

An evaluation of the health risks, antibiotic residue levels and potentially toxic ingredients in Nigerian poultry products

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ABSTRACT

Meat and poultry are rich sources of protein, which is crucial for growth and development of other nutrients the body needs. Poultry meat production at mass level has already been achieved and now the emphasis is on increasing meat quality. The current study aimed to assess the Arsenic (As), Lead (Pb), Cadmium (Cd), and antibiotic residues (chloramphenicol, chlortetracycline, streptomycin, and neomycin) in broiler's albumen, yolk, muscle, and gizzard. A total of 36 samples (12 of each parts of the broiler from each farm) were digested and Pb and Cd concentrations were ascertained with AAS, while concentration was determined by a UV-VIS. Liquid-liquid extraction was used to extract the antibiotics and the concentrations were determined with HPLC. Arsenic (0.0403-0.5970 mg/kg) and Pb (0.2500-0.6670mg/kg) concentrations were above the safe limit in all the samples, while Cd levels (0.0017-0.0672 mg/kg) were within the safe limit for both meat and egg samples. The health risk evaluation of the contaminants revealed that the hazard quotient of Arsenic was above one for both adults and children, indicating that arsenic poses a potential health risk. Except chloramphenicol, all investigated antibiotics were within the residual limit by European Union. The antibiotic distribution between the yolk and albumen showed that chloramphenicol, chlortetracycline, and neomycin were preferentially deposited more in the yolk than albumen. This study therefore indicated that the poultry products (egg and meat) in the studied region are possible means of human exposure to As, Cd, Pb, and antibiotics (chloramphenicol, chlortetracycline, streptomycin, and neomycin). Their usage might be consequential to health hazards, especially, chloramphenicol.

KEYWORDS

Antibiotics, Chloramphenicol, Chlortetracycline, Neomycin, Potential Toxic Elements

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Contamination of the environment and foodstuff by potentially toxic elements (PTEs) is one of the major problems of modern society (Ihedioha *et al.*, 2021, Sana *et al.*, 2016). Unregulated pollution levels most especially in third-world countries have attracted more attention

to the PTEs problem. PTEs of major interest as listed by the U. S. Environmental Protection Agency, in bioavailability studies are Al, Hg, Cd, Cu, Ni, Cr, Sb, As, Se and Pb (Ehiemere *et al.*, 2022, Sveta *et al.*, 2016).

Lead, cadmium and arsenic are included in the major toxic and abundant PTEs which build up in the food chain and are easily absorbed in the digestive tract and from the air (Ehiemere *et al.*, 2022, Sveta *et al.*, 2016). Lead exposure affects the kidney and nervous system (Egwuonwu *et al.*, 2021; Sana *et al.*, 2016). On the other hand, Cd is recognized to be an endocrine disruptor and may cause bone, renal, prostate, and breast cancer in people (Odum *et al.*, 2021, Tajo *et al.*, 2015). PTEs from anthropogenic sources are repeatedly unleashed into water bodies and therefore, the concern about the consequence on the ecosystems is on the increase. PTEs contamination is a grave hazard because of their deleterious, bio magnifications and bioaccumulation in the food chain (Kamal *et al.*, 2013). Because these contaminants are sometimes permanently absorbed into tissues, they frequently have direct physiological harmful consequences (Ihedioha *et al.*, 2021; Maretova *et al.*, 2015). Recently, much attention has been given to the concentration of PTEs in fish and other seafood with little attention to poultry products.

One of the most bizarre medical breakthroughs occurred when Alexander Fleming discovered antibiotics in the early 1920s, and it is acknowledged that these agents have spared humanity from the deadly and undesired powers of pathogenic germs (Eddy *et al.*, 2024; Rosa and Carlos, 2013). The word "antibiotics" describes a broad class of compounds with antibacterial action that are produced naturally, semi-synthetically, or synthetically. These compounds work by either eliminating or preventing the growth of bacterial infections (Macarov *et al.*, 2012). Antibiotics are now frequently administered for the treatment and prevention of microbial illnesses as well as to promote growth in livestock and poultry farms. Foods generated from animals may contain residues from the improper use of these medications in veterinary care (Pratiwi *et al.*, 2023; Makarov *et al.*, 2012). There is strong scientific evidence that the use of antibiotics in animals raised for food contributes to the problems with antibiotic resistance that humans face (Pratiwi *et al.*, 2023; EFSA, 2020; Rosa and Carlos, 2013). Antibiotic growth promoters at sub-therapeutic levels have been added to chicken feed since the 1950s in an effort to increase weight gain and feed conversion.

A 2.5-pound chicken with a 4.7 feed conversion ratio (weight/feed intake) would have required 112 days of growing in 1965; however, a 6-pound chicken now requires only 42 days and a 1.8 feed conversion ratio (NCC, 2015). The application of antibiotic growth promoters is partially to blame for the increase in production rate and efficiency. More frequently used antibiotics in this region are neomycin, streptomycin, chlortetracycline and chloramphenicol. However, most of antibiotics used in Nigerian poultry industries are labeled in different trade names, some of which are the combinations of two or more active pharmaceutical ingredients (APIs) to make them extra potent, while some are incorporated with several multivitamins. Because they provide needed trace elements, poultry meat or products are important for human diet (Ahmad *et al.*, 2018). With an estimated 600 million chickens, Nigeria's poultry sector is worth 600 million dollars (SAHEL, 2015). Nigeria presently produces more than 290,000 metric ton of poultry meat per year and 650,000 metric ton of egg. The poultry industry in Nigeria is highly fragmented, with the majority of chicken raised in "backyards" or on farms with less than 1,000 birds (SAHEL, 2015). Consequentially, this study was designed to determine the levels of selected antibiotic residues and PTEs in the broiler's internal organs (gizzard and muscle) and eggs of chicken from South-Eastern Nigeria and possible health risk from their consumption.

