Merck Veterinary Manual, n.d.). It is mostly prescribing to treat common diseases such as mastitis, gastroenteritis and respiratory tract infection (Mungai & Gathumbi, 2018). Despite its effectiveness, overuse and misuse of oxytetracycline have raised concerns about AMR in both animals and humans (Tufa et al., 2023). The unregulated use in animals, particularly in the absence of veterinary supervision, contributes to the global AMR crisis, prompting calls for stricter regulation and awareness campaigns (Corum et al., 2023). Keeping in views, the investigation was aimed to study the oxytetracycline residues in poultry meat samples obtained from 5 different cities of Kamrup districts of Assam, India.

2. MATERIALS AND METHODS

The experiment was conducted during June–August, 2024 at Instrument Precision Laboratory, Department of Pharmacology and Toxicology, College of Veterinary Science, Khanapara, Guwahati, Assam, India.

2.1 Chromatographic Conditions

Following an existing method (Biswas et al., 2007) with slight modification in the ratio of mobile phases,oxytetracycline levels were analysed using a UHPLC system (Dionex) equipped with an autosampler and quaternary gradient pump with Diode Array Detector (DAD) set to operate at 350 nm. Sample separation was performed on an RP-C18 column with isocratic elution. The mobile phase consisted of 0.01 M oxalic acid, acetonitrile, and methanol in a 50:30:20 v/v ratio. The flow rate was maintained at 1.0 mL min^{-1}, achieving a standard curve with a Coefficient of Determination (R^2) of 99.0%. The method's Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined to be 0.005 µg g^{-1} and 0.008 µg g^{-1}, respectively.

2.2 Chemical and Reagents

Oxytetracycline standard (sigma aldrich), HPLC grade Acetonitrile (Fisher), Methanol (Fisher) and Millipore water were used for the study.

Standard solutions of tetracycline at a concentration of 1 mg ml^{-1} was prepared in methanol and stored in amber coloured bottle at -20°C for future use. A series of working standard solutions with concentrations of 1,2,3,4 and 5 µg mL^{-1} were prepared by diluting the stock solutions using methanol for preparation of calibration curve (Fig. 1).

2.3 Sample Collection

A total of 125 samples of poultry thigh were collected from local vendors of Kamrup districts of Assam for residue analysis. The samples were collected aseptically in a clean sterile container and were transported to the laboratory at -20ºC till further analysis.

2.4 Preparation of Samples

Following an existing method (Mungai & Gathumbi, 2018) with slight modification, 10 g of chicken samples were cut into small pieces with the help of scissors and blended with 10 ml of Millipore water in a tissue blender. An aliquot of 0.5 g of the homogenized samples were transferred to a centrifuge tube. 10 mL of Millipore water was added to the tube. The sample underwent ultrasonication for 15 minutes with probe sonicator (IKA, USA) in order to breakdown the tissue further to release intracellular residues. It was then centrifuged at 3000 rpm for 15 minutes, and the resulting supernatant was filtered with Whatman filter paper no 1. The filtrate was processed through a C18 polymeric cartridge preconditioned with 3 ml methanol and 2 ml water. The loaded cartridge was washed with 5 ml water and tetracycline was eluted 5 ml methanol and further filtered using a 0.22 µm syringe filter before loading 1 ml into HPLC system.

3. RESULTS AND DISCUSSION

The mean retention time for OTC was 3.2 min. A total of 125 tissue samples consisting primarily of thigh muscle were obtained from the road side stalls of 5 major places across the Kamrup Metro district of Assam. These samples were analysed in the laboratory to detect the presence of Tetracycline residue. Table 1 depicts the mean ± SE values for oxytetracyline residues that were detected in the samples analysed.

