

Evaluation of Antibiotic Residual Concentrations and their Effects in Broiler Chicks

Hajara Muhammed-Sadiq; Chibuzo Carole Nweze; Bawa Yusuf Muhammad; Semiu Olalekan Ajiboso; Moses Zira Zaruwa; Halimatu Sadiya Abdullahi; Sarah Tisereh Yohanna; Yusha'u Ibrahim; Rahima Yunusa; Solomon Olubunmi Grace

Department of Biochemistry, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, Nigeria.

Corresponding author: cherryhaajara@gmail.com

DOI: <https://doi.org/10.62154/ajastr.2025.018.010657>

Abstract

Antibiotic resistance occurs when bacteria develop the ability to withstand the effects of an antibiotic. The misuse of antibiotics in poultry production has contributed to antibiotic resistance. Antibiotics are commonly used as growth promoters and for therapeutic purposes. A survey of 80 questionnaires was conducted to identify the most commonly used antibiotics in poultry farms. This was done to recreate how antibiotic administration is carried out in poultry farms, which would indicate whether these drugs are being abused by poultry farmers. Seven antibiotics were selected and administered based on the average body weight of 40 broiler chickens. These included Oxytetracycline (400 mg/kg), Tylosin (500 mg/kg), Gentamycin (330 mg/kg), Penicillin (500 mg/kg), Neomycin (220 mg/kg), Sulfonamide (200 mg/kg), and Fluoroquinolone (300 mg/kg), which were compared with a normal control group. Additionally, 15 chickens were purchased from local markets in Keffi, Nasarawa State, for comparison. Samples from the blood, breast, liver, gizzard, and crop were analyzed to determine antibiotic residual concentrations, their effects on haematological parameters, the presence of resistant bacteria in the antibiotic residue, alongside an antibiotic sensitivity test. The results showed a significant difference ($p < 0.05$) in haematological parameters in the local market group compared to the poultry farm group, indicating immune suppression. Both groups exceeded the recommended maximum residue limits. Pathogenic bacteria like *Salmonella* spp. and *E. coli* were found in the local market group, and antibiotic sensitivity tests showed reduced efficacy of the antibiotics. The misuse of antibiotics as growth promoters and therapeutics affected the blood of chicks, exceeded antibiotic residue limit and also, contributed to the development of antibiotic resistant bacteria. This indicates the misuse of antibiotics in local market chickens during administration, rendering the meat unsafe for human consumption.

Keywords: Antibiotics, Broiler-Chicks, Antibiotic-Resistance, Residual-Concentration, Resistant-Bacteria, Haematological Effect.

Introduction

The term "antibiotic" comes from "antibiosis," which literally means "against life." Originally, antibiotics were defined as organic compounds produced by one microorganism

to inhibit or kill other microorganisms (Russell, 2010). Over time, the definition has broadened to include synthetic antimicrobial agents. Antibiotics that kill bacteria are called "bactericidal," while those that inhibit bacterial growth are known as "bacteriostatic" (Walsh, 2013). At sub-therapeutic doses, the poultry industry extensively uses antibiotics to boost meat production by improving feed conversion, promoting growth, and preventing diseases. The rise of antibiotic resistance has become a significant global health issue, posing serious threats to public health, agriculture, and biosecurity (Holmes et al., 2016). Antibiotic resistance occurs when bacteria develop the ability to withstand the effects of an antibiotic, often due to the misuse or overuse of these drugs, leading to the emergence of resistant strains (Yüce et al., 2011). This resistance complicates the treatment of infections and has caught the attention of the World Health Organization (WHO) and other stakeholders. When these antibiotics are administered, residues can accumulate in animals' cells, tissues, and organs, which may remain pharmaceutically active and end up in consumable food products like milk, meat, and eggs (Botsoglou et al., 2001; Polianciuc et al., 2020). These residues pose direct health risks to consumers, including carcinogenic, mutagenic, and allergic effects (WHO, 2011). Nelson (2011) notes that antimicrobials in livestock can lead to resistant bacteria in treated animals. The gut microbiota and their metabolic products improve nutrient digestion, absorption, metabolism, and overall health and growth performance of poultry. Antibiotic resistance, defined as the ability of microorganisms to thrive despite the presence of antibiotics (WHO, 2011), can result from genetic mutations or the acquisition of resistance genes from mobile genetic elements (Kahne, 2005). For instance, Yüce (2011) found that chickens harbor significant proportions of aminoglycoside-resistant Enterobacteriaceae in their digestive tracts and tetracycline-resistant bacteria in their meat. High resistance levels have also been reported in *Salmonella* (Mahajan, 2012; Mathew, 2019; Martin, 2016), *Enterococcus* (Snel et al., 2020), and *Mycoplasma gallisepticum* (Smith et al., 2018). Excessive use of antibiotics has created a potential health risk that cannot be easily mitigated through food processing methods or increased antibiotic doses during treatment. The regulated administration of antibiotics in sub-lethal concentrations is no longer a viable solution to the problem, as the damage caused by antimicrobial resistance continues to escalate (Mahajan, 2012).

Statement of the Problem

The indiscriminate use of antibiotics in poultry farming to meet consumer demands has resulted in the emergence of resistant microbial strains, posing significant health risks to humans. Despite identifying the causes of antimicrobial resistance, research on eradicating it has been insufficient. Continuous antibiotic use has led to pathogenic bacteria and immunopathological issues, resulting in treatment failures that endanger lives. Current methods, including food processing and increased antibiotic doses, cannot effectively mitigate these risks. Therefore, it is crucial to investigate the emergence, transmission, and harmful effects of antimicrobial-resistant strains in chickens to develop effective strategies to address this pressing public health threat.

