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Original Article

Evaluation of Antibiotic Residues in Commercial Poultry Products in Districts of Faisalabad

Imran Mukhtar[1]*, Akifa Zainab[1], Haleema Sohail[2], Usama Mustafa[3], Amber Sharif[4], Maaz Bin Nasim[5], Raiha Soban[6], Mubeen Fatima[3], Waqas Ahmad[7], Muhammad Shoaib Zafar[7]*

[1] Gut Microbiome Biostructural Research Group, Gut Microbiome Metabolic Physiology Research Lab, Department of Physiology, Government College University, Faisalabad, Pakistan.
[2] Department of Pharmacology, University of Health Sciences, Kala Shah Kaku, Lahore, Pakistan.
[3] Department of Forensic Science, Lahore University of Biological and Applied Sciences, Lahore, Pakistan.
[4] School of Pharmacy, University of Management and Technology, Lahore, Pakistan.
[5] Lords College of Pharmacy, Kot Lakhpat, Pakistan.
[6] Department of Biochemistry, Grand Asian University, Sialkot, Pakistan
[7] College of Pharmacy, University of Health Sciences, Kala Shah Kaku, Lahore, Pakistan

***Corresponding author: Imran Mukhtar, drimranmukhtar@gcuf.edu.pk; Muhammad Shoaib Zafar, shoaibzafar786@gmail.com**

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ABSTRACT

Background: Antibiotic residues in poultry products are an important food safety concern because irrational antimicrobial use and inadequate withdrawal-period compliance may expose consumers to residual antibacterial compounds and contribute to antimicrobial resistance. Objective: This study aimed to screen antibiotic-residue activity in commercially produced poultry products collected from periurban poultry farms in Faisalabad, Pakistan. Methods: An observational cross-sectional laboratory-based screening study was conducted during November–December 2024. Eighty birds, including 40 broilers and 40 layers, and 40 eggs were collected from 20 commercial poultry farms. Serum, breast muscle, liver, egg albumen, and egg yolk samples were tested using the Swab Test on Animal Food method with Bacillus subtilis JS 2004 as the indicator organism. Screening positivity was defined by visible inhibition of bacterial growth around sample swabs. Results: No antibiotic-residue activity was detected in serum or breast muscle samples from broiler or layer birds. Broiler liver showed positivity in 14 of 40 samples (35.0%), while layer liver was positive in 2 of 40 samples (5.0%). Among egg components, egg albumen showed the highest positivity, with 24 of 40 samples positive (60.0%), followed by egg yolk, with 4 of 40 samples positive (10.0%). Overall liver positivity was 16 of 80 samples (20.0%). Conclusion: STAF screening detected antibiotic-residue activity mainly in liver and egg samples, especially egg albumen, while meat and serum were negative. Confirmatory analytical testing and routine residue surveillance are recommended. Keywords: Antibiotic residues; poultry products; STAF; egg albumen; liver; food safety; antimicrobial resistance.

INTRODUCTION

Antimicrobial resistance has emerged as one of the most important public health challenges of the twenty-first century, with inappropriate antimicrobial use in humans, animals, and food-production systems contributing to the selection and dissemination of resistant microorganisms (1). Poultry production represents an important component of this problem because antibiotics are widely used in commercial flocks for therapeutic management, disease prevention, and, in some settings, growth promotion. Although antimicrobial use can reduce morbidity and improve productivity in poultry farming, irrational or poorly regulated use may result in antibiotic residues in edible tissues and poultry products, particularly when recommended withdrawal periods are not followed before slaughter or egg marketing (2).

The presence of antibiotic residues in food of animal origin is a significant food safety concern. Consumers may be exposed to low concentrations of antimicrobial compounds through contaminated meat, liver, eggs, or other poultry products, with potential consequences including hypersensitivity reactions, toxic effects, alteration of gut microbiota, and contribution to antimicrobial resistance through repeated dietary exposure (3,4). The risk is particularly relevant in countries where poultry demand is rising, farm-level antimicrobial use is inadequately monitored, and routine residue surveillance is limited. In Pakistan, poultry meat and eggs are widely consumed and represent an affordable source of animal protein, making residue monitoring important from both clinical and public health perspectives (5).

