**Original Research Article**

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

**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 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**

Agriculture has played a pivotal role in shaping Indian economy and livelihood from ages. More than half of Indian’s population directly or indirectly relies on agriculture for their bread and butter. Apart from acting as source of food the agriculture also contribute to secondary income, employment and export of goods1. Unlike past practice, currently the agricultural sectors also encompass livestock farming alongside cereals, pulses fruits and vegetables. Unique climatic structure and vast geography makes India's agricultural system diverse which favours cultivations of variety of crops2. For instance, for rice cultivation the Gangetic plains are considered best owning to its fertile nature while semi-arid regions of Rajasthan and Gujarat favours growth of crops like groundnut and cotton3. States such as Punjab and Haryana are world known for the vast production of wheat and rice, contributing to India's food security collectively4. The Green Revolution, which began in the late 1960s in Punjab remarkably transformed Indian agriculture. Escalation of agricultural productivity was observed with introduction of high-yielding varieties of seeds, chemical fertilizers, and advanced irrigation techniques during the period of Green Revolution in the latter part of 1960s5. This was the period where India declared itself as self-sufficiency in food grains from food-deficient nation. However, the challenge of the green revolution has been growing concern over the past years due to their environmental and economic sustainability6.

The use of antibiotic in both medical and agri-veterianary sector has taken a 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 world7.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 cases8. 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 India9. 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 direct10.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 availability11. It is mostly prescribing to treat common diseases such as mastitis, gastroenteritis and respiratory tract infection12. Despite its effectiveness, overuse and misuse of oxytetracycline have raised concerns about AMR in both animals and humans13. 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 campaigns14. 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 Kharif (June–August, 2024) at Instrument Precision Laboratory, Department of Pharmacology and Toxicology, College of Veterinary Science, Khanapara, Guwahati-781022, Assam, India.

*2.1. Chromatographic conditions*

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, utilizing an isocratic elution with a mobile phase consisting 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 (R²) 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 (Figure 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 -20⁰C till further analysis.

*2.4. Preparation of samples*

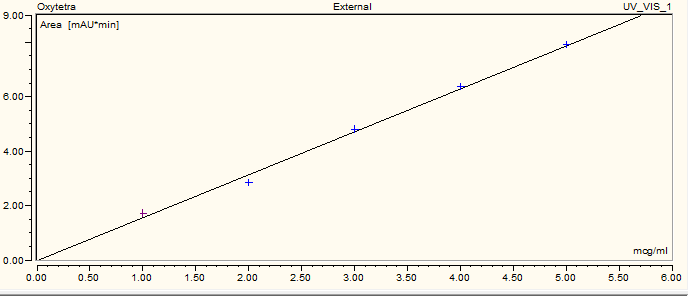
Following an existing method15 with slight modification, 10 g of chicken samples were cut into small pieces with the help of scissors and blended with 10 ml of Millipore water in a tissue blender. An aliquot of 0.5 g of the homogenized samples were transferred to a centrifuge tube. 10 mL of Millipore water was added to the tube. The sample underwent ultrasonication for 15 minutes with probe sonicator (IKA, USA). 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 Discussions**

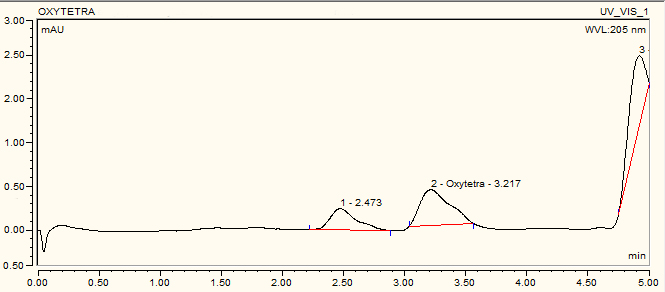
The mean retention time for OTC was 3.2 min. A total of 125 tissue samples consisting primarily of thigh muscle were obtained from the road side stalls of 5 major places across the Kamrup Metro district of Assam. These samples were analysed in the laboratory to detect the presence of Tetracycline residue.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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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 |  |

**Figure 1: Calibration curve of Oxytetracycline**



**Figure 2: Oxytetracycline residue detected in chicken sample**



Only 2 of 125 samples found to be positive for oxytetracycline residue. The average residual concentrations of Oxytetracycline in thigh muscles of Narangi area was found to be 0.021 with μg g-1 with statistical mean of 0.040 μg g-1(Figure 2). None of the sample showed residues above the MRLs i.e, 0.2 μg g-1.

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 efficiency16. The latter has raised significant concerns due to the development of antimicrobial resistance, prompting many countries to implement strict guidelines17. Even for the therapeutic implications, strict following of withdrawal times is mandatory which is 5–14 days depending on route and dose of administration18. 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 safety19. 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/bird20. 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 administration21&22. 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. However, priority must be emphasised on estimation of oxytetracycline residue in muscle tissues as the latter is the primary part of meat being consumed23.

