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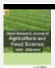

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Assessment of Antibiotic Residues in Broiler and Native Chickens in Delta State, Nigeria

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ABSTRACT

This study was conducted to examine the levels of antibiotic residues in broiler and native chickens reared and sold in Ethiope East, Local Government Area of Delta State, Nigeria. A total 15 mature chickens were purposively selected from 3 locations: Abraka main market 5 Broiler chickens, Poultry farm within Abraka 5 Broiler chicken, and Ovie-orie community 5 native chickens. The birds were slaughtered in a humane manner, eviscerated and cut into parts to obtain samples such as: breast cut, wings, gizzard and liver, for determination of antibiotic residues; Tylosin, Enrofloxacin, Gentamicin, Oxytetracycline following a standard laboratory procedure, and Data were Analyzed using descriptive statistics. Result revealed that samples of broiler and native chickens tested contained various degrees of antibiotic residues. Gentamicin ranged from 18.2 (wings) to 52.8 (liver), Enrofloxacin ranged from 2.0 (liver) to 16.0 (Breast), Tyloxin 0.0 (gizzard) to 40.13 (breast), Oxytetracycline 13.0 (liver) to 52.80 (Breast), and Sulphamethazine 0.4 (wings) to 14.0 (liver) respectively for Broiler chickens sold in Abraka main market. In the native chicken obtained from Ovie-orie, only Oxytetracycline was detected and it ranged from 0.3 (wing) to 38.0 (liver). While, for broiler chicken reared in poultry farms in Abraka, Enrofloxacin varied from 8.6 (wing) to 35 (liver), Tyloxin concentrations ranged from 20.9 (wing) to 50.0 (liver), Oxytetracycline 0.8 (gizzard) to 49.4 (breast), and Sulphamethazine 0.8 (wing) to 13.8 (liver), respectively. The existence of antibiotics in chickens sold and reared in Delta State has raised public health concern, making it imperative to enlighten chicken farmers and regulate antibiotics administration in poultry to ensure consumers safety of poultry products.

Keywords: *Antibiotics residues, Broiler chickens, concentration, Native chickens, Poultry products.*

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INTRODUCTION

The individual needs on the average 65grams of protein daily from animal sources (FAO, 2013). As a result, there is a significant growing demand and supply gap for animal protein. Presently, Nigeria is unable to meet this requirement; as a result, the animal protein consumption in Nigeria is less than 8gm per person per day, which is still very far from the minimum recommendation by FAO (Niang and Jubin, 2017). Poultry has the potential for bridging the protein nutritional gap, due to the availability of high-yielding poultry birds (Niang and Jubin, 2017). Broiler chickens are reared specifically for meat production, and most commercial broilers reach slaughter weight at 6 to 8 weeks of age, however, rearing of broiler is laced with several challenges (Ojo, 2003). Health is of extreme importance in broiler production. Poor chick health hurts all aspects of production and flock management, including growth rate, feed conversion efficiency, livability and processing traits. The use of various veterinary drugs, including antibiotics such as Tylosin, Nitromidazole, Nitrofuran, Enrofloxacin, Amoxicillin, Sulphamethazine, Quinolones, chlortetracycline, gentamicin, furazolidone, tetracycline, etc., growth promoters and feed additives during chicken management, has become crucial to prevent diseases and to enhance growth (Ezenduka *et al.,* 2014). However, this has raised concerns about the potential presence of drug residues in broiler chickens (Muhammad *et al.,* 2017). The most prevalent diseases in poultry influencing growth and production and also contributing considerably to the economic losses due to high mortality and slow growth rate are: typhoid, *mycotoxicosis*, *E. coli* infections, *coccidiosis*, *Salmonellosis*, *enteritis, ascites*, Newcastle disease, Marek's disease, and *Gumboro* disease (Chapman and Jeffers, 2014). Since traditional backyard and intensive poultry farming are commonly practice in various developing nations, poultry farmers have unlimited access to veterinary antibiotics. The indiscriminate and inappropriate use of drugs is common, which eventually leads to drug accumulation resulting in harmful residues in edible tissues and products of treated birds, as these residues eventually become part of the food value chain, which poses a significant threat to public health (Mubito *et al.,* 2014). The public health challenges arising from drug residues include; antibiotic resistance, alteration of micro-flora with possible development of resistant strains, production and proliferation of drug-resistant bacteria in human, which could lead to therapeutic failures among such infected individuals (Alaboudi *et al.,* 2013), risk of allergic reactions particularly those with sensitivities or allergies to certain drugs, gastrointestinal disorders, poor development of fetuses, pro-inflammatory, cytotoxic, and immuno-pathological effects (Palmieri *et al.,* 2014). Also, the effect of antibiotics on White Blood Cell (WBC), Red Blood Cell (RBC), and biochemical parameters such as total protein, albumen, cholesterol, and triglyceride concentrations have been reported (Eslayed *et al.,* 2014). Also, the frequent occurrence of residues of antibiotis such as sulphamethazine, oxytetracycline, and furazolidone in the human body is known to pose immunopathological impacts causing carcinogenicity, autoimmunity, while that of gentamicin and chloramphenicol could be mutagenic, nephropathic, and hepatotoxic, which may lead to reproductive abnormalities or bone marrow toxicity (Nisha, 2008) or adverse effects on consumers over time due to low and continuous exposure to antibiotic residues resulting from long consumption of poultry products produced from chickens subjected to uncontrolled use of antibiotics (Alaboudi *et al.,* 2013, and Mubito *et al.,* 2014). Moreover, prolonged exposure to low levels of drug residues contributes to the accumulation of toxins in the body, potentially leading to long-term health issues (Barbosa *et al.,* 2012). Additionally, the lack of comprehensive studies on drug residues in broiler chickens in Nigeria undermines food safety and creates a gap in understanding the extent of this issue and its potential consequences on animals and human health. There is a need for this study to be conducted on the assessment of antibiotic residues in broiler chickens sold and reared in Delta State.