Antibiotic residues do not distribute uniformly across poultry matrices. Organs involved in metabolism and excretion, particularly the liver, may show higher residue detection than muscle tissue, depending on the class of drug used, dose, route of administration, treatment duration, and withdrawal interval. Eggs may also contain residues, with distribution between albumen and yolk influenced by the physicochemical properties of the antimicrobial compound and the timing of exposure during egg formation (6,7). Therefore, assessing multiple sample types, including serum, muscle, liver, egg albumen, and egg yolk, can provide a broader screening profile than analysis of meat alone.

Several analytical methods are available for detecting antibiotic residues in animal-derived foods, including microbiological inhibition assays, thin-layer chromatography, high-performance liquid chromatography, and liquid chromatography–mass spectrometry. Confirmatory chromatographic methods provide drug-specific quantification, but microbiological screening assays remain useful for preliminary surveillance because they are relatively simple, inexpensive, and capable of detecting broad antibacterial activity in different biological matrices (8,9). The Swab Test on Animal Food is one such screening method and uses inhibition of bacterial growth around the test sample as an indicator of possible antimicrobial residue activity. However, because it does not identify the antibiotic class or quantify residue concentration, positive results should be interpreted as screening evidence requiring confirmatory testing where regulatory or clinical decisions are intended.

Despite increasing concern regarding antimicrobial residues in poultry products, local evidence from periurban poultry production systems in Faisalabad remains limited. Available studies from Pakistan and other regions suggest that residues may be detected in poultry tissues and eggs, but prevalence varies according to production practices, sample type, detection method, and withdrawal-period compliance (10–14). Generating local screening data is important for identifying potential food safety risks, guiding surveillance priorities, and encouraging better antimicrobial stewardship in poultry farming.

The present study was conducted to screen for antibiotic residues in commercially produced poultry products collected from periurban poultry farms in Faisalabad, Pakistan. Specifically, the study assessed serum, meat, liver, egg albumen, and egg yolk samples from broiler and layer sources using the Swab Test on Animal Food method, with the objective of estimating the proportion of residue-positive samples across different poultry matrices and identifying sample types requiring greater monitoring attention.

MATERIALS AND METHODS

This observational cross-sectional laboratory-based screening study was conducted to assess the presence of antibiotic-residue activity in commercially produced poultry products collected from periurban poultry farms in Faisalabad, Pakistan. The study was designed as a descriptive residue-screening survey using the Swab Test on Animal Food method to determine the proportion of screening-positive samples across different poultry matrices.

Sampling was carried out during November and December 2024 in periurban areas of Faisalabad. Twenty commercial poultry farms were included. Four birds were obtained from each farm, giving a

total of 80 birds. The sample included 40 broiler birds and 40 layer birds. In addition, 40 eggs were collected from commercial layer sources for residue screening in egg components.

The study included poultry products intended for commercial consumption. Serum, breast muscle, and liver samples were collected from broiler and layer birds, while egg albumen and egg yolk were analyzed from collected eggs. Birds were selected randomly from participating farms and processed after slaughter for sample collection.

Blood samples were collected and centrifuged at 1000 × g for 10 minutes to separate serum. Breast muscle and liver tissues were collected aseptically and placed in clean polythene bags. Eggs were transported intact to the laboratory and separated into albumen and yolk before testing. All biological samples were transported in cold storage boxes containing ice packs to preserve sample integrity. Tissue and serum samples were stored at −20°C until analysis, while eggs were refrigerated until laboratory processing. Before testing, frozen samples were allowed to thaw completely at room temperature until no ice crystals remained.

Antibiotic residues were screened using the Swab Test on Animal Food method, a microbiological inhibition assay used to detect broad antibacterial activity in food-animal samples. The test organism was Bacillus subtilis JS 2004. A pure colony of B. subtilis was cultured on agar and incubated for 18 hours at 37°C. Gram staining was performed to confirm organism purity and growth. The bacterial growth was harvested in sterile nutrient broth and transferred to freshly prepared nutrient agar for sporulation. The resulting spore suspension was pooled and stored at 4°C. A working spore suspension was prepared using Breed's smear method and adjusted to a final concentration of 2×10^7 spores/mL.

Nutrient agar was used as the assay medium. Twenty-eight grams of nutrient agar were dissolved in one liter of distilled water by boiling and then sterilized. Sterile agar was poured into 6 × 6-inch plates at approximately 20 mL per plate and allowed to solidify on a level surface. Prepared plates were sealed and refrigerated until use. Before inoculation, plates were brought to room temperature and inspected for contamination, cracking, drying, or other defects. The surface of each plate was inoculated uniformly with the prepared B. subtilis suspension using a sterile swab to produce a confluent bacterial lawn.

