Antibiotics use and gentamicin residues in commercial poultry and chicken eggs from Oyo and Lagos States, Nigeria

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Keywords

Poultry, layer chickens, eggs, antibiotic residues, food safety, Nigeria

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Summary

Nigeria poultry industry is fast growing to meet the demand of the increasing population with overdependence on antibiotics for production leading to consumer safety and public health concerns. The antibiotic use in poultry farms and presence of gentamicin residues in chicken eggs from city markets of Southwest Nigeria were investigated. A semi-structured questionnaire was administered to 45 poultry farmers to determine the patterns of antibiotic use, knowledge of withdrawal periods and food safety implications. Furthermore, 270 egg samples from six retail markets in Oyo and Lagos states were analyzed with ELISA for gentamicin residues. Residue concentrations were compared with Student's t test and ANOVA ($p < 0.05$). About 90% of the respondents reported they frequently administered antibiotics, 85% engaged in non-prescribed medication, and about 80% did not observe a withdrawal period before selling the eggs from treated chickens. In addition, 60% and 80% of pooled eggs from Oyo and Lagos states, respectively, contained gentamicin residues with means of 1461 ± 74 and 1350 ± 92 µg/kg, respectively. The mean residues obtained from the two states were higher than the maximum recommended residue limits. High levels of gentamicin residues, from unbridled use of antibiotics in poultry production, detected in retail eggs from markets rendered the eggs unsafe for human consumption. Therefore, regulatory control and veterinary supervision of antibiotic use are advocated to ensure Nigerian consumer protection.

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■ INTRODUCTION

The Nigerian poultry industry constitutes an important agricultural enterprise, contributing significantly to the nation's gross domestic product (GDP) and about 9–10% of the agricultural GDP (Heise et al., 2015). Poultry and poultry products are an important and cheap source of animal protein in Nigeria. Broiler and cockerel chickens are commonly reared for meat and as layers for egg production under intensive and free-range management systems. The Nigerian poultry industry provides employment for a fair proportion of the population. Poultry production is a fast means to bridge the protein deficiency gap common in many developing countries (Heise et al., 2015). It is a good means to supply the fast growing human population with high quality protein (Gueye, 2009).

In developing countries, frequent use of antibiotics, such as tetracycline, gentamicin, neomycin, fluoroquinolones and/or quinolone compounds, tylosine, erythromycin, virginiamycin, ceftiofur, and bacitracin, is a common practice in food poultry. Several authors report that these antibiotics are most commonly used as preventive and chemotherapeutic agents against poultry diseases such as respiratory diseases, necrotic enteritis, fowl typhoid, coryza, pullorum disease, and coccidiosis (Alhendi et al., 2000; Zakeri and Wright, 2008; Soni, 2012; Filazi et al., 2005). Indiscriminate use of antibiotics in livestock production is a common practice in Nigeria that is characterized by their frequently non-prescribed administration by farmers (Adesokan, et al., 2015). They are also used in low and therapeutic levels as part of the contents of commercial poultry and livestock feeds, and extra-label use is very common since the drugs are easily purchased without prescriptions (Lawal et al., 2015). Tetracycline, chloramphenicol, penicillin and gentamicin are the most commonly used antibiotics in poultry production in Nigeria (Adebowale et al., 2016). As a result of their administration, trace quantities of parent drugs and their metabolites may be present as residues in eggs. Moreno-Bondi et al., 2009 wrote: "The residues of these substances, their metabolites in eggs and other foods of animal origin may cause adverse effects on consumers' health."

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Gentamicin is a broad-spectrum aminoglycoside antibiotic commonly used in veterinary medicine against a wide range of bacterial infections (Goetting et al., 2011). It is usually administered by intramuscular injection with poor oral absorption. Its administration usually results in persistent tissue residues thereby requiring prolonged withdrawal times. (Filazi et al., 2005). When gentamicin is administered to laying hens via the intramuscular or subcutaneous route, it is deposited in egg yolk and albumen with the residues persisting longer in the yolk (Filazi et al., 2005). Residues of gentamicin can cause mutagenic, nephropathic, and hepatotoxic effects, as well as they may lead to antibiotic resistance, reproductive abnormalities or bone marrow toxicity in humans (Nisha, 2008). There is no Codex Alimentarius Commission on maximum residue limits (MRL) for gentamicin residues in poultry and poultry products, but the use of gentamicin in laying birds is prohibited by the European Union (Goetting et al., 2011). Consequently, the presence of any gentamicin residues in eggs is not permitted and such eggs are unsafe for human consumption.