**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.

.

**6. 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.

**REFERENCES**

1. Ministry of Statistics and Programme Implementation. Situation assessment of agricultural households and land and livestock holdings of households in rural India: NSS 77th round (January-December 2019). Government of India, 2019. Available at: <https://ruralindiaonline.org/en/library/resource/situation-assessment-of-agricultural-households-and-land-and-livestock-holdings-of-households-in-rural-india>.
2. Sonavale KP, Shaikh MR, Kadam MM, Pokharkar VG. Livestock sector in India: A critical analysis. Asian J Agric Ext Econ Sociol. 2020;38(1):51-62.
3. Buragohain R, Das S. Effect of Bekang, naturally fermented soybean food, as alternative to antibiotic growth promoter on feed efficiency, blood biochemical and gut health parameters of broiler birds. Int J Bio-resource Stress Manag. 2024;15(12):01-09.
4. Mishra S. Agriculture and rural development in India: Post-liberalisation initiatives. Agric Econ Res Rev. 2011;24(2):227-236. Available at: https://doi.org/10.22004/ag.econ.116907. Accessed on: December 3, 2024.
5. Singh J, Kapoor R. Environmental sustainability and economic implications of the Green Revolution in India. Agric Econ. 2018;45(2):234-245.
6. Prasad S. The impact of the Green Revolution on indigenous crops of India. J Ethnic Foods. 2016;3(1):1-7.
7. Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, Laxminarayan R. Global trends in antimicrobial resistance in animals in low- and middle-income countries. Science. 2019;365(6459).
8. Llor C, Bjerrum L. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv Drug Saf. 2014;5(6):229-41.
9. Mongia MK, Kumar S, Joshi P. Antibiotic misuse and resistance in India: A pressing concern. J Glob Health. 2020;10(1):010302.
10. Kumar A, Gupta S. Antibiotic usage in livestock and poultry: A major threat to public health in India. J Environ Sci Health B. 2019;54(5):419-426.
11. Sharma R, Verma S, Tiwari M. Therapeutic use of oxytetracycline in veterinary practice in India. Indian Vet J. 2018;95(8):63-70.
12. Taneja N, Sharma M. Antimicrobial resistance in the environment: The Indian scenario. Indian J Med Res. 2019;149(2):119-128.
13. Wendlandt S, Shen J, Wang Y, Schwarz S, Yang Q. Mechanisms of quinolone resistance in lactic acid bacteria and enterococci. J Antimicrob Chemother. 2015;70(1):4-15.
14. Kumar S, Sharma R, Kumar S. Determination of antibiotic residues in bovine milk by HPLC-DAD: Health risk assessment. Food Biochem. 2021;45(1):e13547.
15. Munk P, Knudsen BE, Lukjancenko O, Duarte ASR, Van Gompel L, Luiken REC, Aarestrup FM. Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. Nat Microbiol. 2018;3(8):898-908.
16. Shao S, Hu Y, Cheng J, Chen Y. Pharmacokinetics and tissue residues of oxytetracycline in pigs after intramuscular administration. J Vet Pharmacol Ther. 2021;44(1):78-85.
17. Attaie R, Bsharat M, Mora-Gutierrez A, Woldesenbet S. Short communication: Determination of withdrawal time for oxytetracycline in different types of goats for milk consumption. J Dairy Sci. 2015;98(7):4370-4376.
18. Kumar SB, Arnipalli SR, Ziouzenkova O. Antibiotics in food chain: The consequences for antibiotic resistance. Antibiotics (Basel). 2020;9(10):688. Available at: https://doi.org/10.3390/antibiotics9100688. Accessed on: December 2, 2024.
19. Merck Veterinary Manual. "Tetracyclines: Use in Animals." Available at: https://www.merckvetmanual.com/pharmacology/antibacterial-agents/tetracyclines-use-in-animals. Accessed on: December 2, 2024.
20. Mungai AK, Gathumbi JK. Pharmacokinetics and tissue residue of oxytetracycline in goats after intramuscular administration. Trop Anim Health Prod. 2018;50(1):171-177.
21. Tufa TB, Regassa F, Amenu K, Stegeman JA, Hogeveen H. Livestock producers' knowledge, attitude, and behaviour (KAB) regarding antimicrobial use in Ethiopia. Front Vet Sci. 2023;10:1167847.
22. Corum O, Durna Corum D, Terzi E, Uney K. Pharmacokinetics, tissue residues, and withdrawal times of oxytetracycline in rainbow trout (Oncorhynchus mykiss) after single- and multiple-dose oral administration. Animals (Basel). 2023;13(24):3845.
23. FAO. Maximum Residue Limits for Veterinary Drugs in Foods (CAC/MRL 2-2015). Rome: Food and Agriculture Organization of the United Nations (FAO), 2015. Available at: https://www.fao.org. Accessed on: December 1, 2024.