Materials and Method

Ethical Approval

All procedures involving animals complied with the specifications and enabling legal provisions captured in the Constitution of the Federal Republic of Nigeria, Criminal Code Act. Cap C38 LFN 2004, Animal Diseases (Control) Act. Cap A17 LFN, 2004 and the Veterinary Surgeon Act Cap V3 LFN 2004. The required ethical approvals were obtained before the research commenced from the Animal Use and Care Committee (AUCC) of University of Nigeria, Nsukka.

Sampling

A total of 16 samples (4 from each farm) of 9 weeks old broiler chicken (the average period from when the broiler consumption starts) and eggs from egg producing chicken layers breed were purchased from four poultry farms located in different areas in Enugu State, Nigeria.

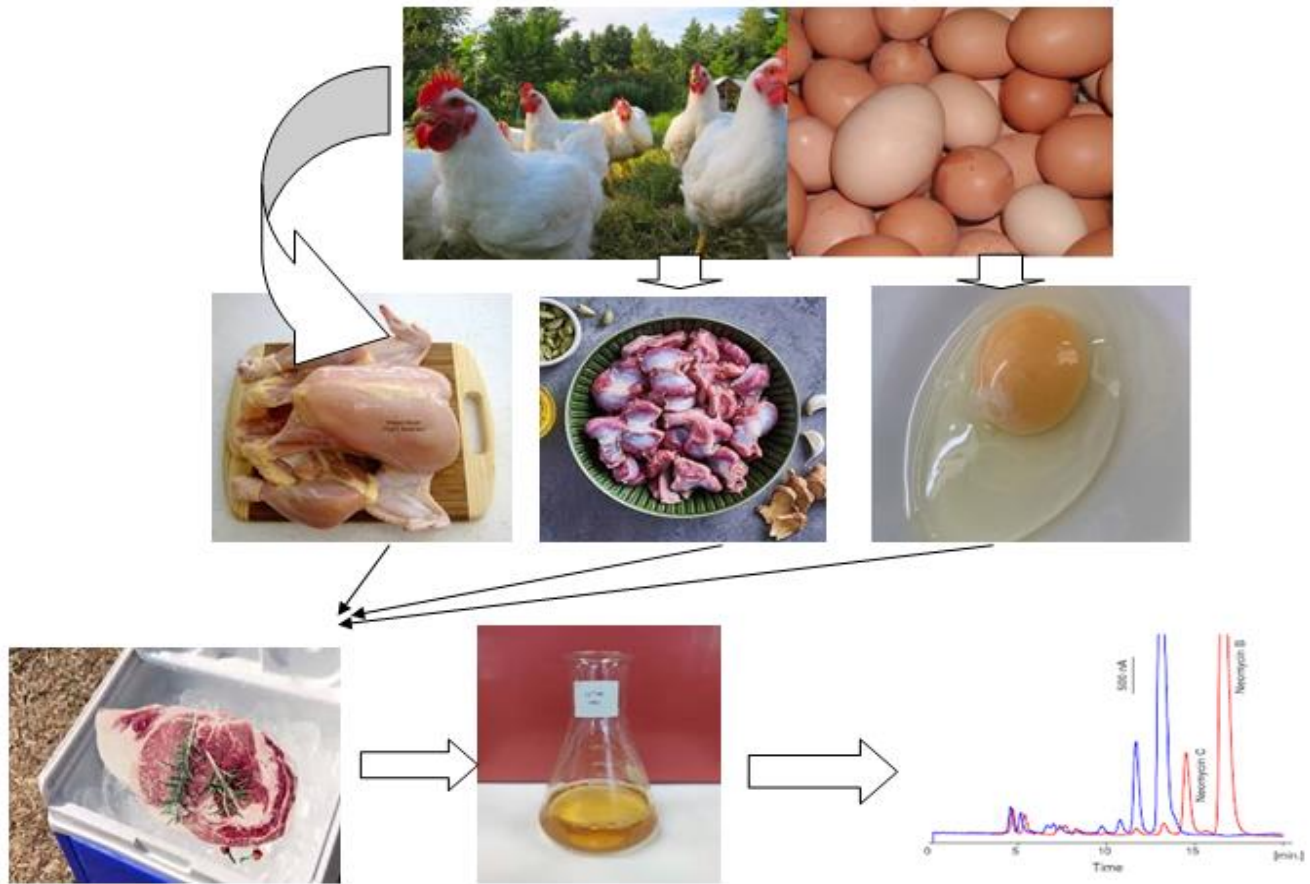


Fig 1. Poultry Process

Four samples of broiler chickens were purchased from each of the four poultry farms and on each of the farms, a composite (to get a true representative of the entire chicken population) sample of muscles and gizzard were collected by carefully dissecting the broiler samples into different parts. This was achieved by carefully collecting a portion of each of the four samples (in each case) and homogenizing them to form a fully represented single sample. Four egg samples of layers were also purchased from each of the poultry farms. The yolks were carefully separated from the albumen using an egg separator (Tupperware, USA) and a composite of the yolks and albumen was formed from each of the farms. A total of 4 composite samples of muscles, gizzards, albumen, and yolks making it a total of 16 composite samples, were carefully prepared for the determination of the PTEs (As, Pb and Cd) and antibiotics residues (chloramphenicol, chlortetracycline, neomycin, and streptomycin). The samples were collected, taken to the laboratory in the ice box within 2 hours, and analyzed within 4 days while refrigerated at 4°C.