Aim and Objectives

Aim

The aim of this research work is to determine the presence and concentration of antibiotic residues as well as, evaluate the potential effects it has on broiler chicks.

Objectives

The specific objectives of this study include evaluation of:

- i. The presence and concentration of antibiotic residues in broiler chicks.
- ii. Haematological parameters of all chicken samples (WBC, RBC, PCV, HB, MCV e.t.c)
- iii. Antibiotic activity on selected microorganisms. (Antibiotic sensitivity test).
- iv. The presence or absence of pathogenic bacterial life in chicken crop and gizzard samples (Microbial tissue culture test).

Literature Review

Antibiotic Resistance

Antibiotics, often referred to as "wonder drugs," have achieved remarkable breakthroughs throughout medical history. They have been employed for both therapeutic and preventive purposes in human health, agriculture, and animal husbandry (CDC, 2019). The phenomenon where microorganisms can survive and thrive even in the presence of antibiotics is termed antibiotic resistance. These microorganisms develop genetic mutations that enable them to resist the effects of antibiotics. This resistance was first observed in Staphylococci, Streptococci, and gonococci following the market introduction of Penicillin, the first discovered antibiotic (WHO, 2020). In 1942, Penicillin-resistant Staphylococcus aureus was identified, which also developed resistance to methicillin. Today, antimicrobial resistance is a major health concern, with over 70% of pathogenic bacteria exhibiting resistance to at least one antibiotic (Laxminarayan *et al.*, 2015).

Early History of Antibiotics

The first antibiotic, mycophenolic acid, was discovered in 1893 by Italian microbiologist Bartolomeo Gosio. Isolated from *P. glaucum*, it was found to inhibit the growth of *Bacillus anthracis* (Mohr *et al.*, 2016). In 1909, Paul Ehrlich and his team discovered Salvarsan (arsphenamine), the first synthetic arsenic-derived antibiotic, effective against *Treponema pallidum*, the causative agent of syphilis (Mohr *et al.*, 2016). Neosalvarsan, introduced in 1913, was less toxic and more effective in treating syphilis than its predecessor, Salvarsan (Di Sykes *et al.*, 2013). Due to the high risks associated with arsenic, these drugs were eventually replaced by Prontosil, a broad-spectrum sulfonamide (sulfamidochrysoidin) discovered by German bacteriologist Gerhard Domagk in 1930. Prontosil was primarily used to treat injured soldiers during World War I, marking a significant milestone in antibiotic research (Golker *et al.*, 2014). Prontosil acted as a bacteriostatic agent against various bacteria by inhibiting dihydropteroate synthetase (DHPS) in the folic acid pathway, thereby blocking bacterial nucleic acid synthesis (Sykes *et al.*, 2013). However, sulfonamides were

eventually superseded by penicillin as bacteria developed resistance to Prontosil due to mutations in the DHPS enzyme (Skold *et al.* , 2000). In 1928, Scottish bacteriologist Alexander Fleming accidentally discovered that a fungus (*Penicillium notatum*) inhibited the growth of *Staphylococcus aureus* colonies. He theorized that the fungus secreted a compound that inhibited the bacteria and successfully isolated the active molecule, naming it 'penicillin,' the first true antibiotic, in 1929. However, it was the work of Howard Walter Florey and Ernst Boris Chain that elucidated the structure of penicillin G in 1939, enabling its purification and large-scale production (Sykes *et al.* , 2013). The introduction of penicillin for treatment in 1945 marked a major breakthrough in antibiotic discovery (Gaynes *et al.* , 2017). Dorothy Crowfoot Hodgkin's X-ray crystallographic analysis of penicillin's structure in the same year allowed it to be classified as the first member of the β -lactam family of naturally occurring antibiotics (Gaynes *et al.* , 2017).

Causes of Antibiotic Resistance

Bacteria naturally evolve to survive antibiotics, but factors like genetic mutations, gene transfer, overuse, misuse, self-medication, and agricultural applications accelerate resistance. Additionally, the slow development of new antibiotics exacerbates the problem.

Mechanisms of Resistance

Antibiotic resistance occurs through natural (innate or mediated) or acquired mechanisms (via gene transfer or mutations). The four main mechanisms include: Limiting drug uptake, Modifying drug targets, Inactivating drugs, Efflux pumps.

Materials and Methods

Ethical Clearance

Ethical approval was obtained from the Ethical Committee, Nasarawa State University, Keffi.

Equipments

The equipments used in the course of the study includes the following: Jenway 7305 Spectrophotometer, Refrigerator, HSGF254 thin layer chromatography silica gel plate, UV lamp AC90-230V (365nm wavelength of visualization), medium scale grinding machine, Microscope, animal cages, surgical blades, weighing balance, Centrifuge, Haematological analyzer (Celtrac G Mek-9100), micropipette, beakers, sample bottles, microscopic slides, Petri dishes, filter paper, Swab sticks, McFarland standard calibration tubes, beakers and rotary evaporator.

Chemicals and Standard Drugs

Methanol (JHD CAS 67-56-1), trichloroacetic acid (TCA), Diethyl ether, Oxytetracycline, gentamycin, sulfonamide, penicillin, tylosin, neomycin, fluroquinolone antibiotics, Acetone, Acetonitrile, McFarland standard solution, Normal saline, Microbial stains and

Ethanol were obtained from various veterinary and pharmaceutical drug stores in Keffi, Nasarawa state. Standard solution was stored in 4°C.

