Thin-Layer Chromatographic Screening of Selected Antibiotic Residues in Animal-Derived Foods in Ondo and Ekiti States, Nigeria

O. T. Owolabi[1]*, I. B. Osho[2], O. E Michael[2] and T. E Adebayo[2]

[1]Department of Animal Health and Production Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria

[2]Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria

***Corresponding Author**

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ABSTRACT

Antibiotic residues in animal-derived foods may pose food-safety concerns when veterinary drugs are used in food-producing animals without adequate observance of recommended withdrawal periods. This study screened for presumptive amoxicillin, chloramphenicol and doxycycline residues in beef, chicken, chevon and eggs sold in Akure, Ondo State, and Ado-Ekiti, Ekiti State, Nigeria. A total of 120 samples, comprising 30 samples of each product type, were reportedly collected from retail markets and analysed using thin-layer chromatography (TLC). Presumptive identification was based on the migration patterns and retention factor ((Rf)) values of sample spots relative to those of antibiotic reference standards. Of the 120 samples, 25 (20.8%) were reported as presumptively positive for at least one antibiotic residue. Chevon recorded the highest proportion of positive samples (30.0%; 9/30), followed by chicken (20.0%; 6/30), while beef and eggs each recorded 16.7% (5/30). No statistically significant association was reported between product type and presumptive residue occurrence (chi^2 =2.173), (df=3), (P=0.537)). Amoxicillin was reported as the most frequently detected antibiotic, followed by chloramphenicol, whereas doxycycline was not detected. The location difference was also not statistically significant (chi^2 =2.476), (df =1), (p =0.116)). These findings provide preliminary evidence of possible antibiotic residues in animal-derived foods sold in the sampled markets. However, because TLC is a screening method, the identities of the suspected residues require confirmation using a validated analytical method, preferably liquid chromatography–tandem mass spectrometry (LC-MS/MS). Further studies should also quantify residue concentrations and assess compliance with applicable maximum residue limits.

Keywords: Antibiotic residues, thin-layer chromatography, screening, animal products

INTRODUCTION

However, antibiotics are critical to the treatment and prevention of bacterial diseases in food animal production and antibiotics are an integral part of intensive livestock production systems across the globe. Use of such agents, however, has led to residues of antibiotics in edible tissues like muscle, liver and fat, especially when they are not withdrawn as recommended (FAO, 2021). These residues have significant public health implications, such as the selection of antimicrobial-resistant bacteria, allergic responses, gastrointestinal dysbiosis, and the potential for carcinogenic or toxic effects (Ghimpețeanu et al., 2022). The issue of antimicrobial resistance (AMR) is especially alarming since the use of antimicrobials in raising livestock has been identified as one of the primary drivers of global burden of AMR, and resistant bacteria and resistance genes are capable of crossing from livestock to humans via the food chain (Oladeji et al., 2025).

Antibiotics are readily available in Nigeria without the need for prescription and are also commonly used in livestock as growth promoters or for disease prevention without veterinary supervision (Emeje et al., 2022).

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This is worsened by inadequate enforcement of the veterinary drug regulations, restricted laboratory testing capabilities to monitor residues, and a general lack of understanding of some farmers about the withdrawal periods (Mesfin et al., 2024). This means that Nigerian food markets may have meat and poultry with traces of antibiotics that were given to the animals in the days leading up to slaughter or egg collection and milk from the animals. These residues are not degraded by typical cooking methods and can be present in the food, at levels that could be toxic to people, especially those with antibiotic allergies or who eat high amounts of animal products (Ghimpețeanu et al., 2022).

An antibiotic residue has been reported in some foods of animal origin in Nigeria such as fresh chicken, fish and beef in Maiduguri (Mustapha et al., 2023), raw meat in Kano (Lateefat et al., 2022) and poultry in Ogun State (Oyedeji et al., 2021). The problem is not unique to Nigeria; evidence from other low and middle income countries confirms its scale. In Bangladesh, doxycycline was detected in poultry meat and feed, though not in poultry eggs, in one study, and another study conducted using TLC, found doxycycline positivity in breast muscle at 26%, in thigh muscle at 28% and in liver at 43% (Sani et al., 2023). In Nepal, doxycycline was identified in 17% of sampled broiler meat by TLC and from survey data, doxycycline was one of the most commonly used poultry antibiotics in both Nepal and Kano (Thapa et al., 2023). Also in the Ugandan beef, TLC-approved penicillin and oxytetracycline were prevalent, which makes the ruminant meat an acceptable residue matrix (Tuhamize and Daniel, 2026). When these studies have been conducted jointly, their prevalence rates have been generally high, and some have found residues in more than 80% of the samples they analysed.