Sample Preparation and Digestion

The meat samples were washed and dried to constant weight at 105°C. The dried samples were pulverized into a powder with a porcelain mortar and pestle. About 2 g of the samples were placed in three round bottom flasks and 20 mL of HNO₃ and HClO₄ mixture (4:1v/v) was added to the sample. This was refluxed at 120-180°C for 20 hours until a clear solution appeared. The digest was filtered into 100 mL volumetric flasks at room temperature and made up to mark with deionized water (Ihedioha *et al.*, 2014). Following that, the sample solutions were examined using a flame-atomization absorption spectrometer to detect lead and cadmium (model AA-7000, Shimadzu, Japan).

Arsenic Evaluation Using Spectrophotometric Method

Arsenic levels in the samples were analyzed using the spectrophotometric method. The technique relied on the release of iodine through the interaction of potassium iodate with arsenic (III) in an acidic media.

In the presence of sodium acetate, the iodine formed a violet-colored species with maximal absorbance at 556 nm by preferentially oxidizing variamine blue (Kamlesh *et al.*, 2015), using a spectrophotometer (Shimadzu Japan, model 1800 double beam).

Extraction of Antibiotics and Analysis

The extraction of antibiotics from all the samples was performed following the modified method as described by (Rosa and Carlos, 2013). Using a pestle and mortar, 4 g of each sample were pulverized. Ten milliliters of phosphate buffer saline (pH 6.5) was then added, and everything was well mixed by overtaxing. It was centrifuged at 6000 rpm for 20 minutes after being mixed with 2 mL of 30% trichloroacetic acid. We gathered and filtered the supernatant. The filtrate was gathered into a falcon tube, to which 2 mL of 30% diethyl ether was added. The mixture was then allowed to stand at room temperature for 10 minutes. After gathering the bottom layer, diethyl ether was used twice more for the extraction process. The extract's final volume was meticulously combined into a screw-cap vial and stored for analysis using high-performance liquid chromatography (HPLC Agilent1200 series, USA). The HPLC was set at a wavelength of 254 nm, with a column (5C18-MS-II) which has a dimension of 5 micrometer, 4.6 x 150 mm, and a Hamilton microliter syringe. 5 µL was injected under a flow rate of 1.0 mL/min at 20 °C using a mobile phase (methanol 20 mmol/L phosphate buffers at pH 3, in the ratio 20:80).

Quality Control Procedure

Deionized water was used all through the analytical work. The Pyrex material was used for all glasses, and before being used, all plastic and glassware containers were cleaned by soaking them in diluted nitric acid for a whole night and then rinsing them multiple times with deionized water. The dilutions were performed using double-deionized water and 65% HNO₃. Recovery analysis was used to validate the correctness of the analytical processes and instrumental methods. Additionally, a known concentration of a separate, independent metal solution was examined on a regular basis. Recalibration for each metal was required if the computed value of the standard metal solution deviated more than 10% from its known concentration.

As a result, throughout the investigation, the analytical data's quality and accuracy were closely adhered to. Different standard solutions of the metal under investigation were added to the samples. The entire experiment involved analyzing the spiked and unspiked samples in triplicate. Formula 1 was used to compute the percentage recovery (Okoye *et al.*, 2011).

$$\text{Recovery (\%)} = \left(\frac{\text{Spiked Sample} - \text{Unspiked Sample}}{\text{Spiking Concentration}} \right) 100 \text{ (Formula 1)}$$

Limit of Detection and Quantification (LOD and LOQ)

The terms "limit of detection" (LOD) and "limit of quantification" (LOQ) refer to the lowest analyte concentration that can be accurately measured using Formulas 2 and 3.

$$\text{LOD} = X_b + 3SD_b \text{ (Formula 2)}$$

$$\text{LOQ} = X_b + 10SD_b \text{ (Formula 3)}$$

Where X_b and SD_b are the mean of blank and standard deviation, respectively (Shrivastava and Gupta, 2011).

Health Risk Analysis

Health risks to the population from poultry meat and egg intake concerning PTEs were determined by evaluation of the non-carcinogenic and carcinogenic effects of the PTEs (US EPA, 2014). The non-carcinogenic and carcinogenic health risks which could emanate from the consumption of poultry products were calculated.

Non-Carcinogenic Risks

The non-carcinogenic risk was evaluated using Formula 4:

$$\text{HR} = \left(\frac{\text{EDI}}{\text{RfD}} \right) \times 10^{-3} \text{ (Formula 4)}$$

Where, EDI is the estimated daily intake; HR is the hazard ratio, RFD is the oral reference dose (0.001, 0.004 and 0.0003 mg/kg/day for Cd, Pb and as, respectively (Oyeyiola *et al.*, 2017). Assuming a ratio of less than one, the samples will not provide any risk; however, if the ratio is one or more, there may be potential health problems for the community. Formula 5 was used to get the EDI of the PTEs in µg/kg/day (Oyeyiola *et al.*, 2017).

$$\text{EDI} = \frac{C \cdot \text{FIR}}{\text{BW}} \text{ (Formula 5)}$$

Where C is the PTEs concentration; FIR is the ingestion rate of food (0.1kg/day for chicken meat and 0.0372kg/day for egg (US EPA, 2014), BW is the body weight (60.7kg for adults, Oyeyiola *et al.*, 2017).

Hazard Index (HI)

HI was created to evaluate the possible danger to human health posed by many PTEs (Marian *et al.*, 2016). The hazard ratio is added up to determine the HI, which is computed using Formula 6:

$$HI = \Sigma HR = HR_{Pb} + HR_{Cd} + HR_{As} \quad (\text{Formula 6})$$

In this case, the hazard ratios for Pb, Cd, and As are represented by HR_{Pb} , HR_{Cd} , and HR_{As} , respectively, while the total is denoted by ΣHR . The size of the negative impact is predicted to be proportionate to the total of several metal exposures. If the HI is less than 1, there is no potential risk to the population, but when it is greater than 1, there is worry about probable health repercussions (Marian *et al.*, 2016).