In Nigeria because of little regulatory control and lack of routine monitoring of veterinary drug use and drug residues in foods of animal origin (Olatoye et al., 2012), several cocktails of antibiotics including gentamicin are being administered to poultry and other livestock indiscriminately. Several studies have reported residues of different antibiotics in these animal products (Ezenduka et al., 2011; Olatoye et al., 2012; Olatoye and Kayode, 2012), but none has reported the presence of gentamicin in commercial chicken eggs. This study therefore assessed the use of antibiotics in poultry and analyzed the levels of gentamicin residues in retail chicken eggs from commercial poultry from city markets of Oyo and Lagos states, Nigeria.

■ MATERIALS AND METHODS

Materials

Study location and sample collection

The study was carried out in selected poultry farms across Oyo and Lagos states. These states are in Southwestern Nigeria at 8° N 4° E. Oyo State is the hub of poultry production in Nigeria; Lagos State is also a major poultry producing state receiving supplies of chickens and eggs from other states of Nigeria to meet the increasing daily demand.

Questionnaire on antibiotic use

A cross-sectional survey was carried out using a pretested semi-structured questionnaire administered to commercial chicken egg producers (poultry farmers) on antibiotic use patterns.

Egg sample collection

A total of 270 chicken egg samples supplied to retail markets from commercial layer farms in Oyo and Lagos states were randomly collected three times at two weeks' intervals. Egg samples were obtained from three major markets in Oyo State (Ibadan, Oyo, and Ogbomosho cities) and in Lagos State (Ikorodu, Agege, Lagos Island). From each market, 45 eggs from 15 retailers were obtained and transported to the Food and Meat Hygiene Laboratory in the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Oyo State.

Equipment and ELISA kit

The equipment used included ELISA plate reader 450/630 nm, a centrifuge, weighing balance, graduated pipette, water bath, homogenizer, vortex mixer, extraction cartridges, micropipettes and glassware. The reagents included deionized water, distilled water, monobasic and dibasic sodium phosphate (NaHPO4.2H2O, NaH2PO4.2H2O).

The gentamicin ELISA test kit comprised microwell strips, six different concentrations of gentamicin standard solutions (1 ml each of 0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb or 4.05 ppb), an enzyme conjugate, an antibody working solution, a substrate A and B solution, a stop solution, a concentrated washing buffer, a concentrated redissolving solution, and Green Spring ELISA software (Green Spring, Shenzhen Lvshiyuan Biotechnology, China).

Methods

Questionnaire administration

The pretested questionnaire was administered to poultry farmers randomly selected from the register of the Poultry Association of Nigeria. Data on reasons for antibiotic administration to poultry and on types of antibiotic used were recorded. In addition, the farmers' knowledge of antibiotic residues and practice of withdrawal periods were assessed.

Sample pretreatment

Sample preparation was carried out according to ELISA kit instructions. Each sample comprised three eggs pooled and homogenized, followed by the addition of 8 ml of 0.2 M phosphate buffer to two grams of each pooled-egg sample. The mixture was thoroughly shaken for 5 min and incubated in a water bath at 56°C for 30 min. The solution was centrifuged at 4000 r/min at room temperature (20–25ºC) for 10 min, then 450 µl of the diluted redissolving solution was added to 50 µl of the supernatant (upper layer) and thoroughly mixed by vortex, after which 50 µl of the solution was used for ELISA test.

ELISA procedure

This test kit is based on the competitive enzyme immunoassay for the detection of gentamicin in the sample. Briefly, according to the manufacturer, washing buffer, samples and standard solutions were prepared, whereas all the microtiter wells were numbered according to samples and standard solutions. Fifty microliters each of sample and standard solutions was dispensed in duplicate into the wells of a microtiter plate with each position recorded. This was followed by dispensing 50 µl of enzyme conjugate into each of standard and sample, and thereafter 50 µl of antibody solution to each well. The plate was gently shaken manually, sealed with a membrane cover and incubated at 25°C for 60 min. Then, the liquid inside the wells was poured out of the wells and 250 µl of washing solution was added to wash each well for 30 s with five repetitions. The plate was flapped and dried with absorbent paper and bubbles were cut with clean tips. Thereafter, 50 µl of substrate A and substrate B solutions were successively added into each well, mixed gently, and incubated for 15 min in the dark. Then, 50 µl of stop solution was added to each well and mixed gently. The plates were measured for the optical density (OD) using ELISA plate reader at 450 nm wavelength. Green Spring ELISA software was used for analysis of gentamicin concentrations in the standard and sample solutions. The test has been validated with ISO Certificate: ISO9001&ISO14001&OHSMS18001; detection limits = 2.5 - 3 ppb; sensitivity = 0.05 ppb; and recovery rate 80 ± 20% - 90 ± 20%.

