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Determination of Oxytetracycline Residues in Table Eggs from the Local Market of San Jose City: Implications for Local Farm Compliance and Food Safety Screening

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Abstract

Antibiotic residues in food of animal origin present an emerging food safety concern due to their potential to promote antimicrobial resistance and cause hypersensitivity reactions in consumers. Eggs, being a staple protein source, require close monitoring for residual veterinary drugs, especially antibacterial, as their use necessitates a withdrawal period. This study aimed to detect oxytetracycline residues in table eggs sold in selected market stalls of San Jose City, Nueva Ecija, using the disc diffusion method with *Escherichia coli* as the indicator organism. Thirty eggs from ten randomly selected market stalls were homogenized and tested on Mueller-Hinton Agar plates. Discs were infused with homogenized egg samples, distilled water (negative control), and oxytetracycline solution (positive control). After incubation at 37 °C for 12 h, inhibition zones were measured using a caliper. The results showed that all egg samples exhibited no detectable inhibition zones, indicating the absence of oxytetracycline residues above the detection threshold (≥ 14 mm for 8 mm discs) for this assay. The findings thus suggest that eggs sold in San Jose City are likely safe from detectable oxytetracycline residues within the operational limits of the disc diffusion method, and that farms may be observing proper withdrawal periods. However, continuous surveillance and confirmatory tests such as the four-plate bioassay or liquid chromatography are recommended to validate and expand these results.

Introduction

The escalating global challenge of antimicrobial resistance (AMR) is a significant public health crisis, intricately linked to the widespread use of antibiotics in human medicine and animal agriculture (Agmas & Adugna, 2018). Antibiotic residues in food of animal origin, including meat and eggs, are a primary concern, as their consumption can directly contribute to AMR development, lead to hypersensitivity reactions, and cause organ toxicity in humans (European Commission, 2010). These residues pose a complex threat, undermining the efficacy of essential medicines and necessitating robust surveillance frameworks under a "One Health" approach that recognizes the interconnectedness of human, animal, and environmental health (Inter-Agency Committee on Antimicrobial Resistance, 2019).

In the Philippines, the poultry sector is a cornerstone of food security, with eggs providing an affordable and vital source of animal protein for millions (Abuel-Ang, 2005). Despite its importance, consumers largely depend on the assumption that poultry producers adhere to responsible antibiotic use and diligently observe recommended withdrawal periods before marketing their products (Chang, 2007). This reliance on voluntary compliance underscores the critical need for localized data on antibiotic residue prevalence.

Previous studies, both international and local, have highlighted the presence of antibiotic residues in various poultry and livestock products. For instance, oxytetracycline residues were detected in 36% of egg samples in Enugu State, Nigeria (Ezenduka *et al*., 2011), and multiple antibiotic classes were found in eggs from Yogyakarta, Indonesia (Widiasih *et al.*, 2019).

While the Bureau of Animal Industry (BAI) in the Philippines reported sporadic residue detections in poultry meat in Central Luzon (BAI, 2021), there remains a notable paucity of research specifically focusing on antibiotic residues in eggs sold in public markets within the country and across the globe (Owusu-Doubreh *et. al.*, 2022). This knowledge gap presents a challenge to assessing the actual food safety status of a widely consumed commodity.

Improper antibiotic administration and the disregard of withdrawal periods are key factors contributing to residue contamination (Muñoz *et al.*, 2014). Oxytetracycline, a broad-spectrum tetracycline antibiotic, is frequently employed in laying hens for therapeutic purposes, particularly given that *Escherichia coli* is a leading cause of mortality in these birds globally (Hendrix Genetics, 2022). While essential for animal health, oxytetracycline requires an approximate nine-day withdrawal period to ensure that residues fall below acceptable limits (Muñoz *et al.*, 2014). However, adherence to these crucial intervals is often uncertain, especially among small-scale and backyard poultry operations (Ezenduka *et al.*, 2011). Moreover, *E. coli* itself has demonstrated escalating antimicrobial resistance worldwide, further complicating infection treatment and emphasizing the need to mitigate residue-contributing practices. (Kibret and Abera, 2011).