Sample Extraction

Sample extraction was performed according to Poppelka *et al.* (2005). Four gram of each sample was cut into small pieces, grinded and blended. 10 mL Phosphate Buffer Saline (pH-6.5) was added and mixed. It was then centrifuged @ 60000 rpm for 20 min after mixing with 2 mL 30% Trichloroacetic acid. Supernatant was collected and filtered by Whatman filter paper and funnel. The filtered chicken extract was concentrated using a rotary evaporator, gentle heat of 40 degrees celcius, allowing the solvent to evaporate. Final volume of the extracts was pooled carefully into screw cap vial and kept into refrigerator for future analysis.

Research Design

A total of Fifty-five (55) chickens were used and subdivided into two groups. Fourty (40) were purchased as day old chicks from a local poultry farm in Nassarawa, State, Keffi. Fifteen (15) were purchased as fully grown chickens from three notable markets (Keffi Old market, Sambisa market and Lambu market) in Nassarawa State, Keffi. The chicks were fed ad libitum. The Chicks were raised until the end of the experimental period (for a total of eight weeks) under the natural summer conditions. The weight of each chicken was monitored every week for two months.

Experimental Design

This study was carried out in Nasarawa State, Keffi. A survey consisting of 80 questionnaires were distributed amongst poultry farmers, veterinary doctors and veterinary practitioners in the poultry sector in the region. The survey was carried out amongst individuals, to assess: the occurrence of diseases; the method of their diagnosis, the commonly used antibiotics in poultry farms and their impact on the health of humans. After the survey, seven of the most commonly used antibiotics were chosen in the order of Oxytetracycline, Penicillin, Neomycin, Tylosin, Gentamicin, Sulfonamide, Fluoroquinolone. The average weight of chickens and recommended dosages of each individual antibiotic was used in each group to ensure even distribution of the drug in drinking water. No withdrawal periods were observed.

GROUP A- The first group consisted of a total of 40 broiler chicks, with 5 chickens in each group, which was divided in subgroups one to eight;

Group A1- The normal control group which were given a normal diet without any antibiotics.

Group A2- Administered Fluoroquinolone (300mg/kg bw) collectively.

Group A3- Administered Sulfonamide (200mg/kg bw) collectively

Group A4- Administered Gentamycin (330mg/kg bw) collectively

Group A5- Administered Oxytetracycline (400mg/kg bw) collectively

Group A6- Administered Neomycin (220mg/kg bw) collectively

Group A7- Administered Tylosin (500mg/kg bw) collectively

Group A8- Administered Penicillin (500mg/kg bw) collectively

Blood, breast, liver, gizzard and crop chicken samples were collected from each chicken

Dosage Calculation

Recommended dosage for Oxytetracycline: 20 grams per 50 kg body weight.

Average weight of 6 chickens in the Oxytetracycline group: 1 kg

The recommended dosage is converted to a per-kilogram basis:

$20 \text{ grams}/50 \text{ kg} = 0.4 \text{ grams/kg}$

The volume of the water the antibiotic is mixed with is one litre (1L)

The concentration of Oxytetracycline (OTC) in the solution is $= 0.4 \text{ grams/kg} \times 1 \text{ kg (chicken weight)} / 1 \text{ litre (water volume)} = 400 \text{ mg/L}$

Hence, the concentration per kg body weight = 400mg/kg bw

All antibiotics had varying recommended dosages and body weights of chickens in each group which explains the different concentration values.

GROUP B

The second group consisted of 15 broiler chickens in total, with 5 chickens in each group. Five chickens were purchased from three different markets in Keffi namely; Keffi New Market, Sambisa Market and Lambu Market and they were referred to as the Local Market Group. The chickens were chosen randomly from poultry sellers. This was done to prove that the administration of antibiotic was being abused in different locations within the state. Blood, breast, liver, gizzard and crop chicken samples were collected from each chicken;

Group B1- Chickens obtained from Lambu Market

Group B2- Chickens obtained from Keffi Old market

Group B3- Chickens obtained Sambisa Market

Chicken samples (blood, liver, breast, gizzard and crop) were collected and stored in normal saline and taken to the laboratory for further analysis.

Collection of chicken samples for analysis (blood, breast, liver, gizzard, crop)

The chicks were grown for the first four weeks and antibiotic administration lasted for four weeks. After which, the animals were sacrificed in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals and other Scientific Purposes ETS-123 (European Treaty Series, 2005). Whole blood was collected using sterile syringe and needle. The blood sample of each chicken was collected into respective capped tubes. The blood samples in the tubes were centrifuged at 1000 rpm for 10 minutes using a bench top centrifuge to separate cells from serum. A necropsy was performed to access the internal organs of the chickens. After which, the breast muscle, liver, gizzard and crop

samples were collected and placed in a sterile container filled with normal saline to preserve the integrity of the samples.

Determination of the presence of Antibiotic residuals in chicken breast and liver samples

High performance thin layer chromatography plate and Ultraviolet lamp were used. Thin layer Chromatography was performed according to Grove and Randal, (1955) with some required adjustments. High performance thin layer chromatography plates with a thin, uniform layer of 100 to 200 micro metres were used. Spots were applied to the plate using micropipettes. A volume of 50 μ l was used for spotting. The plate was placed in Thin layer Chromatography tank (contained mobile phase; Acetone and Acetonitrile: 1:1) and covered by lid and it was left until the mobile phase reached the upper line. Spots were visualized with the UV lamp at 365 nm. Spots marking were done by pencil on the reverse side or on the plate's edge for calculation of retention factor (Rf).