Calibration curve

The mean OD of the duplicate gentamicin standard solution (standard 1–6) was plotted against the logarithms of the mean gentamicin standard concentrations to obtain the calibration curve (Figure 1). The software was integrated with a formula for interpretation of gentamicin concentrations in the samples.

Gentamicin concentrations

OD data of duplicate gentamicin concentrations obtained from ELISA readings were recorded in the software. The OD value of the sample had a negative correlation with the gentamicin in it. Mean sample OD were converted to obtain the logarithms of mean gentamicin

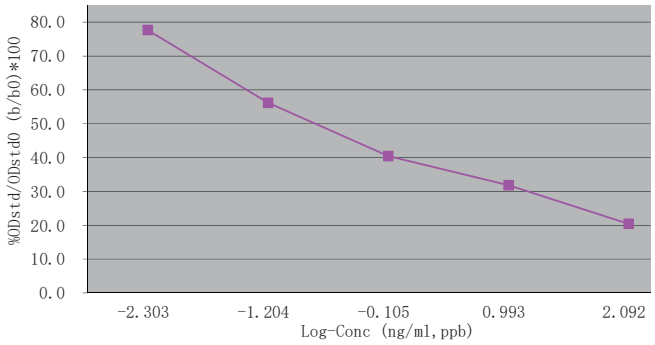

Figure 1: *Standard curve of gentamicin constructed from c-ELISA. b is the optical density (OD) value obtained in the presence of gentamicin standard concentrations, and b0 is the OD value obtained in the absence of gentamicin concentration.*

concentrations through the standard curve in the software. Residue concentrations in the samples were obtained and interpreted by the kit software. The values were compared to the standard curve and gentamicin concentrations were subsequently obtained. According to the software program, residue concentrations above the MRL in eggs (i.e. greater than or equal to 100 ppb) were judged positive for gentamicin residues (Figure 1).

Data analysis

Data collation and management were performed with Microsoft Excel. Responses to the questionnaire were presented in simple frequency tables. The Statistical Package for Social Sciences (SPSS) version 10.0 was used to analyze the mean residue concentrations from different locations. ANOVA Duncan's multiple-range test and the paired-t test were performed to determine the significant differences of gentamicin residues in the chicken eggs from the two states.

■ RESULTS

Antibiotic use pattern in poultry farms in Oyo and Lagos states

Table I shows the antibiotics commonly administered by the interviewed farmers. The most frequently used were tetracycline (98% of the farmers), enrofloxacin (92%) and gentamicin (88%). The farmers routinely used drugs for prevention and treatment of poultry diseases, whereas 86% routinely administered antibiotics as feed additives. Only 15% of the farmers reported treating their poultry on veterinary prescription following specified doses, whereas 85% engaged in non-prescribed medication based on experience and according to the drug instructions on the label. In addition, 20% of respondents claimed to give the antibiotic treatment according to specified periods and had knowledge of withdrawal periods, but they did not discard the eggs because they could not afford to lose the eggs produced during treatment and withdrawal periods. The majority (80%) of the poultry farmers claimed they did not practice a withdrawal period and were not aware of negative health consequences of antibiotic residues.

Gentamicin residues in eggs

Gentamicin residues were detected in 70.0% of all the pooled eggs sampled in this study (Table II). The overall mean gentamicin residue concentrations of 1350 ± 9.2 and 1460 ± 7.4 µg/kg were obtained in egg samples from Lagos and Oyo states, respectively (Table III). There was no significant difference ($p < 0.05$) in the mean gentamicin residue concentrations in eggs from the different market locations and across the two states.

Table I

Antibiotic use pattern in poultry farms (n = 45) in Oyo and Lagos states, Nigeria

Item	Response	Num. of farms	%
Antibiotic use	Yes	40	90.0
	No*	5	10.0
Antibiotics frequently used	Tetracycline	44	98.0
	Gentamicin	40	88.0
	Enrofloxacin	41	92.0
Prescription source	Veterinarian	7	15.0
	Farmer	38	85.0
Reason for administration	Feed additives	39	86.0
	Treatment	6	14.0
Withdrawal period practice	No	36	80.0
	Yes	9	20.0

* Farmers did not use antibiotics directly in drinking water or by injection to birds, but they used antibiotics in feeds.