To address these critical gaps, this study sought to generate local evidence regarding the presence of antibiotic residues in the food supply chain. Specifically, it aimed to detect oxytetracycline residues in table eggs marketed in San Jose City, Nueva Ecija—a locality with active poultry production and egg marketing activities in Central Luzon. Utilizing the disc diffusion method with *Escherichia coli* ATCC 25922 as the indicator organism, this research provides an initial screening assessment to: (1) determine the presence or absence of oxytetracycline residues in table eggs; (2) assess the suitability of eggs as a matrix for antibiotic residue screening in Philippine layer farms; and (3) gather basic information on supplier practices regarding egg sourcing and delivery. While the disc diffusion method serves as a practical and cost-effective screening tool, its findings highlight the need for continuous surveillance and the eventual integration of more sensitive confirmatory techniques, such as the four-plate bioassay or liquid chromatography, to fully ensure public health safety and comprehensive residue assessment.

Materials and Methods

Study Design and Sampling Strategy

A cross-sectional study was conducted in January 2024 to evaluate the presence of antimicrobial residues in table eggs sold in San Jose City, Nueva Ecija. To ensure representativeness of both commercial and backyard poultry suppliers, ten market stalls were randomly selected using a stratified sampling method. A total of 30 table eggs were collected, with three eggs obtained from each selected stall. This sample size exceeded the calculated minimum of 24 eggs (8 stalls × 3 eggs) based on the methodology of Widiasih *et al.* (2019), thereby enhancing the statistical reliability of the findings.

Sample Collection

A total of 30 table eggs (three per stall) were procured from the selected market stalls. To ensure traceability, each egg was assigned a unique identifier linking it to its specific supplier prior to transport to the Veterinary Microbiology Laboratory.

Data Collection

Concurrently, a structured questionnaire was administered to the stall owners to assess their supply chain practices. The survey captured data on delivery volume and frequency, the total number of suppliers utilized, and the owners' knowledge of the source farms.

Preparation of Mueller-Hinton Agar Plates and *Escherichia coli* Inoculum

Mueller-Hinton Agar (MHA) was prepared by dissolving 33.5 g of MHA powder (HiMedia Laboratories Pvt. Ltd., India) in 900 mL of distilled water. The solution was then sterilized by autoclaving at 121 °C for 15 min and subsequently cooled to approximately 45-50 °C. *Escherichia coli* ATCC® 25922 (Microbiologics Inc., USA) served as the indicator organism for the antimicrobial sensitivity test. A standardized inoculum of *E. coli* was prepared and adjusted to a concentration of 1.5×10^1 cells/μL. For plate preparation, 100 μL of this inoculum was aseptically dispensed into sterile Petri dishes, followed by the pour-plate technique to evenly distribute the bacteria within the medium and were allowed to solidify.

Egg Sample Preparation

For each market stall, three collected eggs were aseptically opened. Their entire contents (yolk and albumen) were transferred into separate sterile 150 mL beakers. Using a sterile glass stirring rod, the egg contents from each beaker were thoroughly homogenized to obtain a uniform mixture. This ensured an even distribution of any potential antimicrobial residues present within the samples (Garcia, 2023).

Disc Diffusion Assay

The Kirby-Bauer disc diffusion method, utilized in this study, effectively screens for antimicrobial activity by measuring the inhibition of the chosen indicator organism.

While recognized for its efficiency and cost-effectiveness as an initial screening tool, it offers a qualitative assessment rather than quantitative sensitivity levels (Hudzicki, 2009).

Sterile 8 mm filter paper discs were carefully placed onto the solidified MHA plates, which were already inoculated with *E. coli.* The discs were then infused with 10 μL aliquots of the respective solutions: oxytetracycline solution (100 mg/mL) as a positive control, distilled water as a negative control, and the homogenized egg samples. Each plate contained one disc for the positive control, one for the negative control, and discs for the egg samples. The plates were incubated at 37 °C for 12 h.

Inhibition Zone Measurement and Interpretation

After the 12-h incubation period, the diameter of any inhibition zones formed around the discs was measured using a caliper. According to Widiasih *et al.* (2019), an inhibition zone diameter of ≥14 mm for 8 mm discs indicates a positive result for the presence of antibiotic residues (unidentified class of antibiotics). Additionally, the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines specify that *E. coli* is considered highly susceptible to tetracycline-class antibiotics when the inhibition zone diameter is ≥19 mm. Samples exhibiting inhibition zones meeting or exceeding the 14 mm threshold were interpreted as positive for antibiotic residues. For the purpose of this study, all samples were also evaluated against the 19 mm threshold to specifically assess oxytetracycline susceptibility.

Data Analysis

Descriptive statistical analysis was performed on the inhibition zone measurements and the responses collected from the stall owner questionnaires. The market stall (n=10) was considered the unit of analysis, as the eggs were treated as representative of their source.