Calculation of Rf values: These measurements are the distance travelled by the solvent, and the distance travelled by individual sample spots. Same Rf value of standard and sample were considered as similar compound.

Measurement

1. The distance from the origin (starting point) is measured to the centre of the compound spot.
2. The distance from the origin to the solvent front (farthest point reached by the solvent) is measured.
3. Calculate Rf using the formula.

Interpretation

1. Rf = 0: Compound did not migrate.
2. Rf = 1: Compound migrated with the solvent front.
3. Rf < 1: Compound migrated slower than the solvent.
4. Rf > 0.5: Moderate to high retention.
5. Rf < 0.5: Low retention.

Determination of Antibiotic residual concentration in chicken breast and liver sample

Ultraviolet Spectroscopy was performed for the analysis according to Grove and Randal, (1955).

Haematological Analysis

Haematological analysis was performed according to Williams *et al.*, (1972).

Antibiotic Sensitivity Test

The disk diffusion method was performed according to Bower *et al.*, (1966).

Principle

Sample extracts in agar plates are placed inoculated, allowing the antibiotics to diffuse into the agar and interact with the microorganisms. The growth of the microorganisms is inhibited by the antibiotics creating a clear zone around the disk. The size of the zone of inhibition is measured, indicating the viability of antibiotic residue present in the sample.

Procedure

The Mueller-Hinton agar is poured into a sterile plate and allowed to solidify. The desired bacterial culture (staphylococcus aureus, bacillus, Escherichia coli and Streptococcus Pneumoniae) is adjusted to a desired density (McFarland standard turbidity) to prepare the inoculum. The inoculum is spread evenly over the agar surface using a sterile swab and then the antibiotic discs are placed into the inoculated agar surface and spread evenly apart. The plate is placed into an incubator at 35degree Celsius+/-2degree Celsius for 24 hours. The diameter of clear zones around each disc are measured using a ruler or caliper. The zone diameters are compared to established breakpoints to determine the level of antibiotic susceptibility. These zones are referred to as zones of inhibition.

Antibiotic Sensitivity Test Parameters

1. Minimum Inhibitory Concentration (MIC): Lowest antibiotic concentration inhibiting bacterial growth.
2. Minimum Bactericidal Concentration (MBC): Lowest antibiotic concentration killing bacteria.
3. Zone of Inhibition (ZOI): Diameter of clear zone around antibiotic disk, indicating bacterial growth inhibition.
4. Incubation Time: Time bacteria are exposed to antibiotics (e.g., 18-24 hours).
5. Temperature: Optimal temperature for bacterial growth (e.g., 35-37°C).
6. pH: Optimal pH for bacterial growth (e.g., 7.2-7.4).
7. Zone diameter: The size of the zone of inhibition correlated with the level of antibiotic susceptibility
8. Breakpoints: Established zone diameter breakpoints determine the level of susceptibility
9. Susceptible(S)- Zone diameter greater than /equal to breakpoint
10. Intermediate(I)- Zone diameter=breakpoint plus/minus 1mm
11. Resistant(R)- Zone diameter less than breakpoint

Microbial Screening and Antibiotic Sensitivity test

The microbial screening and tissue culture test was performed according to Gale *et al.* , (1972).

Statistical Analysis

The data obtained were analyzed using one-way ANOVA in IBM SPSS version 23.0 to get the means and standard deviations ($n=5$). Further test for levels of significance was considered statistically at a level of $p<0.05$. (Duncan, 1957). The presence of antibiotics was indicated by their standard retention factors and antibiotics were quantified by their individual concentrations.

Results

Detection of the Presence of Antibiotics Residues in Group A and Group B Samples

The results for the presence of antibiotic residues in all chicken groups are depicted in Table 1, 2, 3 and 4. No antibiotic residue was detected in the normal control group, but fluoroquinolone, sulfonamide, gentamicin, oxytetracycline, Neomycin, Tylosin and penicillin antibiotic residues were present in group two to group eight accordingly for both breast and liver samples. In contrast, the local market chicken breast (chest) samples showed presence of penicillin for group one, oxytetracycline for group two, sulfonamide and fluoroquinolone antibiotic residues for group three. The liver samples showed the presence of penicillin and oxytetracycline in group one, penicillin and fluoroquinolone in group two, sulfonamide and fluoroquinolone antibiotic residues in group three.

Table 1: Antibiotics Residues in Group A (Breast Samples)

Groups	Retention factor	Present	Antibiotic type
Group A1(Control)	ND	-	ND
Group A2	0.71	+	Fluoroquinolones
Group A3	0.65	+	Sulfonamide
Group A4	0.55	+	Gentamicin
Group A5	0.57	+	Oxytetracycline
Group A6	0.75	+	Neomycin
Group A7	0.57	+	Tylosin
Group A8	0.69	+	Penicillin

ND=Not detected, + =present, - =Absent

Table 2: Antibiotics Residues Group A (Liver Samples)

Groups	Retention factor	Present	Antibiotic type
Group A1(Control)	ND	-	ND
Group A2	0.71	+	Fluoroquinolones
Group A3	0.65	+	Sulfonamide
Group A4	0.55	+	Gentamicin
Group A5	0.57	+	Oxytetracycline
Group A6	0.75	+	Neomycin
Group A7	0.57	+	Tylosin
Group A8	0.69	+	Penicillin

ND=Not detected, + =present, - =Absent

Table 3: Antibiotic Residues in Group B (Breast samples)