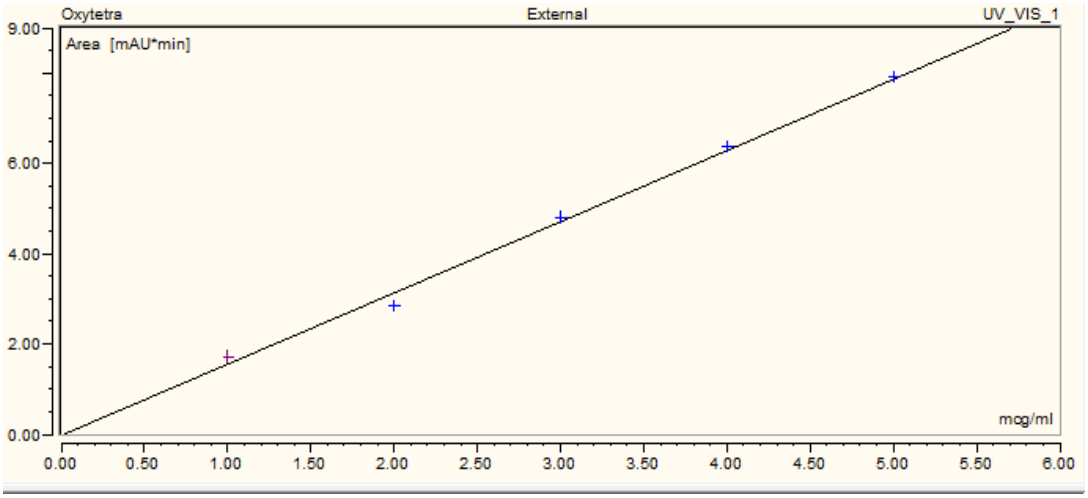

Fig. 1. Calibration curve of Oxytetracycline

Table 1. Oxytetracycline residue in chicken samples

Place of sample collection	Sample origin	No. of samples analysed	No. of+ve samples	Average conc. (µg g^{-1})
Azara	Muscle	25	ND	
Kamakhya	Muscle	25	ND	
Khanapara	Muscle	25	ND	
Narangi	Muscle	25	2	0.021
Noonmati	Muscle	25	ND	

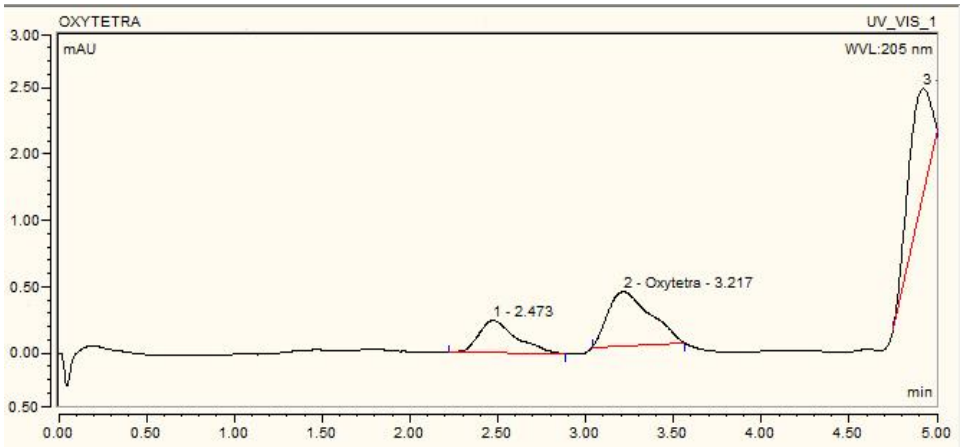

Fig. 2. Oxytetracycline residue detected in chicken sample

Out of 125 samples analysed for oxytetracycline residues, only 2 samples were found to contain oxytetracycline residue.

The average residual concentration of oxytetracycline in the thigh muscles of samples collected from the Narangi area was determined to be0.021 µg g^{-1} with statistical mean of 0.040 µg g^{-1}(Fig. 2). Importantly, none of the tested samples exceeded the maximum residue limit (MRL) of 0.2 µg/g, (FAO, 2015)).

Oxytetracycline (OTC) has been widely used in the treatment and prevention of bacterial infections such as respiratory and callibacillosis in poultry. However, sub-therapeutic doses of oxytetracylines are also routinely used as feed additive in the poultry sector to promote growth and improve feed efficiency (Shao et al., 2021). The latter has raised significant concerns due to the development of antimicrobial resistance, prompting many countries to implement strict guidelines (Attaie et al., 2015). Even for the therapeutic implications, strict following of withdrawal times is mandatory which is 5–14 days depending on route and dose of administration (Kumar et al., 2020). Factors contributing to retention of oxytetracycline in meat and implementing effecting control measures is utmost necessity to minimize the potential risk such as drug resistance, drug allergy and alteration in human microbiome to mankind in order to ensure food safety (Merck Veterinary Manual, n.d.). The presence of oxytetracycline residue in tissue depends on several factors such as dosage, route of administration, withdrawal period and metabolic build-up of the animal/bird (Mungai & Gathumbi, 2018). Owning to their longer absorption and distribution time, orally administered oxytetracycline tends to remain in tissue for longer duration of time when compared to parenteral administration (Tufa et al., 2023) strict adherence to the withdrawal period i.e, time required for the antibiotic to be metabolized and excreted to safe levels is essential. Slaughter of animal/bird before the withdrawal period ends up with oxytetracycline residue in meat and meat products. Oxytetracyline within the body is mostly concentrated in liver and kidney due to their role in drug metabolism and excretion (Pame et al., 2024). However, priority must be emphasised on estimation of oxytetracycline residue in muscle tissues as the latter is the primary part of meat being consumed. Current study concludes that there is low prevalence of oxytetracycline residue in the areas monitored which suggest that the presence of oxytetracycline residues in the

examined tissue samples is minimal and well within the permissible safety limits, reducing potential concerns regarding antibiotic contamination in chicken meat intended for human consumption.

4. CONCLUSION

The present investigation concludes that the levels of Oxytetracycline residue in the screened samples were below the permissible limits. The findings possibly reflect a strict compliance with the withdrawal period before slaughtering of the animals. Nevertheless, effects such as drug resistance, drug allergy, disturbance to enteral microbiome and toxicity are inevitably encounter on chronic exposure to antibiotic residues. In order to mitigate the risk strict regulatory measures, such as withdrawal periods compliance and rational use of antimicrobials are crucial.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

DATA AVAILABILITY STATEMENT

Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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