Ethics Approval

The research protocol was reviewed by the CLSU Ethics Review Committee (ERC Code 2024-551). All stall owners were properly informed of the study's purpose, the nature of the questionnaire, and the intended use of the data.

Results and Discussion

Table 1. Antimicrobial sensitivity test (AST) results of the egg samples in stall 1-10, which show egg samples with or without the presence of antimicrobial residues.

Stall	Positive/Negative
Sample_01	Negative
Sample_02	Negative
Sample_03	Negative
Sample_04	Negative
Sample_05	Negative
Sample_06	Negative
Sample_07	Negative
Sample_08	Negative
Sample_09	Negative
Sample_10	Negative

Table 2. Measurement of inhibition zones of positive and negative controls in stall 1-10.

Stall	Positive control inhibition zone size	Negative control inhibition zone size
Sample_01	22.0 mm	No inhibition zone
Sample_02	20.5 mm	No inhibition zone
Sample_03	21.0 mm	No inhibition zone
Sample_04	19.7 mm	No inhibition zone
Sample_05	20.0 mm	No inhibition zone
Sample_06	19.5 mm	No inhibition zone
Sample_07	23.5 mm	No inhibition zone
Sample_08	22.1 mm	No inhibition zone
Sample_09	23.3 mm	No inhibition zone
Sample_10	22.4 mm	No inhibition zone

All egg samples collected from the ten stalls in San Jose City consistently tested negative for the presence of detectable oxytetracycline residues (Table 1), as they showed no measurable inhibition zones beyond the 8 mm disc diameter, signifying the absence of detectable residues above these thresholds. The validity and responsiveness of the assay were consistently affirmed by the positive control discs (oxytetracycline), which produced clear inhibition zones measuring between 19.0–23.5 mm, while negative controls showed no inhibition whatsoever (Table 2, Figures 1-10). This finding was confirmed by a microbiologist during the study. As summarized in Table 2, the positive controls of sample_01 to sample_10 measured 22.0 mm, 20.5 mm, 21mm, 21.0mm, 19.7mm, 20.0 mm, 19.5 mm, 23.5 mm, 22.1 mm, 23.3 mm, and 22.4 mm, respectively.

The absence of detectable oxytetracycline residues in this study contrasts with findings from other countries. For instance, Widiasih *et al.* (2019) reported a higher prevalence of antibiotic residues, including penicillin (75%), oxytetracycline (12.5%), and aminoglycosides (12.5%), in eggs from traditional markets in Yogyakarta City, Indonesia, using the Four-Plate method, wherein they tested with four different indicator organisms and different classes of antibiotics. This disparity may be attributed to differences in regional and national regulatory enforcement, such as biosecurity protocols and disease control to prevent the use of antimicrobials, which involves proper veterinary drug usage and adherence to withdrawal periods among farming practices. Our results, suggesting the absence of detectable residues, are favorable for consumers and align with reports indicating improved compliance within Central Luzon poultry farms.

This experiment also demonstrated the suitability of eggs as a biological matrix for the initial screening of antibiotic residues. Their homogeneous composition and ability to reflect systemic exposure in layer hens make them a practical choice for preliminary residue monitoring. However, it is crucial to acknowledge the limitations of the disc diffusion method; it is primarily effective for detecting residues strong enough to inhibit bacterial growth, and trace residues below 0.001 mg/kg may remain undetected (Almashhadany, 2020). Therefore, while a useful screening tool, it necessitates follow-up with more advanced confirmatory techniques, such as the four-plate bioassay or liquid chromatography, to ensure a comprehensive and highly sensitive detection of a broader spectrum of antimicrobial compounds.

Regarding farm management practices, only one out of ten surveyed market stalls was able to disclose the specific origin of their eggs, which came from a known Tarlac-based poultry farm. Other vendors reported suppliers from various provinces, including Pangasinan, Pampanga, Tarlac, and Bulacan, but often lacked detailed knowledge of the specific farm practices or antibiotic usage. This observed lack of transparency underscores a significant gap in consumer knowledge and, more importantly, in farm-to-market traceability. Such opacity makes it challenging to verify adherence to proper antibiotic use and withdrawal periods, highlighting the importance of rigorous monitoring systems.