Carcinogenic Risk Assessment

The carcinogenic risk levels were assessed by the detection of incremental lifetime cancer risk (ILCR) Formula 7.

$$ILCR = CSF \times CDI \quad (\text{Formula 7})$$

Where CSF = cancer slope factor (CSF values are 8.5×10^{-3} , 1.5×10^{-3} , and 6.3×10^{-2} mg/kg/day for Pb, As, and Cd, respectively) (US EPA, 2014). CDI = metals' chronic daily intake in mg/kg/day, Formula 8.

$$CDI = \frac{EDI * EFr * ED_{tot}}{AT} \quad (\text{Formula 8})$$

Where EF = exposure frequency (365 days/year), EDI = estimated daily intake, ED_{tot} is the exposure duration (70 years, Bortey-sam *et al.*, 2015), and AT is the exposure period of carcinogenic effect (70 years lifetime x 365 days for adults and 20.5 years, 365 days for children). The ILCR is acceptable when the value is in the range of 1×10^{-6} - 1×10^{-4} (Kong *et al.*, 2012).

Statistical Analysis

Descriptive statistics were used and the results were presented as mean \pm standard deviation. The data obtained were also subjected to correlation analysis at a 0.05% significance level. This was to determine any relationship between the PTEs within each farm sample and antibiotics. All statistics were performed with SPSS Version 23 for windows.

Results and Discussion

Table 1 presents the percentage recovery and LOD and LOQ for the PTEs and antibiotics. The percentage of recovery ranged from 87.50% for Cd to 99.85% for As. Table 2 showed the mean concentrations of all the studied parameters in albumen, yolk, muscles, and gizzard. The mean concentrations of arsenic were in the range of 0.0403-0.1213 mg/kg, the muscles had the least concentration (0.0403 ± 0.0038 mg/kg), while the yolk had the highest level of arsenic (0.1213 ± 0.0025 mg/kg). Lead concentration was not detected in the gizzard but had 0.333 ± 0.0144 mg/kg in the yolk, while albumen and muscles both had a concentration of 0.2500 ± 0.000 mg/kg. In the case of cadmium, the albumen had the highest concentration (0.0672 ± 0.014 mg/kg), while the lowest concentration was observed in the muscles with a mean value of 0.0086 mg/kg. The yolk and gizzard recorded Cd concentration of 0.0126 ± 0.0003 mg/kg and 0.0103 ± 0.0005 mg/kg, respectively.

Comparing the levels of arsenic detected in the analyzed samples from farm A with the safe limit of 0.01 mg/kg set for arsenic in poultry products, all the samples were found to be above the safe limit, while the values for cadmium in the analyzed samples were within the safe limits 0.5 mg/kg (FAO/WHO, 2000). This supports the proposition that arsenic accumulates more in the internal organs (Ratnaik, 2003). The results for arsenic obtained in this study were comparable to the levels reported in different poultry products by (Iwegbue *et al.*, 2008), in the southern part of Nigeria, however, it was far lower than that reported by (Mariam *et al.*, 2004) (40.80-52.44 mg/kg) for poultry products in Lahore market, Pakistan. The major source of As contamination in animals is the drinking water prepared from natural geological sources rather than from smelting, agricultural sources and mining (Ihedioha *et al.*, 2021; Matschullat, 2000).

Table 1. Percentage recovery of potential toxic elements in broiler chicken, limit of detection and limit of quantification

PTEs / Antibiotics	Spiked Sample (mg/kg)	Unspiked Sample (mg/kg)	Mean Recovery Value (mg/kg)	Recovery (%)	LOD (mg/kg)	LOQ (mg/kg)
As	2.081	0.084	1.997 ± 0.002	99.85	0.00016	0.00048
Pb	2.079	0.080	1.999 ± 0.001	99.95	0.00012	0.00036
Cd	1.750	0.000	1.750 ± 0.001	87.50	0.00010	0.00030
Chloramphenicol	59.410	57.600	1.810 ± 0.081	90.50	0.09000	0.27000
Chlortetracycline	22.150	20.300	1.850 ± 0.023	92.50	0.07200	0.21600
Neomycin	6.5000	4.6700	1.900 ± 0.020	95.00	0.01400	0.04200
Streptomycin	5.3100	3.4700	1.840 ± 0.100	92.00	0.01700	0.05100

Note: As: Arsenic, Pb: Lead, Cd: Cadmium, LOQ: Limit of quantification, LOD: Limit of detection, PTEs: potential toxic metals

Table 2. Mean concentration of the albumin, yolk, muscle, and gizzard in broiler chicken