Nevertheless, reviewed of past studies show that there are mixed findings and they also differ by geographic locations and sampling techniques whose results cannot be generalised or apply to other communities.

Although livestock production (poultry, cattle, goats and egg production) is one of the major economic activities in Ondo and Ekiti States, however, there is very limited available information on the presence of antibiotic residues in animal derived food products in both the states. Without local data, state health and agriculture officials have a poor chance of designing targeted interventions, or evaluating effectiveness of current regulations.

Thin-layer chromatography (TLC) is the method adopted for the screening of antibiotic residues in food of animal origin is simple, relatively inexpensive and practical which is useful for preliminary analysis especially in the absence of sophisticated analytical instruments (Getahun et al., 2023). TLC can be used to separate antibiotics residue from animal derived foods due their differential migration on stationary phase and can be used as preliminary screening (Sani et al., 2023). It is easy to use, can test several samples at once, and can be used in the field or in a resource-poor laboratory. TLC is not a confirmatory method, however, and positive results must be confirmed by other methods, such as HPLC or LC-MS/MS, to make a regulatory or public health decision. The use of TLC in this study is therefore appropriate, as it is a first line screening technique that can provide preliminary data, and help design more strenuous confirmatory studies.

This study was conducted to screen the presence of amoxicillin (beta-lactam antibiotic), chloramphenicol (amphenicol antibiotic banned for use in food producing animals) and doxycycline (tetracycline) in beef, chicken, chevon and eggs sold in retail markets within Akure and Ado-Ekiti, South-Western Nigeria using TLC. These three antibiotics were chosen because they are representative of three different classes of antimicrobials that are frequently used in Nigerian livestock production and because chloramphenicol is a substance that is banned from food production, the presence of which in the food chain would be a clear sign of a big gap in regulation. The findings are expected to supply preliminary surveillance data to help direct additional confirmatory investigations, direct laboratory resources, and draw regulatory attention to marketing and use of veterinary drugs in the study area.

MATERIALS AND METHODS

Study Area and Sample Collection

This study was carried out in Ondo and Ekiti States respectively in the South-Western part of Nigeria from

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which samples were collected from Akure and Ado-Ekiti respectively. A total of 120 samples were collected (30 eggs, 30 beef, 30 chicken and 30 chevon samples) with 15 samples from each of the states. Samples were taken from retail markets and brought to the laboratory in ice-cooled sample boxes for analysis.

Sample preparation and extraction protocol

All the samples (10 g) were chopped and homogenized in a sterile blender. 7ml of phosphate buffer (pH 6.5) was added and well shaken. The mixture was set for 10-15 min so that the antibiotic residue can be released. For precipitation of protein, 2 ml of 30% trichloroacetic acid was added and then centrifuged at 7,000 rpm for 15 minutes. The liquid above the sediment was poured off and strained. Diethyl ether was added to the filtrate in equal volume, shaken gently and separated into two layers and the filtrate was defatted. The top organic layer was discarded, and the defatting conducted two times. The defatted extract was further concentrated to about 2 ml using rotary vacuum evaporator at 50°C until TLC analysis was done, after which, it was stored in refrigerator (Thapa et al., 2023).

Antibiotic Standard Preparation

Stock solutions of 10mg/ml were prepared for antibiotic drugs amoxicillin, doxycycline and chloramphenicol by dissolving 0.05g of each drug in 5ml of dilute acetic acid, methanol and distilled water respectively. Working concentrations were made by serial dilution, and the one which produced a clear, compact and well-defined spot was chosen for further TLC analysis.