For sample application, sterile swabs were inserted into liver and breast muscle tissues to allow contact with tissue fluid, with gentle movement in muscle samples to release tissue fluid. For serum samples, swabs were dipped directly into separated serum. For egg analysis, separate swabs were soaked in egg albumen and egg yolk. Each sample swab was placed on the surface of the inoculated STAF plate, ensuring uniform contact with the agar surface without damaging the medium. A neomycin 5 µg control disc was placed on each plate at a standardized distance from the sample swab to confirm appropriate bacterial inhibition response. Plates were incubated at 30°C for 16–18 hours and then examined for inhibition of B. subtilis growth around the sample swab.

The primary outcome was the presence or absence of antibiotic-residue activity in each tested sample matrix. A sample was considered screening-positive when a clear zone of inhibition was observed around the sample swab, indicating antibacterial activity against B. subtilis. Samples without a visible inhibition zone were classified as screening-negative. The neomycin control disc was used to assess plate validity; inhibition around the control disc was expected to fall within the acceptable range of 10–16 mm. Plates outside the acceptable control range were considered unsuitable for interpretation and required repeat testing.

Data were recorded separately for broiler and layer samples and for each biological matrix, including serum, breast muscle, liver, egg albumen, and egg yolk. Descriptive statistics were used to summarize the findings. Frequencies and percentages were calculated for antibiotic-residue-positive and residue-negative samples using the relevant denominator for each matrix. Monthly distributions for November and December were also summarized descriptively.

To improve laboratory reliability, samples were transported under cold-chain conditions, stored under controlled temperature conditions, and tested using a standardized microbiological inhibition protocol. Culture purity of the test organism was checked before preparation of the working spore suspension, and control antibiotic discs were used during plate interpretation. All sample categories were processed using the same screening approach to maintain procedural consistency across matrices.

Because STAF is a screening assay, positive findings were interpreted as evidence of possible antimicrobial-residue activity rather than drug-specific confirmation or quantitative residue concentration. No confirmatory chromatographic analysis was performed; therefore, the study did not determine the specific antibiotic class, residue concentration, or whether detected residues exceeded maximum residue limits.

The study was limited to commercially available poultry products from periurban Faisalabad and was designed to estimate screening positivity across sample types rather than to infer farm-level causality, antimicrobial-use practices, or withdrawal-period compliance.

RESULTS

A total of 80 poultry birds and 40 eggs were screened for antibiotic-residue activity using the Swab Test on Animal Food method. The bird samples included 40 broilers and 40 layers collected from 20 commercial poultry farms in periurban areas of Faisalabad. Serum, breast muscle, and liver samples were obtained from both broiler and layer birds, while egg albumen and egg yolk were analyzed from 40 eggs collected from commercial layer sources.

Table 1. Distribution of Samples Included in STAF Screening

Sample Category	Matrix Tested	Number Tested
Broiler birds	Serum	40
Broiler birds	Breast muscle	40
Broiler birds	Liver	40
Layer birds	Serum	40
Layer birds	Breast muscle	40
Layer birds	Liver	40
Eggs	Albumen	40
Eggs	Yolk	40

Broiler Samples Among broiler samples, no antibiotic-residue activity was detected in serum or breast muscle samples. All 40 serum samples and all 40 breast muscle samples were screening-negative. In contrast, liver samples showed detectable antibacterial activity in 14 of 40 samples, giving a positivity rate of 35.0%.

Table 2. STAF Results in Broiler Samples

Sample Type	Positive, n (%)	Negative, n (%)	Total
Serum	0 (0.0)	40 (100.0)	40
Breast muscle	0 (0.0)	40 (100.0)	40
Liver	14 (35.0)	26 (65.0)	40

Layer Samples and Eggs Among layer samples, serum and breast muscle were also negative for antibiotic-residue activity, with 0 of 40 samples positive in each matrix. Layer liver samples showed lower positivity than broiler liver, with 2 of 40 samples positive, corresponding to 5.0%. Egg albumen showed the highest screening positivity among all tested matrices, with 24 of 40 samples positive (60.0%). Egg yolk was positive in 4 of 40 samples (10.0%).