Parameter	Albumin	Yolk	Muscle	Gizzard
Farm A				
Arsenic (mg/kg)	0.0880 ± 0.003	0.1213 ± 0.0025	0.0403 ± 0.0038	0.1017 ± 0.00513
Lead (mg/kg)	0.2500 ± 0.000	0.3333 ± 0.0144	0.2500 ± 0.0000	ND
Cadmium (mg/kg)	0.0672 ± 0.014	0.0126 ± 0.0003	0.0086 ± 0.0014	0.0103 ± 0.0005
Chloramphenicol (mg/g)	54.900 0± 0.004	71.3100 ± 0.0080	60.0000 ± 0.0020	76.4000 ± 0.0050
Chlortetracycline (mg/g)	23.000 0± 0.004	32.2700 ± 0.0027	21.6800 ± 0.0039	37.1500 ± 0.0018
Streptomycin (mg/g)	4.6900 ± 0.0000	4.6800 ± 0.0005	5.2200 ± 0.0005	5.4200 ± 0.0003
Neomycin (mg/g)	4.0400 ± 0.0008	7.2600 ± 0.0012	3.5500 ± 0.0006	7.3200 ± 0.0014
Farm B				
Arsenic (mg/kg)	0.0977 ± 0.0038	0.1453 ± 0.0040	0.5970 ± 0.0021	0.1163 ± 0.0025
Lead (mg/kg)	0.4167 ± 0.1443	ND	0.2500 ± 0.0000	ND
Cadmium (mg/kg)	0.0089 ± 0.0004	0.0105 ± 0.0011	0.0092 ± 0.0018	0.0119 ± 0.0001
Chloramphenicol (mg/g)	59.2000 ± 0.0064	77.5000 ± 0.0080	69.1700 ± 0.0020	80.0000 ± 0.0070
Chlortetracycline (mg/g)	25.7000 ± 0.0072	34.1000 ± 0.0030	24.4500 ± 0.0010	38.0800 ± 0.0029
Streptomycin (mg/g)	4.9100 ± 0.0003	5.0100 ± 0.0005	5.6000 ± 0.0005	5.6100 ± 0.0008
Neomycin (mg/g)	4.1000 ± 0.0001	8.4022 ± 0.0015	4.0 ± 0.0015	9.60 ± 0.0027
Farm C				
Arsenic (mg/kg)	0.0777 ± 0.0021	0.1187 ± 0.0021	0.0433 ± 0.0025	0.1043 ± 0.0021
Lead (mg/kg)	ND	0.2500 ± 0.0000	0.6670 ± 0.0144	0.5830 ± 0.0144
Cadmium (mg/kg)	0.0092 ± 0.0012	0.0114 ± 0.0017	0.0092 ± 0.0012	0.0105 ± 0.0002
Chloramphenicol (mg/g)	57.6000 ± 0.0032	65.7000 ± 0.0060	58.8600 ± 0.0030	72.4000 ± 0.0040
Chlortetracycline (mg/g)	20.3000 ± 0.0034	39.2000 ± 0.0033	29.6200 ± 0.0063	35.4200 ± 0.002
Streptomycin (mg/g)	4.6700± 0.00002	4.4000 ± 0.0002	4.9000 ± 0.0008	5.2200 ± 0.00027
Neomycin (mg/g)	3.4700± 0.00089	7.800 ± 0.0006	3.1000 ± 0.0006	6.3600 ± 0.0018
Farm D				
Arsenic (mg/kg)	0.1043 ± 0.0015	0.1173 ± 0.0031	0.0433 ± 0.0025	0.101 ± 0.002
Lead (mg/kg)	ND	0.2500 ± 0.0000	ND	ND
Cadmium (mg/kg)	0.0078 ± 0.0006	0.0112 ± 0.0023	0.0089 ± 0.0014	0.0113 ± 0.0002
Chloramphenicol (mg/g)	57.05 ± 0.0064	74.41 ± 0.0080	64.5 ± 0.0020	78.88 ± 0.0070
Chlortetracycline (mg/g)	24.35 ± 0.0037	36.11 ± 0.0030	23.05 ± 0.0039	39.50 ± 0.0029
Streptomycin (mg/g)	4.71 ± 0.0000	4.89 ± 0.0010	5.41 ± 0.0005	5.53 ± 0.0003
Neomycin (mg/g)	4.09 ± 0.0005	7.88 ± 0.0010	3.75 ± 0.0006	8.45 ± 0.0027

Note: ND: Not detected

Many industrialized and less industrialized countries have drinking water contaminated with As (Iheioha *et al.*, 2021; Gebel, 2000). The high level of as could be from its being an additive in animal feeds as observed by (Ratnaik, 2003). Absorption and accumulation of Cd in tissue seem to depend on several factors such as iron, nutritional and vitamin status, sex, and age. Comparing the concentration of Cd in our results with the reported metal level in poultry products agrees with (Mariam *et al.*, 2004) report. It can also be noticed that Cd level was higher than those reported by (Oforka *et al.*, 2012) for muscle and liver. With few exceptions like gizzards where the concentration was not detected, all other samples had Pb concentrations above the safe limit of 0.01 mg/kg. Overexposure to lead has been linked to decreased cognitive development and intellectual performance in children as well as elevated blood pressure and an increased risk of cardiovascular disease in adults (EC, 2001).

An excessive amount of Pb in chicken meat could not be attributed to industrialization alone. The primary sources of high PTE levels in poultry products are contaminated diets and water sources. Values above the permissible limit have been detected for lead in various brands of poultry feeds sold in Southeastern Nigeria (Okoye *et al.*, 2011). The values recorded for lead in the various poultry products in this study ranged 0.00-0.333 mg/kg; 0.00-0.4167 mg/kg; 0.00-0.667 mg/kg and 0.00-0.25 mg/kg for farm A, B, C and D, respectively. Pb concentration from these poultry products was lower than those reported by (Okoye *et al.*, 2011) but agrees with the report of (Mariam *et al.*, 2004). The mean concentration of chloramphenicol ranged from 76.40 mg/g in the gizzard to 54.9 mg/g in albumen, although yolk had concentration of 71.311 mg/g while muscle had 60 mg/g. The mean concentrations of chlortetracycline, streptomycin, and neomycin, were in the range of 37.15 mg/g for chlortetracycline in the gizzard, to 3.55 mg/g for neomycin in muscles. Compared with the minimum residual levels of 100, 200, and 100 mg/g set for chlortetracycline, streptomycin, and neomycin, respectively, they are all within the safe limits. There is a zero-safe limit for chloramphenicol in the poultry products under consideration. Animal products like milk, eggs, and meat contain antibiotic residues from the usage of these drugs on food-producing animals.