Thin-Layer Chromatography

TLC plates (10 x 5 cm) were silica gel coated and moisture was removed by heating at 100-110°C for 10min. A line was drawn on the lower edge of the plate about 1.5 cm away from the edge, for use as an application line. The antibiotic solutions and extracted samples were placed on this line with a micropipette, with the spots being approximately 1 cm apart from each other and the size of the spot being 2–3 mm in diameter.

Mobile phases were chosen depending on the antibiotic to be analysed. Hexane and ethyl acetate were mixed in 1:1 ratio for amoxicillin. In the case of doxycycline, a ratio of 59:35:6 dichloromethane: methanol: distilled water was used. The ratio of the solvents used for chloramphenicol was 85:14:1 for chloroform, methanol, and acetic acid. For about 1 hour the TLC chamber was equilibrated with the mobile phase used for the development. The spotted plate was gently placed into the chamber so that the sample spots were above the level of the solvent. The solvent was allowed to flow up the plate by the capillary action until it reached close to the top of the plate. The plate was removed, the solvent front was marked and the plate was left to dry at room temperature for 30 min.

Detection and Presumptive Identification. Presumptive Detection and Identification

The developed plate was inspected under the UV light at 254 nm and the location of the spots that were visible were noted. The distance covered by the antibiotic compound was divided by the distance covered by the solvent front to give the retention factor (Rf). Presumptive identification was made by a sample that had a chromatographic spot that was in close proximity to the relevant antibiotic standard in terms of migration position and Rf value (Islam et al., 2022). It is stressed that this classification is only a screening result and not a confirmed identification, and that in this study confirmatory analysis by HPLC or LC-MS/MS was not carried out.

Statistical Analysis

The data were subjected to statistical analysis using SPSS version 25. The frequencies and percentages were obtained by descriptive data. To examine the associations between residue occurrence, product type and location, Pearson's Chi-square test was used and the significance level was taken at 5% probability level. All of the chi-square tests report degrees of freedom. The chi-square values are descriptive in nature and because of the exploratory nature of the study and the small sample size, cannot conclusively be interpreted as tests of association.

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RESULTS

Presumptive Antibiotic Residues in Animal Products from Pinnipeds and Other Marine Mammals.

Table 1 shows the TLC screening result of the samples obtained from Akure. The mean Rf values of amoxicillin, chloramphenicol and doxycycline were all below the standard reference values in the beef, chicken and egg samples respectively. The concentrations of chloramphenicol (0.65 ± 0.02) and doxycycline (0.64 ± 0.02) were found to be below the standard reference value, whilst amoxicillin (0.60 ± 0.02) was above the standard reference value in goat meat.

Table 1: TLC Screening of Antibiotic Residues in Animal Products from Akure

Product	Antibiotic	Mean Rf	SEM	Status vs Standard
Beef	Amoxicillin	0.55	0.03	Below Standard
Beef	Chloramphenicol	0.64	0.02	Below Standard
Beef	Doxycycline	0.65	0.02	Below Standard
Chicken	Amoxicillin	0.54	0.02	Below Standard
Chicken	Chloramphenicol	0.65	0.02	Below Standard
Chicken	Doxycycline	0.65	0.02	Below Standard
Egg	Amoxicillin	0.54	0.03	Below Standard
Egg	Chloramphenicol	0.64	0.02	Below Standard
Egg	Doxycycline	0.67	0.02	Below Standard
Goat	Amoxicillin	0.6	0.02	Above Standard
Goat	Chloramphenicol	0.65	0.02	Below Standard
Goat	Doxycycline	0.64	0.02	Below Standard

Values represent mean Rf values of antibiotic residues detected in animal products using thin-layer chromatography (TLC). Data are expressed as mean ± standard error of mean (SEM).

Source: Author's computation, 2026

Presumptive Antibiotic Residues in Animal Products from Ado-Ekiti

Table 2 presents the TLC results for samples from Ado-Ekiti. Beef samples recorded mean Rf values of 0.56 ± 0.02 for amoxicillin, 0.63 ± 0.01 for chloramphenicol, and 0.65 ± 0.02 for doxycycline, all below their respective standards. Chicken and egg samples followed the same pattern, with all values below the standard reference values. As observed in Akure, goat meat was the only product in which amoxicillin (0.61 ± 0.01) exceeded the standard reference value.