Despite these encouraging findings regarding the absence of detectable oxytetracycline, this study's scope was limited to one indicator organism (*E. coli*) and one antibiotic class proxy (tetracyclines/oxytetracycline). To further strengthen the monitoring of antibiotic residues and provide a more comprehensive assessment of food safety, future research should integrate several key improvements:

1. Broader Antibiotic Screening: Incorporate multiple indicator organisms and employ more sensitive confirmatory methods, such as the four-plate bioassay or advanced chromatographic techniques (e.g., Liquid Chromatography-Mass Spectrometry), to detect a wider range of antibiotic classes and their metabolites at lower concentrations.

2. Farm-Level Data and Traceability: Expand surveillance to include direct farm-level data collection. This would involve assessing antibiotic usage protocols, verifying withdrawal period compliance, and establishing stronger farm-to-market traceability systems to enhance accountability and consumer confidence.

3. Risk Assessment: Further investigate the potential residue adverse effects on human and animal health, and the associated risks of antimicrobial resistance (AMR), even at levels below the current detection thresholds.

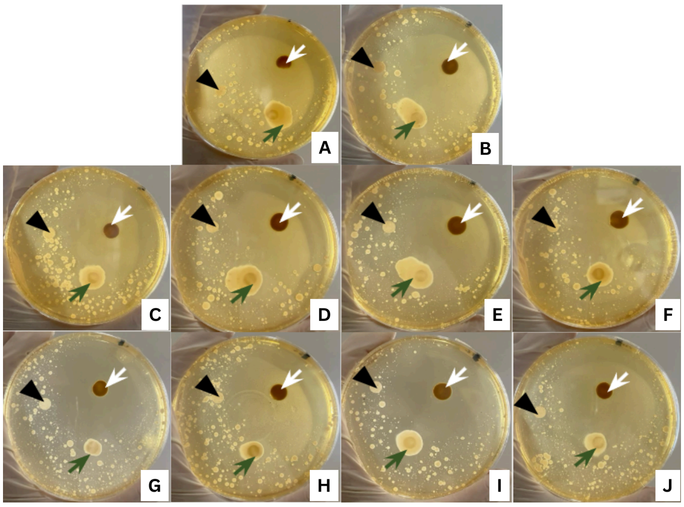

Figure 2. Antibiotic Sensitivity Testing results of market stall 1-10 (A-J, respectively) where the white arrow represents positive control; the black arrow, which corresponds to negative control; and the green arrow, which corresponds to disc infused with egg sample.

Conclusion

This study definitively concludes that no detectable oxytetracycline residues were present in table eggs sold in the selected market stalls of San Jose City, Nueva Ecija. The consistent absence of inhibition zones in all egg samples, coupled with the clear responsiveness of the assay to positive controls and the lack of false inhibition zones, validates the suitability of table eggs as a matrix for microbial inhibition-based screening. These results are highly encouraging, suggesting a likely observance of proper antibiotic withdrawal periods by local poultry farms, which is a positive indicator for food safety. While the disc diffusion test proved to be a practical and efficient initial screening tool for residue detection, its qualitative nature and limitations in detecting trace residues or a wide spectrum of antibiotic classes necessitate further action. Therefore, to provide a more comprehensive and robust assessment of antimicrobial residues and uphold public health standards, future efforts should prioritize:

1. Confirmation and Broader Screening: Implementing more advanced analytical methods such as the four-plate bioassay for detecting a wider variety of antibiotics, or chromatographic techniques (e.g., liquid chromatography-mass spectrometry) for higher sensitivity and quantification of residues. This also includes incorporating multiple indicator organisms to screen for diverse antibiotic classes beyond tetracyclines.

2. Enhanced Farm-to-Market Traceability: Strengthening mechanisms to obtain direct farm-level data on antibiotic usage and withdrawal period compliance, and improving transparency in the supply chain to ensure accountability and build greater consumer trust.

3. Comprehensive Risk Assessment: Further investigations into potential residue adverse effects on human and animal health, as well as the associated risks of antimicrobial resistance (AMR), even at levels below current detection thresholds.

Ethical Statement

The authors used a questionnaire survey which clearly contains the participants' informed consent before and during the conduct of this study, confidentiality of the information about the market stalls, and minimizing harm among others.

Conflict of Interest Statement

The authors declare no conflict of interest related to the conduct and publication of this research. All procedures followed were in accordance with institutional and ethical standards, and there were no financial or personal relationships that could have influenced the outcomes of this study.

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Declaration of Generative AI and AI-Assisted Technologies

During the preparation of this work, the authors utilized ChatGPT for grammar checking and revisions of ideas to make a clear statement from the main ideas of the author. Following the use of this tool/service, the authors conducted a review and made necessary modifications, assuming full responsibility for the content of the publication.