The existence of these residues may be the consequence of not adhering to the drug's withdrawal periods, treating animals' excrement getting into animal feed, giving animals doses that are higher than recommended on the label, or using antibiotics that are not authorized. The mean chloramphenicol concentrations in all the studied poultry products are in the range of 54.9-76.40, 59.2-80.00, 57.60-72.4, and 57.05-78.88 mg/g for farms A, B, C and D, respectively. The result of the chloramphenicol residue recorded and this study agreed with the report of (Adewuyi *et al.*, 2011). This is of great concern as there is no standard safe limit for chloramphenicol residues in food. Studies by (Omeiza *et al.*, 2012 and Nonga *et al.*, 2010) also reported detectable levels of Chloramphenicol residue in egg samples in Nigeria and Tanzania, respectively. The values of chlortetracycline obtained ranged between 21.68-37.15 mg/g, 24.45-38.08 mg/g, 20.3-39.2 mg/g, and 23.05-39.50 mg/g for farm A, B, C and D, respectively. These results concurred with the work of (Salama *et al.*, 2011), who assessed the residue of chlortetracycline in poultry meat and eggs in Egypt.

Olufemi and Agboola (2011) reported higher values of tetracycline residue in poultry meats and eggs consumed in Nigeria. Nevertheless, with the exception of chloramphenicol, all other studied antibiotics were within the safe limit proposed by European Union. The detectable levels of all the studied antibiotics in the poultry products suggested that the poultry farmers in this region use veterinary drugs which comprise of three or more antibiotics. The result of this study is a clear indication that chloramphenicol despite been banned in Nigeria, is still very much in use in this locality and this supports the claims of (Adewuyi *et al.*, 2011) who emphasized the predominant use of chloramphenicol in poultry farming in Nigeria. Similar studies have reported detectable levels of chlortetracycline, chloramphenicol, and Streptomycin in poultry products in Tanzania (Nonga *et al.*, 2010). The mean concentrations of arsenic in the entire poultry products of farm B were in the range of 0.5970 to 0.0970 mg/kg, farm C was in the range of 0.0433-0.1187 mg/kg, while farm D was in the range of 0.0113-0.0078 mg/kg (table 2). The muscle recorded the highest mean concentration of 0.5970 mg/kg and 0.043mg/kg in farms A and C which was higher than 0.1163 mg/kg and 0.1187 mg/kg recorded for yolk, respectively.

Table 3. Non-carcinogenic risk assessment of potential toxic elements in broiler chicken products from different farms (A-D)

Samples / Parameters	Albumen				Yolk				Muscle				Gizzard				RFD (mg/kg g/ day)	
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D		
EDIA (µg/kg /day)	As	0.054	0.060	0.050	0.060	0.074	0.090	0.073	0.070	0.066	0.980	0.071	0.080	0.168	0.200	0.172	0.170	0.0003
	Pb	0.15	0.260	0.000	0.000	0.20	0.000	0.150	0.150	0.41	0.410	1.090	0.000	0.000	0.000	0.960	0.000	0.004
	Cd	0.040	0.005	0.006	0.005	0.008	0.006	0.007	0.007	0.010	0.015	0.015	0.015	0.020	0.019	0.017	0.019	0.001
EDIC (µg/kg /day)	As	0.160	0.177	0.140	0.190	0.220	0.264	0.220	0.210	0.196	2.884	0.211	0.240	0.496	0.570	0.508	0.490	0.0003
	Pb	0.27	0.760	0.000	0.000	0.60	0.000	0.270	0.270	1.22	1.220	3.250	0.000	0.000	0.000	2.850	0.000	0.004
	Cd	0.120	0.020	0.017	0.014	0.020	0.020	0.200	0.020	0.040	0.045	0.045	0.043	0.050	0.060	0.050	0.055	0.001
HRA	As	0.18	0.200	0.160	0.200	0.25	0.300	0.240	0.240	0.220	3.270	0.240	0.270	0.560	0.640	0.570	0.550	0.0003
	Pb	0.04	0.060	0.006	0.000	0.05	0.000	0.04	0.040	0.100	0.100	0.270	0.000	0.000	0.000	0.240	0.000	0.004
	Cd	0.040	0.005	0.166	0.005	0.008	0.006	0.007	0.069	0.010	0.015	0.015	0.015	0.020	0.010	0.017	0.019	0.001
HRC	As	0.50	0.590	0.470	0.630	0.73	0.880	0.720	0.700	0.650	9.610	0.740	0.800	1.650	1.900	1.690	1.640	0.0003
	Pb	0.06	0.190	0.000	0.000	0.15	0.000	0.006	0.060	0.300	0.300	0.810	0.000	0.00	0.000	0.710	0.000	0.004
	Cd	0.120	0.020	0.017	0.014	0.020	0.020	0.020	0.020	0.040	0.045	0.045	0.043	0.050	0.060	0.050	0.055	0.001
HIA		0.60	0.265	0.166	0.2047	0.308	0.306	0.287	0.349	0.330	3.385	0.525	0.285	0.580	0.640	0.827	0.569	
HIC		0.710	0.800	0.487	0.6442	0.900	0.900	0.800	0.780	0.990	9.955	1.595	1.230	1.700	1.960	2.450	1.695	

Note: EDIA: Estimated daily intake for an adult, EDIC: Estimated daily intake for children in µg/kg/day, HRA: Hazard risk for an adult, HRC: Hazard risk for children, RFD: Oral reference dose, HIA: Hazard index for an adult, HIC: Hazard index for children, A-D: farms sampled were collected