METHODOLOGY

Location of study and Sample Collection

This study was conducted in Ethiope East, Local Government Area of Delta State. A total of fifteen (15) mature chickens were used for this study. 5 mature broiler chickens were purposively collected from different locations of Abraka main market, 5 local chickens from Ovie-orie community market, and 5 chickens from the poultry farm, around Abraka, Delta State. All birds were slaughtered humanely, following ethical practices, and cut parts and organs, such as breast cut, wings, gizzard, and liver, were harvested and stored in a freezer at -18^{o}C, for the determination of antibiotic residues (NOUWS *et al*., 1985).

Laboratory Procedures and Chemicals Analysis

This study was undertaken at the Poultry unit, Teaching and Research farm, and the Animal Science Laboratory, Faculty of Agriculture, Delta State University, Abraka Delta State, Nigeria. The antibiotics analyzed for in the selected chickens' samples and organs were: Gentamicin, Tylosin, Enrofloxacin, Oxytetracycline, and sulphamethazine. The working standard solutions were of high purity grades (>99%) and contained all analytes except the lactams group with variable concentrations, according to their MRLs, and was prepared as dilution of the stock solution in water /acetonitrile ratio of 70:30 (*v/v*), and the working standard solutions were kept at −20°C in glasss. HPLC-grade water, HPLC-grade acetonitrile, trifluoroacetic acid (TFA; 0.2%), disodium ethylenediaminetetracetate (Na_2EDTA), and magnesium sulfate ($MgSO_4$) were also used. Individual stock solutions were prepared at 1 g/L in acetonitrile and stored at −18 °C.