Table 2: TLC Screening of Antibiotic Residues in Animal Products from Ado-Ekiti

Product	Antibiotic	Mean Rf	SEM	Status vs Standard
Beef	Amoxicillin	0.56	0.02	Below Standard
Beef	Chloramphenicol	0.63	0.01	Below Standard
Beef	Doxycycline	0.65	0.02	Below Standard
Chicken	Amoxicillin	0.54	0.02	Below Standard
Chicken	Chloramphenicol	0.65	0.01	Below Standard
Chicken	Doxycycline	0.64	0.02	Below Standard
Egg	Amoxicillin	0.52	0.02	Below Standard
Egg	Chloramphenicol	0.62	0.01	Below Standard

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Egg	Doxycycline	0.67	0.02	Below Standard
Goat	Amoxicillin	0.61	0.01	Above Standard
Goat	Chloramphenicol	0.64	0.01	Below Standard
Goat	Doxycycline	0.64	0.01	Below Standard

Values represent mean Rf values of antibiotic residues detected in animal products using thin layer chromatography (TLC). Data are expressed as mean ± standard error of mean (SEM).

Source: Author's computation, 2026

Distribution of Presumptively Positive Samples

Table 3 shows the distribution of presumptively positive samples by product type, antibiotic type and location. Of the 120 samples screened, 25 (20.8%) were positive for at least one of the antibiotics tested. Goat meat had the highest proportion of positive samples (30.0%; 9/30), followed by chicken (20.0%; 6/30), with beef and eggs recording the same proportion of 16.7% (5/30). There was no significant difference in positive samples by product type ($\chi2 = 2.173$, df = 3, P = 0.537). Amoxicillin was the most commonly detected antibiotics, with 22 of 40 (55.0%) of the samples testing positive, compared with chloramphenicol (7.5%; 3/40) and doxycycline (0.0%; 0/40). Since each sample was screened for all three antibiotics, these proportions are not independent, but the chi2 value for antibiotic type ($\chi2 = 43.150$, df = 2, $P < 0.001$) should be interpreted only as an indication of the higher frequency of the amoxicillin positive samples as opposed to an independent statistically significant association. A higher proportion of presumptively positive samples was recorded in Akure (26.7%; 16/60) than Ado-Ekiti (15.0%; 9/60), but this was not statistically significant ($\chi2 = 2.476$, df = 1, P = 0.116).

Table 3: Distribution of Presumptively Positive Samples across Product Type, Antibiotic Type, and Locations

Variable	**Negative**	**Positive**	**Total**	**% Positive**
Product				
Beef	25	5	30	16.7
Chicken	24	6	30	20
Egg	25	5	30	16.7
Goat	21	9	30	30
Total	95	25	120	20.8
Antibiotic				
Amoxicillin	18	22	40	55
Chloramphenicol	37	3	40	7.5
Doxycycline	40	0	40	0
Total	95	25	120	20.8
Location				
Ado-Ekiti	51	9	60	15
Akure	44	16	60	26.7
Total	95	25	120	20.8

Source: Author's computation, 2026

Pearson's chi-square test was used to assess associations between residue occurrence and product type ($\chi^2 = 2.173$, df = 3, P = 0.537) and location ($\chi^2 = 2.476$, df = 1, P = 0.116). The chi-square value for antibiotic type ($\chi^2 = 43.150$, df = 2, $P < 0.001$) is reported descriptively because the same samples were screened for all three antibiotics, violating the assumption of independence.

DISCUSSION

The presumptive detection of antibiotic residues in 20.8% of the 120 animal-derived food samples screened in

this study represents an important food safety concern. This finding is lower than the prevalence reported in other parts of Nigeria, where antibiotic residues were detected in 84.7% of fresh chicken, fish, and beef samples in Maiduguri, including 74% of beef samples (Mustapha et al., 2023). The lower prevalence observed in the present study may reflect differences in antibiotic usage patterns, sample types, detection methods, or the screening (rather than confirmatory) nature of the TLC approach used here. Some samples assumed to be positive in this study might have failed confirmation by other analytical methods of higher specificity. Likewise some samples considered negative might have residues that are below the TLC detection limit.