Table II

Presence of gentamicin residues in chicken eggs from Oyo and Lagos states, Nigeria

Location	Total num. of samples	Num. of positive samples
Oyo State	45	27 (60%)
Lagos State	45	36 (80%)
Total	90	63 (70%)

Table III

Gentamicin concentrations in chicken eggs from markets in Oyo and Lagos states, Nigeria

Location	Total pooled eggs	Total positive samples	Mean gentamicin concentration ± SD (µg/kg)
Oyo State			
Ibadan	15	9	964 ± 43.0
Oyo	15	10	1807 ± 69.0
Ogbomosho	15	8	1587 ± 78.0
Total	45	27	1460 ± 74.0
Lagos State			
Lagos Island	15	12	1147 ± 9.2
Agege	15	12	1148 ± 6.4
Ikorodu	15	12	1783 ± 10.1
Total	45	36	1350 ± 9.2

SD: standard deviation

■ DISCUSSION

The global challenge of efficacy of antibiotics is a major threat to public health, and their use in livestock has been under critical scrutiny for the control of antimicrobial resistance. "Drug residues in chicken eggs are of concern because relatively few drugs are labeled for laying hens, although several medications are approved for other production classes of poultry" (Hofacre, 2006; Castanon, 2007). The dependence

on antibiotics for poultry production without stringent regulations on use and residue monitoring portend food safety risks. In the present study, residues of such antibiotic use may present a risk as none of the farmers discarded the eggs from treated chickens during treatment but rather offered them for sale and human consumption due to economic consideration. This supports the report of Donoghue (2003) that antimicrobials are used by poultry farms to enhance growth, feed efficiency, and to reduce bacterial diseases.

The majority of the egg sampled in this study had detectable gentamicin residues. The mean residues detected in eggs from within each state and across the two states were not significantly different ($p < 0.05$) but were above the permissible level. The detection of gentamicin in these samples correlated with the results of antibiotic use by the poultry farmers surveyed who mostly engaged in misuse and failed to observe withdrawal periods without consideration for consumers' protection. These results agree with the observation that non-adherence to antibiotic withdrawal periods is the major cause of antimicrobial residues in food animals (Donoghue, 2003; Young et al., 2010). The detection of gentamicin in eggs is of further concern as it persists in both the yoke and albumen. However, residues are rapidly accumulated in the yolk and persist longer, i.e. 10 or more days following treatment (Etches, 1996). Most of the laying chickens could have been treated parenterally with injectable gentamicin against common bacterial diseases of poultry such as salmonellosis, pasteurellosis and colibacillosis that are enzootic in the study area. Although Brown and Riviere (1991) and Bennett et al. (2001) reported that orally administered gentamicin in birds were excreted in the feces and thus that it was rare to find aminoglycoside residues in eggs following oral administration, Filazi et al. (2005) observed persistent residues of gentamicin in eggs from laying hens following administration of gentamicin for chemotherapy. Gentamicin accumulates for a long period at a high concentration in egg yolk as a result of accumulation of the yolk material during the intense laying period of hens with a direct correlation between dosage increase and drug residue concentration in eggs (Filazi et al., 2005).

Thus, the indiscriminate use of gentamicin by egg producers without consideration for consumer safety and adherence to a withdrawal period causes the residues to enter the human food chain with public health consequences. The majority of the eggs sampled in this study contained concentrations of gentamicin residues higher than those recommended by European Union MRL, i.e. levels unsafe for human consumption.

■ CONCLUSION

These findings established an unregulated antibiotic use, and the detection of gentamicin in eggs with a high proportion containing residue levels in breach with international food standards. The majority of poultry farmers in the study areas and by implication across Nigeria are not adhering to the prudent use of antibiotics and are not applying withdrawal periods thereby producing and selling eggs with antibiotic residues. The laws regulating the sale and administration of antibiotics should be strengthened and enforced to prevent indiscriminate administration of antibiotics to livestock. Veterinary supervision of drug administration to poultry and routine residue testing are also critical to ensure food safety and consumer protection in Nigeria.

Author contributions statement

OIO and YJA engaged in the conception and design of the study; YJA collected samples; OIO and TOO participated in sample preparation; OIO and YJA performed the laboratory analysis and drafted the manuscript first version; OIO performed data analysis and interpretation; OIO critically reviewed the manuscript.