Table 4. Cancer risk assessment of Arsenic, Lead and Cadmium in poultry products of the different farms (A-D)

Samples/ Parameters	Albumen				Yolk				Muscle				Gizzard				
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
ILCRA	As	8.5	9.0	7.5	9.0	1.1	1.4	1.1	1.1	9.9	1.5	1.1	1.2	2.5	3.0	2.6	2.6
		E-5	E-5	E-5	E-5	E-4	E-4	E-4	E-4	E-5	E-3	E-4	E-6	E-4	E-4	E-4	E-4
	Pb	1.3	2.2	0.00	0.00	1.7	0.00	1.3	1.3	3.5	8.5	9.3	0.00	0.00	0.00	8.2	0.00
		E-5	E-6			E-6		E-6	E-6	E-6	E-6	E-6				E-6	
ILCRC	As	2.4	2.7	2.1	2.9	3.3	4.0	3.3	3.2	2.94	4.3	3.2	3.6	7.8	8.3	7.6	7.4
		E-4	E-4	E-4	E-4	E-4	E-4	E-4	E-4	E-4	E-3	E-4	E-4	E-4	E-4	E-4	E-4
	Pb	2.3	6.5	0.00	0.00	5.1	0.00	2.3	2.3	1.1	1.0	2.8	0.00	0.00	0.00	2.4	0.00
		E-6	E-6			E-6		E-6	E-6	E-5	E-5	E-5				E-4	
Cd		7.6	1.3	1.1	9.0	1.3E	1.3	1.3	1.3	2.5	2.8	2.8	2.7	3.2	3.8	3.2	3.5
		E-4	E-3	E-3	E-5	-4	E-4	E-4	E-4	E-4	E-4	E-4	E-4	E-4	E-4	E-4	E-4

The mean concentration of arsenic residue was highest in the yolk with 0.117 mg/kg and lowest in muscles with the concentration of 0.0433 mg/kg, while the albumen and gizzard had concentrations of 0.1043 and 0.101 mg/kg, respectively in farm D. For Pb, 0.4160 mg/kg and 0.25 mg/kg were recorded for albumen and muscle, respectively, but it was undetected in yolk and gizzard for farm A. The highest concentration of Pb was observed in muscle with 0.6670 mg/kg in farm C, followed by gizzard and yolk with the mean concentrations of 0.5830 mg/kg and 0.2500 mg/kg, respectively for farm C. Lead in albumen was undetected in farm C. The levels of lead in albumen, muscles and gizzard in farm D were undetected, while the yolks had the mean concentration of 0.2500 mg/kg. With exception of albumen whose concentration was not detected, the entire samples were all above the safe limit of 0.01 mg/kg. The mean concentrations of Cd in all the poultry products from farm D were in the range of 0.0113-0.0078 mg/kg. The gizzards had the highest mean concentration of Cd-0.0113 mg/kg which was higher than 0.0112 mg/kg and 0.0089 mg/kg recorded for yolk and muscles, respectively, while albumen had the lowest concentration of 0.0078 mg/kg. Gizzard had more deposit of Cd with the concentration of 0.0119 mg/kg, while the lowest Cd concentration was found in albumen with 0.0089 mg/kg in farm B. In farm C, the yolk had the highest concentration of Cd-0.0114 mg/kg, while the lowest concentration was observed in the muscles with a mean concentration of 0.0092 mg/kg.

Albumen and gizzard had Cd concentrations of 0.0093 mg/kg and 0.0105 mg/kg, respectively. Comparing the levels of arsenic recorded in the analyzed samples from farm C with the safe limit of 0.01 mg/kg for arsenic in poultry products, all the samples were found to be above the safe limit, while the values for cadmium in the analyzed samples were within the safe limits 0.5 mg/kg (FAO/ WHO, 2000). Arsenic concentration in all the samples from farm B was above the safe limits, while for cadmium; all the samples were within the safe limit. The concentrations of Pb in albumen and muscle in farm B was above the safe limit. However, for yolk and gizzard the concentrations were within the safe limit. A detectable level of chloramphenicol was found in the entire sample in farm B within the range of 80 mg/g in gizzard to 59.2 mg/g in albumen.

The residual levels of chlortetracycline, streptomycin and neomycin were in the range of 38.08 - 4.00 mg/g. When compared with the safe limit of 100, 200 and 100 mg/g for chlortetracycline, streptomycin and neomycin, respectively. It was observed that all the samples from farm B were within the safe limit. The mean concentrations of chloramphenicol ranged from 72.40 mg/g in gizzard to 57.6 mg/g in albumin. The yolk had a concentration of 65.7 mg/g, while muscle had 58.86 mg/g. The mean concentrations of chlortetracycline, streptomycin, and neomycin, were in the range of 39.2 mg/g for chlortetracycline in yolk to 3.10 mg/g for neomycin in muscle. Comparing with the minimum residual levels of 100, 200 and 100 mg/g set for chlortetracycline, streptomycin and neomycin, respectively, the antibiotics were all within the safe limits.

Non-Carcinogenic Health Risk Assessment

Human exposure to PTEs is related to many health effects and toxicological implications which are more pronounced in children. One main function of food hygienists is to maintain safety standards of foods given to populace. Therefore, dietary exposure and risk evaluation resulting from the intake of PTEs polluted meat were assessed. The hazard ratio, estimated daily intakes and hazard index corresponding to health risk indices associated with arsenic, cadmium, and lead in the studied poultry products in both adults and children are presented in table 3. In farm A, the EDI values ($\mu\text{g}/\text{kg}/\text{day}$) were As (0.054-0.220), Pb not detectable (ND-1.22) and Cd (0.008-0.12) while in farm B were (0.060-2.884), Pb (0.00-1.22) and Cd (0.005-0.02).