Groups	Retention factor	Present	Antibiotic present
Group B ₁ (Aguwan Lambu)	0.69	+	Penicillin
Group B ₂ (Keffi Old market)	0.57	+	Oxytetracycline
Group B ₃ (Sambisa market)	0.65 and 0.71	+	Sulfonamide and fluroquinolne

ND=Not detected, + =present, - =Absent

Table 4: Antibiotic Residues in Group B (Liver Samples)

Groups	Retention factor	Present	Antibiotic present
Group B ₁ (Aguwan Lambu)	0.69 and 0.57	+	Penicillin and oxytetracycline
Group B ₂ (Keffi Old market)	0.69 and 0.71	+	Penicillin and fluoroquinolone
Group B ₃ (Sambisa market)	0.65 and 0.71	+	Sulfonamide and fluroquinolne

ND=Not detected, + =present, - =Absent

Detection of Antibiotics Residual concentration in Group A and Group B samples

The results of residual antibiotics detected in chickens from poultry farm and local market (Angwan Lambu Market, Keffi Old Market and Sambisa Market) were shown in Figure 1 and 2 for breast and liver samples respectively. The analysis revealed the penicillin group showed the highest concentration in both liver and breast samples for the poultry farm group. In contrast, the local market group showed higher concentrations. Oxytetracycline had the highest concentration for both breast samples Group one (Keffi Old market) and in the liver samples which was prominent in Group two (Angwan Lambu Market). All detected antibiotic residues were above the Maximum Residue Limits (MRL) recommended by the United State (US) and Europe (EU) standards.

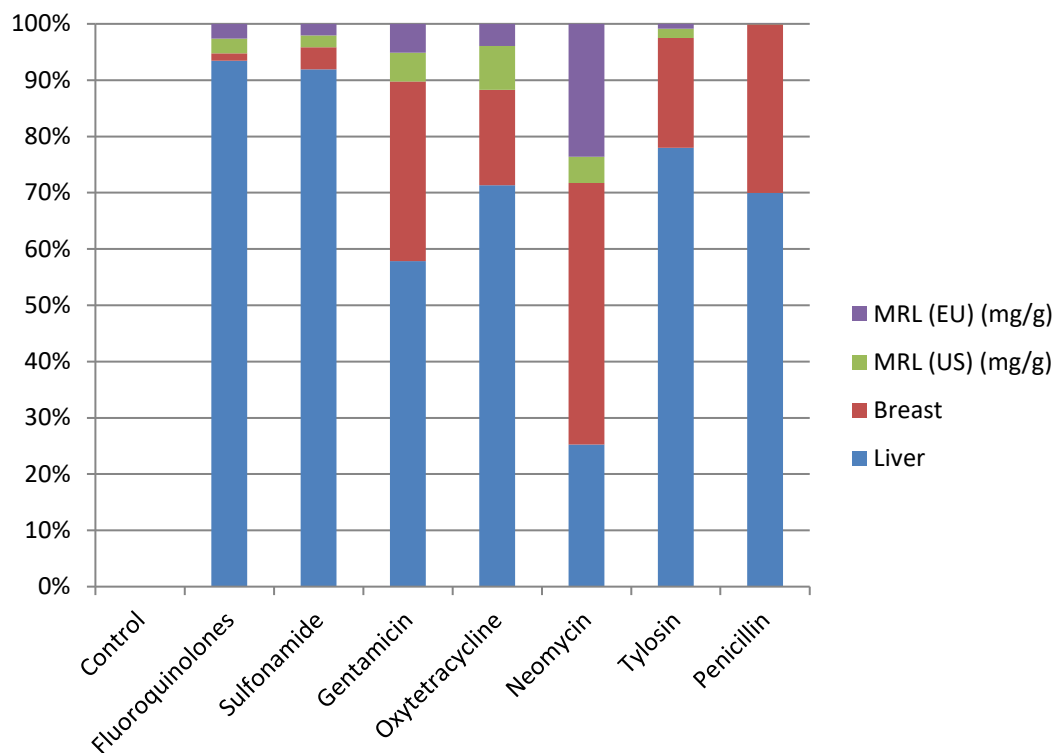


Figure 1: Residual concentration of Antibiotics from GROUP A

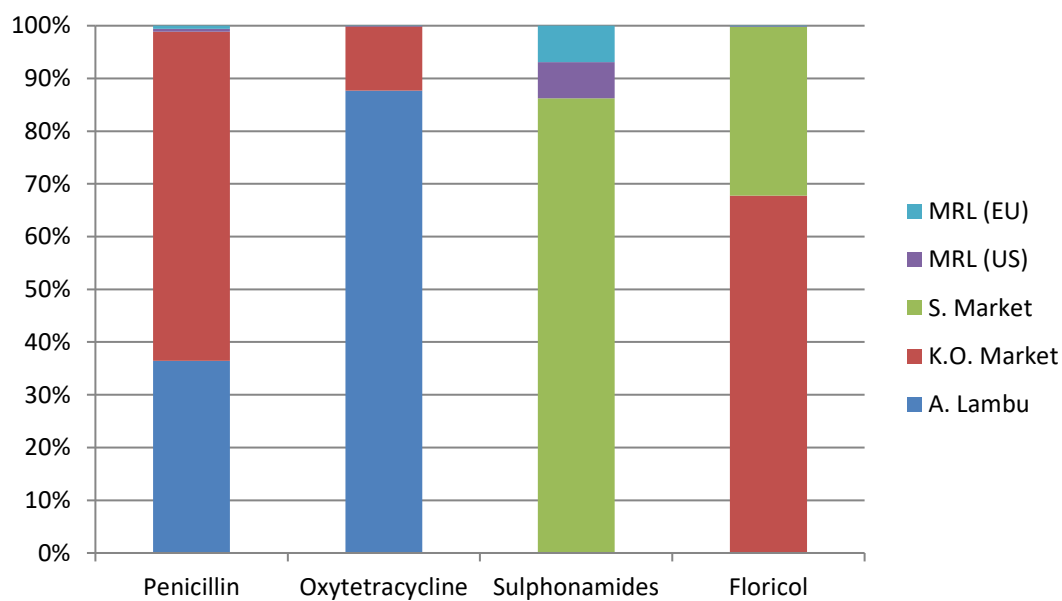


Figure 2: Residual concentration of Antibiotics from GROUP B

Effect of antibiotic administration the Haematological parameters of both chicken groups