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Résumé

Olatoye O.I., Ojomo T.O., Adeseko Y.J. Utilisation d'antibiotiques et résidus de gentamicine dans la volaille commercialisée et les œufs de poule des états d'Oyo et de Lagos, Nigeria

L'industrie avicole nigériane se développe rapidement pour répondre à la demande d'une population croissante et dépendante des antibiotiques pour la production, ce qui entraîne des problèmes de sécurité des consommateurs et de santé publique. L'utilisation d'antibiotiques dans les élevages avicoles et la présence de résidus de gentamicine dans les œufs de poulets de marchés urbains du sud-ouest du Nigeria ont été étudiées. Un questionnaire semi-structuré a été proposé à 45 aviculteurs afin de déterminer leurs habitudes relatives à l'utilisation d'antibiotiques, leur connaissance des délais de retrait de la vente, et les conséquences pour la sécurité sanitaire des aliments. En outre, 270 échantillons d'œufs provenant de six marchés de détail des états d'Oyo et de Lagos ont été analysés avec Elisa pour détecter la présence de résidus de gentamicine. Les concentrations de résidus ont été comparées avec le test t de Student et Anova ($p < 0,05$). Environ 90 % des répondants ont déclaré administrer fréquemment des antibiotiques, 85 % le faisaient sans prescription et environ 80 % n'observaient pas de période de retrait des œufs des poulets traités. En outre, respectivement 60 % et 80 % des œufs mis en commun dans les états d'Oyo et de Lagos contenaient des résidus de gentamicine avec des moyennes de 1461 ± 74 et 1350 ± 92 µg/kg. Les résidus moyens obtenus dans les deux états étaient supérieurs aux limites maximales de résidus recommandées. Les niveaux élevés de résidus de gentamicine, liés à l'utilisation incontrôlée d'antibiotiques dans la production avicole et détectés dans les œufs vendus au détail sur les marchés, rendaient les œufs impropres à la consommation humaine. Par conséquent, le contrôle réglementaire et la supervision vétérinaire dans l'utilisation d'antibiotiques sont indispensables pour assurer la protection des consommateurs nigérians.

Mots-clés : volaille, poule pondeuse, œuf, résidu d'antibiotique, sécurité sanitaire des aliments, Nigeria

Resumen

Olatoye O.I., Ojomo T.O., Adeseko Y.J. Uso de antibióticos y residuos de gentamicina en aves comerciales y huevos de gallina en los estados de Oyo y Lagos, Nigeria

La industria avícola de Nigeria esta creciendo rápidamente, para satisfacer la demanda de una población creciente, con una dependencia excesiva en los antibióticos para la producción, generando problemas de seguridad y salud pública para el consumidor. Se investigó el uso de antibióticos en granjas avícolas y la presencia de residuos de gentamicina en huevos de gallina en los mercados citadinos del suroeste de Nigeria. Se administró un cuestionario semi estructurado a 45 avicultores, con el fin de determinar patrones de uso de antibióticos, conocimiento sobre periodos de abstinencia e implicaciones para la seguridad alimenticia. Se analizaron además mediante ELISA 270 muestras de huevos provenientes de seis mercados públicos en los estados de Oyo y Lagos, con el fin de detectar residuos de gentamicina. Las concentraciones de residuos se compararon mediante la prueba t de Student y ANOVA ($p < 0,05$). Alrededor de 90% de los encuestados informaron que frecuentemente administraban antibióticos, con 85% utilizando antibióticos sin prescripción medica y 80% sin respetar períodos de abstinencia antes de vender los huevos de las aves medicadas. Además, 60% y 80% de los huevos examinados en Oyo y Lagos, respectivamente, contenían residuos de gentamicina, con medias de 1461 ± 74 y 1350 ± 92 µg/kg, respectivamente. Los residuos medios obtenidos en ambos estados fueron superiores a los límites máximos de residuos recomendados. Los altos niveles de residuos de gentamicina, provenientes de un uso excesivo de antibióticos en la producción avícola, detectados en los huevos vendidos en mercados, los hicieron inseguros para el consumo humano. Por lo tanto, se recomienda reglamentación de control y supervisión veterinaria del uso de antibióticos para garantizar la protección del consumidor nigeriano.

Palabras clave: aves de corral, gallina ponedora, huevos, residuos de antibióticos, inocuidad alimentaria, Nigeria