The EDI values obtained were within the tolerance daily intake of 2.1, 3.57 and 1 for As, Pb and Cd, respectively as established by EFSA (2006), all the samples with the exception of arsenic in muscles (children) that had the EDI value of 2.884 $\mu\text{g}/\text{kg}/\text{day}$ in farm B. The HR values of Cd and Pb did not surpass unity for the samples of farm A in both children and adult. Nevertheless, children had HR value of 1.65 for arsenic in the case of gizzard meat; consequentially, children had the HI values of 1.7 in gizzard depicting a potential risk. These observations are in alignment with the report of (Akan *et al.*, 2010).

The HR values of Pb and Cd was less than one for the samples from farm B in both children and adult, while the HR values determined for children was high than one (9.61 and 1.90) for arsenic in the case of muscles and gizzard meat, respectively. Adults had HR value of 3.27 for arsenic in muscles and children had HI values of 1.96 and 9.955 for gizzard and muscles, respectively, while adult had HI value of 3.385 for muscles which suggests a potential health risk. The EDI values in $\mu\text{g}/\text{kg}/\text{day}$ for farm C ranged between 0.050-0.508 for As, ND-3.25 for Pb and 0.006-0.02 for Cd while in farm D, the EDI values for As, Pb and Cd ranged between 0.060-0.49, ND-0.27 and 0.0047-0.02 (table 3). The EDI values for As, Pb, and Cd were less than the accepted tolerable daily intake and the HR values of Pb and Cd were less than one for the samples in farm C and D in both children and adult. Nonetheless, children had HR value of 1.69 and 1.64 for arsenic in muscles and gizzard for farm C and D, respectively. The calculated hazard index values for children in muscles and gizzard were above one for farm C. Also, the hazard index values of gizzard and muscles for children are both above one, having the values of 1.70 and 1.23, respectively in farm D.

Carcinogenic Risks Assessment

Table 4 presents the carcinogenic risk assessment of the PTEs in the analyzed samples. The incremental lifetime cancer risk (ILCR) is acceptable within the range of 10^{-6} - 10^{-4} , the cancer risk assessment revealed that cancer risk value for lead in all the samples were within the safe limit, however, values above the safe limit were observed for arsenic and cadmium in albumen and muscle in farms B and C (table 4). The highest cancer risk (4.3×10^{-3}) by arsenic for children was observed in muscle. This indicates approximately 43 cancer cases will result per 10,000 children that consumes the chicken muscles and this signifies eminent risk as PTEs concentration in poultry products is approaching a critical level with respect to its carcinogenic tendency (Ihedioha *et al.*, 2021; Omeje *et al.*, 2022). There is no quantitative model to assay the health risk effect associated with antibiotic residues at the moment. It is important to emphasize that some antibiotics prohibited in livestock production are still very much in use in Nigeria (Omeiza *et al.*, 2012) and are incorporated with the

legitimate ones during production. Regardless of the fact that chloramphenicol has been banned; it is still mixed alongside other antibiotics and sold in the study region. ILCR was dissatisfactory mostly for arsenic; nonetheless, lower values of Arsenic were reported by (Bortey-sam *et al.*, 2015, table 4). International Agency for Research on Cancer (IARC) categorized Pb, Cd and As as carcinogenic toxicants (IARC, 2017), and the highest cancer risk was observed at a level (4.3×10^{-3}), which shows that the consumption of chicken meat (muscles) may result in 43 cancer cases per 10,000 children.

Potential Toxic Elements and Antibiotics Correlations

The PTEs concentration in the albumen and muscle had a perfect correlation ($p < 0.05$) at 0.05 significant levels indicating that increase in the PTEs concentrations in the muscles of the chicken leads to increase in concentrations in the albumin as well. That was not the case for yolk and gizzard since there was no positive correlation. The correlations of the antibiotics indicate that there was a strong correlation ($p > 0.9$) at 0.01 significant level between all the parts of the chicken supporting the earlier suggestion that the antibiotics (both the restricted and allowed) are mixed before administering it to the poultry products. A similar result was obtained for the antibiotics usage in all the farms under consideration. With respect to PTEs correlation in farm B, C and D, it was observed that there was perfect positive correlation between yolk and gizzard for farm B and strong positive correlation ($p < 0.05$) for farm D at significant level of 0.05 but there was no positive correlation for farm C.

Conclusion

The result of this study indicates that the poultry products (egg and meat) in the studied region are possible means of human exposure to As, Cd, Pb and antibiotics (chloramphenicol, chlortetracycline, streptomycin, and neomycin). Their usage might be consequential to health hazard especially for chloramphenicol. Therefore stringent regulatory measures should be put in place by the responsible agencies to ameliorate the suffering resulting from human exposure to antibiotics and heavy metals.

Therefore it is necessary that the government agency responsible for regulating poultry products to be up and doing in carrying out their mandate of monitoring agro-products sold in the region.

Data Availability Statement

All the associated data are within the manuscript

Author Contributions

Ezike, C. C. and Ekere, N. R.: conceptualization, experiments, Literature Writing, H. O. A. and Ihedioha, J. N.: data analysis, Odum, P. U. and Nwoke, S. U.: manuscript writing and AAS analysis, Akpomie, K. G.: data analysis, proofreading, reviewing, Interpretation. All authors contributed to the revisions of the manuscript and approved it for publication. All authors have read and agreed to the published version of the manuscript.

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There is no conflict of interest.

Ethical Consideration

The authors complied with all ethical issues required.

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