Preparation of Sample

Chicken samples: breast cut, wings, gizzard, and liver, of about 20 g, were homogenized using a blender to increase the surface area for the detection of antibiotic residues. Afterwards, 2 g of chicken mixture was weighed into a 20 mL glass centrifuge tube, 4mL of distilled water was added, and was agitated and swirled to mix thoroughly for a few seconds and allowed to stay in the dark for a few minutes. 1 mL of EDTA was added as a binding agent to compete with drugs such as oxytetracycline. This compound improved, as well as the performance of these drugs, and to avoid their losses (Freitas *et al*., 2014). 10mL of acetic acid (1%) in acetonitrile was added to the prepared mixture, and then shaken vigorously for 1 min. After which, magnesium sulfate ($MgSO_4$; 4 g) and sodium chloride (NaCl: 1 g) were added to the solution. The tubes were subjected to centrifugation at 5000 rpm at 4°C for 5 min and allowed to rest for 30 minutes (Freitas *et al*., 2014). 4mL of the supernatant was mixed with 50 mg of primary-secondary amine (PSA), 150 mg of C18, and 900 mg of $MgSO_4$. A second centrifugation was conducted at 5000 rpm at 4°C for 5 minutes. Four milliliters of the supernatant obtained were transferred to a new tube and evaporated under a gentle stream of nitrogen at 50°C. The residue was redissolved in 1 mL solution with a mobile-phase water/acetonitrile ratio (70:30, *v/v*), and then subjected to filtration through a 0.45 µm polyvinyliodene fluoride (PVDF) filter for further LC–MS/MS analysis under Multiple Reaction Monitoring (MRM)-optimized conditions for each compound, which is a quick, easy, cheap, effective, rugged, and safe methodology (Anastassiades *et al*., 2003).

Antibiotics Samples Extraction

A multi-class method for identification and quantification of several drugs from five different chemical classes (sulfonamides, tetracyclines, quinolones, and beta-lactams) has been developed by using liquid chromatography–mass spectrometry. The method was optimized for the detection of antibiotics in chicken meat. The optimized method was validated according to the European Commission Directive 2002/657/EC.

Determination of Drug Residues

Quantification of the five drugs in chicken samples was performed by measuring peak areas in the MRM chromatogram and comparing them with the relevant matrix-matched calibration curves. A calibration curve ranging between 5 and 200 µg/L was used to verify the linearity. The separation of sulfamides, tetracyclines, and quinolones was accomplished at 40 °C. The flow rate and injection volume were 0.3 mL/min and 10 µL. The mobile phases used were (A) TFA (0.1%) in water and (B) acetonitrile. The gradient elution program was as follows: A (90%) (3 min), A (25%) (5 min), and A (90%) (1min); the final run time of the method was 15 min.

Data Analysis

Data were subjected to Descriptive Statistical Analysis using SPSS version 23, 2024.

RESULTS

Antibiotic residue (%) in broiler chicken sold in Abraka Market

Table 1 revealed the result of antibiotic residues (%) in broiler chicken sold in Abraka Market. Results revealed that cut parts and organs of broiler chickens contained Gentamicine 32.4% in breast cut, 18.2% in wings, 40.13% in gizzard, and 52.84% in liver. Enrofloxacin: 16.0% in breast cut, 6.0% in wings, 4.0% in gizzard, and 2.0% liver. Tyloxin: 40.13% Breast cut, 38.0% in wings, 2.0% gizzard, 0% liver. Oxytetracycline: 52.84% in breast cut, 48.0% in wings, 29.1% gizzard, and 13.0% in liver. Sulphamenthazine was 0.0% in breast cut, 0.4% in wings, 8.0% gizzard, and 14.0% in liver, respectively.

Antibiotic Residues (%) in Native Chickens reared in Ovie-orie community

Table 2 presents the antibiotic residues (%) in native chicken reared in Ovie-orie rural community. The result showed that there were no traces of Gentamicin, Enrofloxacin, Tyloxin, and Sulphamentazine residues in the breast cut, wing part, and organs; gizzard and liver of native chicken. However, tested samples revealed that there was oxytetracycline, 25.0% residue in the breast cut, 0.3% in the wing, 0.5% in the gizzard, and 38.0% in the liver of native chicken reared in the study area.