Amoxicillin was the most commonly presumptively identified antibiotic residue found, with 55.0% of samples tested for amoxicillin being positive. The frequent findings of amoxicillin residues implies that this class of antibiotic, which belongs to the beta-lactam class of drugs, is widely used in animal production in the study area. Beta-lactam antibiotics are primarily used in food animals, but the residues may be found in meat, milk, and eggs and can affect food quality and safety (Mesfin, 2024).The finding of amoxicillin Rf values above the standard reference value in goat meat from both Akure and Ado-Ekiti is particularly noteworthy and may reflect the common practice of treating goats with injectable amoxicillin shortly before slaughter without observing withdrawal periods. However, without confirmatory analysis, this observation should be treated as hypothesis-generating rather than definitive.

The presumptive detection of chloramphenicol in three samples (7.5%) is concerning, as chloramphenicol has been banned for use in food-producing animals in Nigeria and many other countries due to its association with dose-independent aplastic anaemia. Despite this ban, the continued detection of chloramphenicol residues in animal products has been reported in several African countries, suggesting ongoing unauthorised use (Hassan et al., 2021). However, the present finding is based on TLC screening only, and confirmation by LC-MS/MS is essential before any regulatory conclusion can be drawn. If proven, the detection of this banned compound in the food chain would constitute a major regulatory failure.

No doxycycline residues were found in any of the 40 samples screened, which was somewhat surprising given tetracyclines are one of the most commonly used antibiotics in the livestock sector in Nigeria, although Lateefat et al. (2022) found doxycycline was below the limit of detection in catfish samples from Lagos and Ogun States. However, it is possible that the use of this drug is not as common in the other areas or that the residual amount is quickly reduced in tissues, or that the extraction and TLC procedures may not have been sensitive enough for this particular tetracycline.

The non-significant relationship between sample type and residue positive status ($P = 0.537$) indicates that contamination is not specific to only certain animal products but has been observed in all animal products. Nevertheless, the higher overall positive rate in goat meat (30.0%) and the observation of amoxicillin Rf values above the cutoff in all locations in goat meat should be investigated further using other confirmatory techniques.

The detection of presumptive antibiotic residues, which included a prohibited substance, in animal produce from various retail markets throughout South-Western Nigeria raises concerns. It implies that rules governing the use of veterinary drugs and suggested withdrawal periods may not have been adhered to during animal rearing, the consumption of food with residues of these antibiotics may also affect the health of individuals by altering the profile of beneficial bacteria in the gut and favoring the emergence and sustainment of antibiotic resistant bacteria (Oladeji et al, 2025). This motivates confirmatory follow-up studies and surveillance.

Limitations

This study has important limitations. To start, TLC is a screening method and does not identify residues in complex matrices because co-extracted substances may form spots with Rf values equivalent to those of antibiotics. The limit of detection of the TLC method was not established and recovery experiments in blank spiked samples were not conducted. In addition, the sample size ($n = 120$) was small to ensure statistically significant associations. Furthermore, samples were obtained only from retail markets in the two capital cities and did not necessarily reflect conditions in the immediate regions. Moreover, no quantitative determination

was carried out and the concentrations of residues cannot be compared to MRLs. Future studies with validated HPLC or LC-MS/MS methods with quality control are encouraged.

CONCLUSION

This study has established the presence of presumptively positive levels of antibiotic residues, especially amoxicillin, in beef, chevon, chicken, and eggs, offered for sale at retail outlets in South-Western Nigeria. Presumptive detection of chloramphenicol at 7.5% of samples screened was of regulatory importance and requires confirmation using alternative quantification methods (HPLC or LC-MS/MS). Higher Rf values obtained for amoxicillin in chevon and chicken, obtained from the two locations in the two states indicate the need for targeted intervention. It must be pointed out that all these results represent the preliminary screening levels, and require further validation using the more sensitive methods before any regulatory steps are taken. This study reveals the dire need for the implementation of routine screening programmes, oversight on withdrawal periods, validation of screening methods for these matrices, and awareness campaign on prudent use of antibiotics in livestock production to safeguard public health.

Ethical Approval: The study did not directly involve human participants or experimental procedure on live animals as confirmed by the institution committee on ethical committee.

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