As shown in Table 5 and 6 below, there was a significant decrease ($p < 0.05$) in all haematological parameters values for the local market group when compared to the normal control indicating anaemia and immune suppression. Haematological function slightly improved in the poultry farm group.

Table 5: Effects of Antibiotics on Hematological Parameters in Group A samples

Groups	AI	AII	AIII	AIV	AV	AVI	AVII	AVIII
WBC ($\times 10^9/L$)	4.10 $\pm$ 0.42 ^a	6.35 $\pm$ 2.33 ^a	4.80 $\pm$ 0.99 ^a	4.60 $\pm$ 2.26 ^a	6.45 $\pm$ 0.92 ^a	7.30 $\pm$ 0.99 ^a	5.40 $\pm$ 1.41 ^a	4.50 $\pm$ 0.42 ^a
PCV (%)	37.00 $\pm$ 1.41 ^a	40.50 $\pm$ 2.12 ^b	45.00 $\pm$ 5.66 ^c	46.50 $\pm$ 2.12 ^c	51.50 $\pm$ 3.53 ^c	49.50 $\pm$ 2.12 ^c	47.00 $\pm$ 5.66 ^c	43.00 $\pm$ 1.41 ^c
PLT ($\times 10^9/L$)	135.00 $\pm$ 18.38 ^c	116.50 $\pm$ 10.61 ^c	91.50 $\pm$ 13.44 ^b	72.00 $\pm$ 2.83 ^a	77.00 $\pm$ 18.38 ^b	81.00 $\pm$ 29.70 ^b	106.00 $\pm$ 9.90 ^c	110.50 $\pm$ 13.43 ^c
HB (g/dl)	11.55 $\pm$ 0.49 ^a	12.60 $\pm$ 1.41 ^b	14.65 $\pm$ 2.05 ^c	15.40 $\pm$ 1.13 ^c	17.25 $\pm$ 1.20 ^c	16.35 $\pm$ 0.49 ^c	15.45 $\pm$ 2.19 ^c	14.05 $\pm$ 0.21 ^c
RBC ($\times 10^{12}/L$)	4.40 $\pm$ 0.14 ^a	4.75 $\pm$ 0.21 ^b	5.40 $\pm$ 0.90 ^c	5.50 $\pm$ 0.42 ^c	6.35 $\pm$ 0.35 ^c	6.70 $\pm$ 1.13 ^c	5.50 $\pm$ 0.90 ^c	5.45 $\pm$ 0.78 ^c

Results are expressed as mean $\pm$ standard deviation, n=5. Mean values with different alphabets superscripts across the groups indicates significant differences at $P < 0.05$. I=Control; II= Fluoroquinolones; III=Sulfonamide; IV=Gentamicin; V=Oxytetracycline; VI=Neomycin; VII=Tylosin; VIII=Penicillin

Table 6: Effects of Antibiotics on Haematological Parameters in Group B samples

Groups	Normal control group	B1 Lambu Market	B2 Keffi Old Market	B3 Sambisa Market
WBC	4.10 $\pm$ 0.42 ^a	2.10 $\pm$ 0.14 ^b	2.20 $\pm$ 0.42 ^b	3.05 $\pm$ 0.07 ^b
PCV	37.00 $\pm$ 1.41 ^a	29.00 $\pm$ 1.41 ^b	31.50 $\pm$ 0.71 ^b	33.50 $\pm$ 0.71 ^b
PLT	135.00 $\pm$ 18.38 ^a	62.50 $\pm$ 4.95 ^b	53.50 $\pm$ 0.71 ^b	63.50 $\pm$ 4.95 ^b
HB	11.55 $\pm$ 0.49 ^a	7.55 $\pm$ 1.48 ^b	8.05 $\pm$ 0.07 ^b	7.45 $\pm$ 0.07 ^b
RBC	4.40 $\pm$ 0.14 ^a	3.15 $\pm$ 0.21 ^b	3.45 $\pm$ 0.07 ^b	3.15 $\pm$ 0.64 ^b

Results are expressed as means $\pm$ standard deviation. Means value with different alphabets at the superscripts across the groups indicates significant difference at $P < 0.05$

Microbial Identification test for Group A and Group B Crop and gizzard samples

Lactobacilli, Citrobacter, E. coli were found in all chicken samples. Lactobacilli and Citrobacter colonies were found in the normal control, Fluoroquinolone, Gentamycine, Tylosin and Penicillin groups, Citrobacter was found in the Sulfonamide, and Neomycine groups only, E. Coli and Citrobacter was found in Oxytetracycline group only and no

Salmonella spp were found in all eight groups. For the local market groups, Lactobacilli, Citrobacter, E. coli and Salmonella spp were found in all chicken samples. Salmonella spp and E.coli were found in all chicken samples. Lactobacilli was found only in Lambu market crop sample, Citrobacter was found only in Lambu market and Keffi Old market crop and gizzard samples.