Antibiotic residue (%) in broiler chicken reared in poultry farms in Abraka

Table 3 presents the results of antibiotics residues (%) in broiler chicken reared in poultry farms within Abraka. The result showed that Gentamicin residue was not detected in the breast, wing, gizzard, and liver of broiler chicken. However, Emofloxacin was found at 28.0% in breast muscle, 8.6% in wings, 26.0% in gizzard, and 35% in the liver. Tyloxin concentrations were 47.5% in the breast muscle, 20.9% in wings, 30.0% in the gizzard, and 50.0% in the liver of broiler chickens reared in Abraka. The breast muscle, wing, gizzard, and liver of broiler chickens contained 49.4%, 13.0%, 0.8% and 19.6% oxytetracycline. While Sulphamentazine residues detected in the breast muscle were 11.5%, others were 0.8% in wings, 8.06 in % gizzard, and 13.8% in the liver of broiler chicken reared in poultry farms within Abraka.

DISCUSSION

Results of broiler chicken samples revealed corresponding high residual levels of different antibiotics across different parts and organs of chickens. The result from this study also indicated that the breast, wing, gizzard, and liver of broiler chickens sold in Abraka, contained 32.4%, 18.2%, 40.13% and 52.84% Gentamicin, 16.0%, 6.0%, 4.0%, and 2.0% Emofloxacin, respectively. Tyloxin and Oxytetracycline have corresponding values of 40.13%, 38.0%, 2.0%, 10.0%, and 52.84%, 48.0%, 29.1% and 13.0%, respectively. Sulphamenthazine was found to have 0.0%, 0.4%, 8.0% and 14.0%. This result is an indication of the significant use and routine administration of these antibiotics during poultry rearing in the area, and this may be due to their efficacy in treating infections or preventing diseases in poultry. This realization aligns with the work of Sattar *et al.* (2014), who reported accumulation of enrofloxacin (22%), and tetracycline (20%) residues in broiler meat, ciprofloxacin (30%), tetracycline (24%), and enrofloxacin (18%) residues in breast muscles of laying hens.

In contrast, the breast muscle, wings, gizzard and liver of native chickens showed a drastically lower concentration of Oxytetracycline, with only 25%, 0.3%, 0.5% and 38.0% residues, highlighting a disparity in residue levels among different parts and organs of the chicken, which may be attributed to differences in tissue absorption and metabolism (Salehzadeh *et al.,* 2006 and Shahid *et al.,* 2007). Notably, no detectable residue of Gentamicine, Enrofloxacin, Tyloxin, or Sulphamenthazine

Table 1: Antibiotic residues (%) in broiler chicken sold in Abraka Market.

Parameter	Breast Cut	Wing	Gizzard	Liver
Gentamicin	32.4	18.2	40.13	52.84
Enrofloxacin	16.0	6.0	4.0	2.0
Tyloxin	40.13	38.0	2.0	0
Oxytetracyclin	52.84	48.0	29.1	13.0
Sulphamethazine	0.0	0.4	8.0	14.0

Table 2: Antibiotic residues (%) in Native chickens reared in Ovie-orie.

Parameter	Breast Cut	Wing	Gizzard	Liver
Gentamicin	0.0	0.0	0.0	0.0
Enrofloxacin	0.0	0.0	0.0	0.0
Tyloxin	0.0	0.0	0.0	0.0
Oxytetracyclin	25.0	0.3	0.5	38.0
Sulphamethazine	0.0	0.0	0.0	0.0

Table 3: Antibiotic residues (%) in broiler chickens reared in poultry farms in Abraka

Parameter	Breast Cut	Wing	Gizzard	Liver
Gentamicin	0.0	0.0	0.0	0.0
Enrofloxacin	28	8.6	26	35
Tyloxin	47.5	20.9	30	50
Oxytetracyclin	49.4	13.0	0.8	19.6
Sulphamethazine	11.5	0.8	8.06	13.8

was found in the cut parts and organs of native chickens, suggesting either no use or an effective withdrawal time for these antibiotics. The absence of these residues in the chicken could reflect more traditional rearing methods employed by local farmers, emphasizing the reduced use of antibiotics in native chickens compared to their commercial counterparts. This finding underscores the importance of monitoring antibiotic usage and implementing strict regulations to ensure food safety and maintain the health benefits of consuming native chicken meat over conventionally raised broiler chickens (Salehzadeh *et al.,* 2006).