Table 3. STAF Results in Layer and Egg Samples

Sample Type	Positive, n (%)	Negative, n (%)	Total
Serum	0 (0.0)	40 (100.0)	40
Breast muscle	0 (0.0)	40 (100.0)	40
Liver	2 (5.0)	38 (95.0)	40

Sample Type	Positive, n (%)	Negative, n (%)	Total
Egg albumen	24 (60.0)	16 (40.0)	40
Egg yolk	4 (10.0)	36 (90.0)	40

Monthly Distribution Screening positivity differed descriptively between November and December. In broiler liver samples, positivity was 5 of 16 samples in November (31.3%) and 9 of 24 samples in December (37.5%). Layer liver samples were negative in November but showed 2 of 14 positive samples in December (14.3%). Egg albumen positivity increased from 6 of 15 samples in November (40.0%) to 18 of 25 samples in December (72.0%). Egg yolk positivity was 1 of 15 samples in November (6.7%) and 3 of 25 samples in December (12.0%).

Table 4. Monthly Distribution of STAF-Positive Samples

Sample Type	November Positive/Total (%)	December Positive/Total (%)
Broiler serum	0/16 (0.0)	0/24 (0.0)
Broiler breast muscle	0/16 (0.0)	0/24 (0.0)
Broiler liver	5/16 (31.3)	9/24 (37.5)
Layer serum	0/26 (0.0)	0/14 (0.0)
Layer breast muscle	0/26 (0.0)	0/14 (0.0)
Layer liver	0/26 (0.0)	2/14 (14.3)
Egg albumen	6/15 (40.0)	18/25 (72.0)
Egg yolk	1/15 (6.7)	3/25 (12.0)

Overall Screening Positivity Overall, antibiotic-residue activity was not detected in serum or breast muscle samples from either broilers or layers. Liver samples showed 16 positive results out of 80 tested samples, giving an overall liver positivity rate of 20.0%. Among egg components, albumen had the highest positivity rate, with 24 of 40 samples positive (60.0%), while egg yolk showed 4 of 40 positive samples (10.0%).

Table 5. Overall STAF Positivity by Sample Matrix

Sample Matrix	Positive, n (%)	Negative, n (%)	Total
Serum	0 (0.0)	80 (100.0)	80
Breast muscle	0 (0.0)	80 (100.0)	80
Liver	16 (20.0)	64 (80.0)	80
Egg albumen	24 (60.0)	16 (40.0)	40
Egg yolk	4 (10.0)	36 (90.0)	40

These findings indicate that STAF screening positivity was concentrated mainly in liver and egg samples, particularly egg albumen, while serum and breast muscle samples remained negative across both broiler and layer groups.

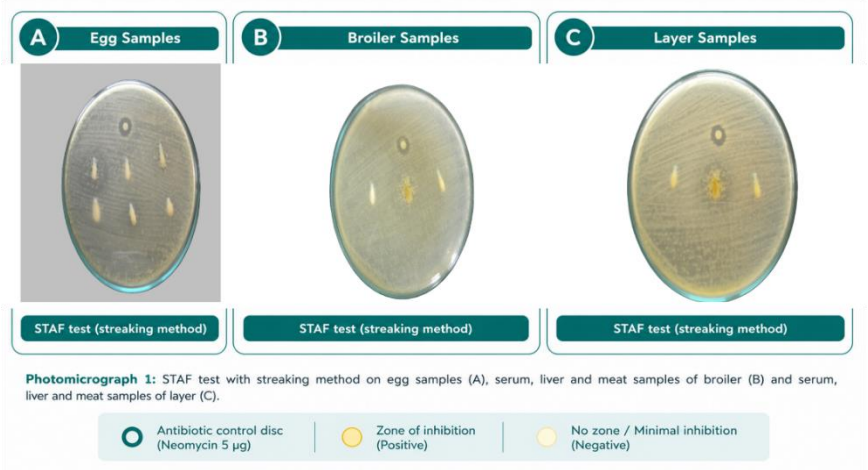

Figure 1. Swab Test on Animal Food (STAF) using the streaking method for detection of antibiotic-residue activity in poultry products.

Panel A shows egg samples, Panel B shows serum, liver, and meat samples from broiler birds, and Panel C shows serum, liver, and meat samples from layer birds. The neomycin 5 µg disc served as the antibiotic

control, while visible inhibition around sample swabs indicated screening-positive antibacterial activity against Bacillus subtilis.