Table 7: Microbial screening of Group A samples

GROUPS	LACTOBACILLI		E. COLI		CITROBACTER		SALMONELLA	
	A	B	A	B	A	B	A	B
Group A1(Control)	+	+	-	-	+	+	-	-
Group A2	+	+	-	-	+	+	-	-
Group A3	-	-	-	-	+	+	-	-
Group A4	+	+	-	-	+	+	•	-
Group A5	-	-	+	+	+	+	•	-
Group A6	-	-	-	-	+	+	•	-
Group A7	+	+	-	-	+	+	•	-
Group A8	+	+	-	-	+	+	•	-

- + interprets present
- Interprets absent
- A- Crop samples
- B- Gizzard samples

Table 8: Microbial screening of Group B samples

GROUPS		LACTOBACILLI		E. COLI		CITRO		SALMONELLA	
		A	B	A	B	A	B	A	B
B1	LAMBU MARKET	+	-	•	+	+	+	+	+
B2	KEFFI OLD MARKET	•	-	+	+	+	+	+	-
B3	SAMBISA	•	-	+	+	•	-	+	+

- + interprets present
- Interprets absent
- A- Crop samples
- B- Gizzard samples

Antibiotic sensitivity test on Group A and Group B samples

Antimicrobial activities of Antibiotics in Poultry farm and Local market chicken samples were represented in table 9 and 10. E. coli, B.subtilis, S. pneumoniae and Staphylococcus Aureus were used as test organisms for the antibiotic sensitivity test. Bacterial growth inhibitions were found in all eight antibiotic residues respectively. Bacillus Subtilis growth

inhibition were found in Fluoroquinolone, Oxytetracycline and neomycin residues, showing no inhibition zones in Sulfonamide, Gentamycin, Penicillin and Tylosin antibiotic residues. Staphylococcus Aureus growth inhibition zones were found in Fluoroquinolone and Gentamycin residues only, while no inhibition zones were present in Oxytetracycline, Tylosin, Sulfonamide, Neomycin and Penicillin groups. E. coli bacterial growth inhibitions was found in all eight antibiotic residues and Streptococcus Pneumoniae growth inhibition zones were found in fluoroquinolone, getamycin, Oxytetracycline and Tylosin antibiotic residues with no growth in Penicillin, Sulfonamide and Neomycin residues. Bacterial growth inhibitions were found only in Lambu and Keffi Old market samples. Bacillus Subtillis growth inhibition were found in Lambu market samples only. Staphylococcus Aureus growth inhibition zones were found in Sambisa market samples only. No growth inhibition zones were found for E.coli and Streptococcus Pneumoniae in all three groups.

TABLE 9: Antimicrobial activities of Antibiotics in Group A samples

Organisms	A1.	A2	GROUPS A3	A4	A5
Bacillus	a 00 b 00	28mm 26mm	00 00	00 00	27mm 30mm
Staphylococcus Aureus	a 00 b 00	36mm 27mm	00 00	21mm 23mm	00 00
Escherichia Coli	a 00 b 00	30mm 24mm	26mm 34mm	27mm 34mm	34mm 30mm
Streptococcus Pneumonia	a 00 b 00	33mm 30mm	00 00	27mm 21mm	18mm 21mm
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Organisms	A6	GROUPS A7	A8
Bacillus	a 18mm b 20mm	00 00	00 00
Staphylococcus Aureus	a 00 b 00	00 00	00 00
Escherichia Coli	a 33mm b 30mm	24mm 33mm	18mm 20mm
Streptococcus pneumonia	a 00 b 00	20mm 24mm	00 00
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Numbers in mm- Zones of inhibition

- Breast sample
- Liver sample

Table 10: Antimicrobial activities of Antibiotics in Group B samples

Organisms	B ₁ Lambu.M	B ₂ K.O market	B ₃ Sambisa market
Bacillus	a 23	00	00
	b 20	00	00
Staphylococcus Aureus	a 00	18	00
	b 00	20	00
Escherichia Coli	a 00	00	00
	b 00	00	00
Streptococcus Pneumonia	a 00	00	00
	b 00	00	00

- Breast sample
- Liver samples

Numbers in mm- Zones of Inhibition, Lambu. M- lambu market, K.O market- Keffi Old market

Discussion

Detection of Residual Antibiotics in Chicken Samples

The study detected residual antibiotics in the breast and liver samples of chickens from both poultry farms and local markets, as detailed in Tables 1, 2, 3, and 4. The normal control group showed no detectable antibiotic residues, validating the control setup since the chickens were not administered antibiotics, ensuring a baseline for comparison. In contrast, various penicillin, sulfonamide, oxytetracycline, and fluoroquinolone residues were found in all three local market groups. These findings align with previous studies that reported multiple antibiotic residues in chicken samples from different markets (Donoghue, 2003; Sarmah et al., 2006).

Antibiotic Residue Concentrations in Liver and Breast Tissues

Notable differences in concentration and distribution were observed between the liver and breast tissues. Although both groups exceeded the recommended Maximum Residue Limits (MRLs), the highest concentration of penicillin was found in both liver and breast samples from the poultry farm group. This high concentration highlights the need for strict monitoring to ensure levels remain below the MRL to mitigate potential public health risks.

Since the liver plays a key role in drug metabolism and detoxification, antibiotics tend to accumulate there.

Findings from the local market group revealed penicillin, oxytetracycline, and fluoroquinolones above the MRL recommendation, while gentamicin and neomycin were not detected. These results emphasize the importance of adhering to withdrawal periods and MRLs to minimize public health risks. They also suggest that antibiotics in the poultry farm group were administered without proper consultation.