Similarly, the results of broiler chicken samples from Abraka Main Market revealed that breast muscle, wings, gizzard, and liver contained antibiotic residues at various levels of 28%, 8.6%, 26%, and 33.0% for Enrofloxacin. 47.5%, 20.9%, 30% and 50% for Tyloxin. These results, which showed substantial residual levels of antibiotics, supported their prevalent use in the management of broiler chickens in the study area. Also, Oxytetracyclin residues at 49.5% (Breast muscle), 13.0% (Wings), 0.8% (gizzard), 19.0% (liver) in broiler chickens, in line with the report of Salehzadeh *et al.* (2006) and Shahid *et al.* (2007), which indicated non-compliance to established Maximum Recommended Levels (MRLs) for antibiotics. The results from this study aligned with those of Mehtabuddin *et al.* (2012), who found corresponding high concentrations of tetracycline (48%), ciprofloxacin (44%), amoxicillin (42%), and enrofloxacin (40%) residues in livers of boilers and laying hens. In the same vein, the occurrence of high levels of nicarbazin residues (dinitrocarbanilide) has been reported in broiler chicken livers (Sattar *et al.,* 2014). Similarly, Morshdy *et al.* (2015) reported the incidence of liver and gizzard samples contaminated with tilmicosin in chickens obtained from the market and poultry farms. Sulphamenthazine was detected at 11.5% (breast muscle), 0.8% (wings), 8.06% (gizzard), and 13.8% (liver) in broiler chickens. The results of Sulphamenthazine in broiler chicken appeared relatively low when compared with those of other antibiotics present in the chicken. This occurrence could be due to its reduced application or faster metabolization of this antibiotic. This result is in accordance with Karmi (2014) who also confirmed the presence of tetracycline, quinolone, and sulfonamide residues in fresh, local frozen, and imported frozen samples from thighs and breast cuts of chickens. Similarly, widespread misuse of antimicrobial agents due to non-compliance to withdrawal periods has also revealed oxytetracycline residues in chicken fillets (Hussein and Khalil, 2013).

Conclusion

Broiler and native chicken samples revealed antibiotic residues in the liver and breast muscles with high concentrations, particularly for Gentamicin, Enrofloxacin, and Tyloxin, suggesting extensive antibiotic usage in commercial poultry farming. The presence of antibiotic residues in the chicken samples suggested a reliance on antibiotics to maintain poultry health and enhanced productivity, which has raised concerns about consumer safety and antibiotic resistance. In contrast, native chickens showed minimal antibiotic residue, except for Oxytetracycline in the liver, which was 38.0%. This difference indicated that native chickens were reared with minimal use of antibiotics, and are a safer alternative for consumers who are concerned about antibiotic exposure. The presence of these antibiotics in the chicken samples examined highlights the need for careful administration and strict adherence to antibiotic use in poultry, to prevent health risks associated with high residual levels and ensure the consumption of safe chicken products. The withdrawal period of different drugs should be strictly adhered to, during which chicken products should not be consumed. Antibiotic utilization as growth enhancers and therapeutic purposes must follow the recommended administration and proper doses at the proper time. The high residue levels in broilers, notably in the liver, raised public health concerns and issues about food safety, including potential antibiotic resistance.

Therefore, it is imperative to regulate antibiotic usage in poultry farms during management and enforce stringent monitoring to ensure consumer safety. Promoting native chicken rearing practices might provide a healthier meat source while reducing antibiotic dependency in the poultry industry. To prevent future occurrences and ensure a safer way of producing chickens, stricter regulations and enforcement regarding the use of drugs in poultry farming are essential. Additionally, educating poultry farmers about the potential risks associated with drug residues and providing support for the adoption of alternative practices can facilitate the transition to safer and more sustainable poultry production methods.

REFERENCES

Alaboudi, A.; Basha, E.A.; Musallam, I (2013). Chlortetracycline and Sulfanilamide Residues in Table Eggs: Prevalence, Distribution between Yolk and White, and Effect of Refrigeration and Heat Treatment. Food Contaminants, 33, 281–286.

Anastassiades M., Lehotay S.J., Stajbaher D., Schenck F.J. (2003). Fast and Easy Multiresidue Method Employing Acetonitrile. *J. AOAC Int.*; 86:412–431.