DISCUSSION

This study screened commercially produced poultry products from periurban poultry farms in Faisalabad and found that antibiotic-residue activity was concentrated mainly in liver and egg samples, while serum and breast muscle samples were negative across both broiler and layer birds. The highest screening positivity was observed in egg albumen, followed by broiler liver, egg yolk, and layer liver. These findings suggest that edible poultry products other than muscle meat, particularly liver and eggs, may serve as important matrices for residue surveillance in commercial poultry systems. The absence of detectable activity in breast muscle does not exclude prior antimicrobial exposure, but it indicates that, under the conditions of the STAF assay, antibacterial activity was not detected in meat samples at the time of testing.

The higher positivity in liver samples is biologically plausible because the liver is a major site for drug metabolism and may retain antimicrobial compounds or metabolites depending on the drug class, dosage, duration of treatment, and withdrawal interval. In the present study, broiler liver showed substantially higher positivity than layer liver, which may reflect differences in production purpose, medication practices, flock turnover, or timing of antimicrobial exposure before slaughter. However, because treatment history and withdrawal-period records were not collected, these explanations should be interpreted cautiously. The detection of antibacterial activity in egg albumen and yolk is also relevant from a consumer safety perspective, as eggs are widely consumed and may enter the market soon after laying. The higher positivity in albumen than yolk may relate to differences in drug transfer, timing of egg formation, and physicochemical properties of administered antimicrobials, although the STAF method cannot identify the specific antimicrobial class responsible for inhibition.

The results are broadly consistent with previous reports showing antibiotic residues in poultry tissues and eggs from different settings. Earlier studies have reported detectable residues in liver, kidney, muscle, meat, milk, and eggs, with variation depending on sample type and analytical method used (10–16). Studies using chromatographic methods such as HPLC or LC-MS/MS provide drug-specific quantification, whereas microbiological screening assays such as STAF detect antibacterial activity without identifying the compound or concentration. Therefore, the present findings should be regarded as preliminary screening evidence that indicates possible residue contamination and justifies confirmatory testing rather than definitive evidence of residues exceeding maximum residue limits.

The monthly pattern showed higher screening positivity in December than November for broiler liver, layer liver, egg albumen, and egg yolk. This descriptive variation may reflect differences in farm-level antimicrobial use, disease pressure, production cycle, or sample distribution between months. However, the study was not designed to test seasonal trends, and the sample size was limited. Therefore, monthly differences should be interpreted as descriptive observations that may guide future surveillance rather than conclusive evidence of temporal variation.

The findings have important implications for poultry residue monitoring and antimicrobial stewardship. If poultry products are marketed before adequate drug depletion, consumers may be exposed to antimicrobial residues, which may contribute to hypersensitivity reactions, toxicity concerns, disruption of gut microbiota, and broader antimicrobial resistance risks (1,2,7,8). Routine screening of poultry matrices, especially liver and eggs, may help identify high-risk products and farms requiring further evaluation. However, screening programs should ideally be linked with confirmatory analytical methods, farmer education, veterinary prescription oversight, and enforcement of appropriate withdrawal intervals.

This study has several limitations. First, STAF is a broad microbiological screening method and cannot identify the antibiotic class, quantify residue concentration, or determine whether residues exceed regulatory maximum residue limits. Second, no confirmatory chromatographic testing was performed. Third, farm-level antimicrobial-use records, treatment timing, feed contamination history, and withdrawal-period compliance were not assessed, limiting causal interpretation. Fourth, the study was restricted to periurban Faisalabad and used a modest sample size, so findings may not be generalizable to all poultry production systems in Pakistan. Despite these limitations, the study provides useful local screening evidence and highlights the need for structured residue surveillance in commercially available poultry products.

CONCLUSION

Antibiotic-residue activity detected by STAF screening was present mainly in poultry liver and eggs collected from periurban commercial farms in Faisalabad, with egg albumen showing the highest positivity, followed by broiler liver, egg yolk, and layer liver, while serum and breast muscle samples remained negative. These findings indicate that poultry products beyond meat, particularly liver and eggs, require greater attention in residue-monitoring programs. Because STAF is a screening assay, confirmatory chromatographic testing is needed to identify specific antimicrobial compounds, quantify residue levels, and determine compliance with maximum residue limits. Strengthening routine surveillance, farmer education, veterinary oversight, and withdrawal-period implementation may reduce potential consumer exposure to antibiotic residues.

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