Hematological Effects of Antibiotic Residues

There was no significant difference in the seven antibiotic groups when compared to the normal control group. However, a significant decrease ($P < 0.05$) in White Blood Cell (WBC) count, Packed Cell Volume (PCV), Red Blood Cell (RBC) count, and hemoglobin (HB) concentration was observed in the local market group when compared to the normal control group. This suggests immune suppression or severe anemia, likely caused by the random and unregulated use of antibiotics.

Conversely, an increase in WBC count was observed in the poultry farm group, supporting the findings of Smith et al. (2020) that antibiotic administration can lead to leukopenia in chickens, suggesting immunosuppression. The increased PCV in the poultry farm group may be due to the stimulatory effect of antibiotics on erythropoiesis or reduced destruction of red blood cells, possibly as a compensatory mechanism to maintain oxygen transport capacity in the presence of subclinical infections. Similar increases in PCV have been noted by Davis et al. (2019), who reported enhanced erythropoiesis in antibiotic-treated poultry. A significant decrease ($P < 0.05$) in hemoglobin concentration was recorded in all three local market groups—Lambu Market, Keffi Old Market, and Sambisa Market—compared to the normal control and poultry farm groups. This aligns with Khane et al. (2005), who reported that antibiotic administration can lead to decreased PCV, increasing susceptibility to infections and diseases. The rise in hemoglobin concentration in the poultry farm group may be attributed to stimulated erythropoiesis or reduced red blood cell destruction due to improved health status and fewer subclinical infections. Robinson et al. (2017) observed similar increases in hemoglobin levels, suggesting enhanced erythropoiesis or reduced hemolysis following antibiotic treatment.

The increase in RBC count in the poultry farm group may result from enhanced erythropoiesis or improved overall health, leading to better red blood cell production and survival. Sommer et al. (2017) also reported increased RBC counts in antibiotic-treated poultry, suggesting that antibiotics may stimulate erythropoiesis or reduce hemolysis. Increased RBC counts enhance oxygen-carrying capacity, promoting better metabolic efficiency and overall health. However, a significant decrease ($P < 0.05$) in RBC count was observed in all three local market groups compared to the normal control and poultry farm groups, further supporting Khane et al. (2005), who found that antibiotic administration can decrease RBC count, leading to anemia and greater susceptibility to infections.

Antibiotic Sensitivity Test Results

The antibiotic sensitivity test revealed significant differences in the susceptibility of antibiotic residues isolated from poultry farm and local market chicken samples. Specifically, antibiotic residues from poultry farm samples exhibited larger zones of inhibition compared to those from market samples. This indicates that the antibiotic residues in poultry farm samples were more viable and effective in inhibiting bacterial growth (WHO, 2011).

Similar findings have been reported in other studies, where market samples showed smaller zones of inhibition compared to farm samples (Journal of Food Protection, 2019). A study in Nigeria found that *Escherichia coli* isolates from market chicken samples were more resistant to antibiotics than those from farm samples (Journal of Infection in Developing Countries, 2018). Likewise, a study in India reported higher resistance levels in market chicken samples compared to farm samples (Indian Journal of Medical Microbiology, 2020). This discrepancy could be attributed to the overuse or misuse of antibiotics in poultry production, which promotes the selection and spread of antibiotic-resistant bacteria (FAO, 2018).

Microbial Identification and Bacterial Populations

The microbial identification test revealed distinct differences in bacterial populations between poultry farm and local market samples. Poultry farm and Lambu Market samples were dominated by *Lactobacilli*, which are generally considered beneficial or commensal bacteria (Kohlar et al., 2018). *Citrobacter* was also present in farm samples, but as an opportunistic pathogen, its presence is less concerning than that of *Salmonella* and *E. coli* (Clark et al., 2015).

In contrast, market samples contained a higher presence of harmful and pathogenic bacteria, with *Salmonella* and *E. coli* found in all crop and gizzard samples. These findings underscore the increased risk of contamination in market chicken samples, likely due to inadequate hygiene practices and uncontrolled antibiotic use.

This study highlights the presence of antibiotic residues in chicken samples, with notable differences in concentration, hematological effects, antibiotic sensitivity, and bacterial populations between poultry farm and local market samples. The findings emphasize the urgent need for strict antibiotic monitoring, adherence to MRLs, and proper withdrawal periods to minimize public health risks.

Conclusion and Recommendation

Conclusion

There was presence of antibiotic residues in all chicken groups. The residual concentration indicated higher levels of antibiotic residues in the market survey samples exceeding the poultry farm group. Both groups exceeded maximum residue limits. The local market group showed a significant difference ($p < 0.05$) on liver, kidney, and hematological functions when compared to the poultry farm group indicating impaired liver and kidney function as well

as, anemic tendencies on the blood parameters. The antibiotic sensitivity test and microbial identification revealed that the integrity of the antibiotics in the local market group had been compromised when compared to the poultry group. The local market group harboured more pathogenic bacteria and higher antibiotic resistance. Hence, proving that antibiotic residues were lodged in both chicken groups, increasing the possibility of resistance transfer during consumption thereby, rendering the chicken meat unsafe for human consumption.

Recommendations

- i. To evaluate adverse effects from consumption of chicken meat consisting of antibiotic residuals.
- ii. To determine the antibiotic residual concentration from other sources of chicken production like supermarket sold chickens.
- iii. To determine of the effectiveness of locally produced drugs as less harmful alternatives to antibiotic use in poultry production.

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