Barbosa, J.; Freitas, A.; Mourão, J.L.; da Silveira, M.I.N.; Ramos, F (2012). Determination of Furaltadone and Nifursol Residues in Poultry Eggs by Liquid Chromatography−Electrospray Ionization Tandem Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, 60, 4227–4234

Chapman, H.D.; Jeffers, T.K. (2014). Vaccination of Chickens Against Coccidiosis Ameliorates Drug Resistance in Commercial Poultry Production. *International Journal of Parasitology: Drugs and Drug Resistance*, 4, 214–217.

Eslayed, M.; Elkomy, A.; Aboubakr, M.; Morad, M (2014). Tissue Residues, Hematological and Biochemical Effects of Tilmicosin in Broiler Chicken. *Veterinary Medicine International,* 1–6. DOI:http://dx.doi.org/ 10.1155/2014/502872

Ezenduka, E.V.; Ike, O.S.; Anaelom, N.J (2014). Rapid Detection of Antimicrobial Residues in Poultry: A Consequence of Non-Prudent Use of Antimicrobials. *Health*, 6(2), 149–152.

Food and Agriculture Organizations (2013). "Food Security Concepts and Measurements". In Trade Reforms and Food Security: Conceptualizing the Linkages. *Experts Consultative Forum held in Rome*, Italy, 11 – 12

Hussein, M.A. and Khalil, S (2013). Screening of Some Antibiotics and Anabolic Steroids Residues in Broiler Fillet Marketed in El-Sharkia Governorate. *Life Science Journal*, 10(1), 2111–2118.

Karmi, M (2014). Detection and Presumptive Identification of Antibiotic Residues in Poultry Meat by Using FPT. *Global Journal of Pharmacology*, 8(2), 160–165

Morshdy, A.E.; El-Atabany, A.; Hussein, M.A. (2015). Abdelaziz, S.; Ahmed, A.M. Antibiotic Residues in Poultry Edible Offal. 2nd Conference of Food Safety, Suez Canal University, Faculty of Veterinary Medicine, 1, 188–195

Mubito, E.P.; Shahada, F.; Kimanya, M.E.; Buza, J.J (2014). Antimicrobial Use in the Poultry Industry in Dar-Es-Salaam, Tanzania, and Public Health Implications. *American Journal of Research Communication*, 2(4), 51–63.

Muhammad D. M., Umair H. K., Uruj T., Bahar-EMustafa, A. F. (2017). Antimicrobial drug residues in poultry products and implications on public health: *A review, International Journal of Food Properties*, 20:7, 1433-1446,

Niang, T. and Jubrin, S. (2017). Quarterly Newsletter of the Nigeria Agriculture Question and Answer Service, vol. 1, No. 3

Nisha, A.R. (2008). Antibiotic Residues—A Global Health Hazard. Veterinary World, 1(12), 375–377.

Palmieri, M.D.; Cerbo, A.D.; Laurino, C (2014). Antibiotic Treatments in Zootechnology and Effects Induced on the Food Chain of Domestic Species and Comparatively, the Human Species. *Nutricion Hospitalaria*, 29, 1427–1433

Salehzadeh, F.; Madani, R.; Salehzadeh, A.; Rokni, N.; Golchinefar, F (2006). Oxytetracycline Residue in Chicken Tissues from Tehran Slaughterhouses in Iran. *Pakistan Journal of Nutrition*, 5(4), 377–381.

Sattar, S.; Hassan, M.M.; Islam, S.K.M.A.; Alam, M.; Faruk, M.S.A.; Chowdhury, S.; Saifuddin, A.K.M (2014). Antibiotic Residues in Broiler and Layer Meat in Chittagong District of Bangladesh. Veterinary World, 7(9), 738–743. DOI:10.14202/vetworld 2014.738-743

Shahid, M.A.; Siddique, M.; Abubakar, M.; Arshed, M.J.; Asif, M.; Ahmad, A. (2007). Status of Oxytetracycline Residues in Chicken Meat in Rawalpindi/Islamabad Area of Pakistan. *Asian Journal of Poultry Sciences*, 1(1), 8–15.