Data Availability

All data supporting the findings of this study are available within the paper.

Author Contributions

JASSP: Investigation, Resources, Writing (Original Draft and Editing), Visualization, and Project administration; **AGA:** Conceptualization, Methodology, and Supervision

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References

Abuel-Ang, P. (2005). *Philippines: Poultry and products. Annual 2005 (GAIN Report No. RP5033). United States Department of Agriculture, Foreign Agricultural Service.* http://fas.usda.gov/gainfiles/200509/146130789

Agmas, B., & Adugna, M. (2018). Antimicrobial residue occurrence and its public health risk of beef meat in Debre Tabor and Bahir Dar, Northwest Ethiopia. *Veterinary World*, 11(7), 902–908. https://doi.org/10.14202/vetworld.2018.902-908

Almashhadany, D. (2020). Screening of Antimicrobial Residued among Table Eggs Using Disc Diffusion Assay at Erbil Governorate, Kurdistan Region, Iraq. *Bulletin UASVM Animal Science and Biotechnologies*, 77(2), 64. https://pdfs.semanticscholar.org/48b2/09f37b27a15bcdc0300d165c7650ee47a198.pdf

Bureau of Animal Industry. (2021). *[Unpublished data on chemical residue detections in poultry meat in Central Luzon]*. Republic of the Philippines.

Chang, H. (2007). Analysis of the Philippine chicken industry: Commercial versus backyard sectors. *Asian Journal of Agriculture and Development*, 4(1), 41–56. https://doi.org/10.22004/.ag.econ.165854

Clinical and Laboratory Standards Institute. (2020). *Performance standards for antimicrobial susceptibility testing* (30th ed., CLSI Supplement M100). Clinical and Laboratory Standards Institute.

Ezenduka, E. V., Oboegbulem, S. I., Nwanta, J. A., & Onunkwo, J. (2011). Prevalence of antimicrobial residues in raw table eggs from farms and retail outlets in Enugu State, Nigeria. *Tropical Animal Health and Production*, 43, 557–559. https://doi.org/10.1007/s11250-010-9730-z

European Commission. (2010). Commission Regulation (EU) No. 27/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official Journal of the European Union*, L 15, 1–72. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:015:0001:0072:en:PDF

Garcia, G. (2023). *Preparation of Mueller-Hinton agar plates together with E. Coli inoculum: Antimicrobial susceptibility testing protocol* [Unpublished protocol]. College of Veterinary Science and Medicine, Central Luzon State University

Hendrix Genetics. (2022). *E. Coli in laying hens*. https://layinghens.hendrix-genetics.com/en/articles/e-coli-in-laying-hens/

Hudzicki, J. (2009). *Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology.* https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf

Inter-Agency Committee on Antimicrobial Resistance (IACAR). (2019). *The Philippine Action Plan to Combat Antimicrobial Resistance 2019–2023: One Health approach*. https://cdn.who.int/media/docs/default-source/antimicrobial-resistance/amr-spc-npm/nap-library/philippine-national-action-plan-on-amr-2019-2023-final.pdf

Kibret, M., & Abera, B. (2011). Antimicrobial susceptibility patterns of *E. Coli* from clinical sources in northeast Ethiopia. *African Health Sciences*, 11(Suppl 1), S40–S45. https://doi.org/10.4314/ahs.v11i3.70069

Muñoz, R., Cornejo, J., Maddaleno, A., Araya-Jordan, C., Iraguen, D., Pizarro, N., & Martin, B. (2014). Withdrawal times of oxytetracycline and tylosin in eggs of laying hens after oral administration. *Journal of Food Protection*, 77(6), 1017–1021. https://doi.org/10.4315/0362-028X.JFP-13-440

Owusu-Doubreh, B., Appaw, W., & Abe-Inge, V. (2022). Antibiotic residues in poultry eggs and its implications on public health: A review. *Scientific African*, 19, e01456. https://doi.org/10.1016/j.sciaf.2022.e01456

Widiasih, D., Drastini, Y., Yudhabuntara, D., Daru Maya, F., Sivalingham, P., Susetya, H., Nugroho, W., Putri, M. Th. K., Primatika, R., & Sumiarto, B. (2019). Detection of antibiotic residues in chicken meat and eggs from traditional markets at Yogyakarta City using bioassay method. *Acta Veterinaria Indonesiana*, Special Issue, 1–6. https://doi.org/10.29244/avi.0.0.1-6