



## Detection of antibiotic residues in poultry products in Al Bayda, Libya.

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## Abstract

As a result of the absence of health control on poultry farms by the competent authorities and our knowledge of the excessive use of antibiotics without taking into account the drug withdrawal period, an HPLC device was used, which was conducted in the central laboratory of the Veterinary Teaching Hospital at Omar Al-Mukhtar University, to study and identify four types of antibiotic residues ( Tylosin, erythromycin, colistin, enrofloxacin). 120 birds were collected and marketed at sales points in the city of Al-Bayda and its suburbs, designated for selling poultry. After the slaughtering and processing process, all samples were examined. The results showed 70 (58%) positive samples. Of the 40 chest muscle, liver, and kidney samples tested, 17 (24%) were positive for the antibiotic tylosin, 11 (15%) were positive for the antibiotic erythromycin, and 7 (15%) were positive for the antibiotic Erythromycin. For the antibiotic erythromycin. 7 (10%) of the kidney samples were positive for erythromycin and 19 (27%) of the samples were positive for tylosin, while 14 (20%) of the chest muscles were positive for tylosin and 2 (2.8%) were positive for erythromycin. No colistin or enrofloxacin residues were detected. All samples were analyzed by HPLC. Among the four antibiotics detected, tylosin was the most abundant antibiotic in poultry samples (71.4%), followed by erythromycin (28.5%). The highest percentage of antibiotic residues was in the liver at 28 (40%), followed by the kidneys at 26 (37%), then the chest muscles at 16 (22.8%).

**Keywords:** poultry, antibiotic, chest muscles, HPLC,

## INTRODUCTION

In animal husbandry, veterinary medications are frequently used to treat and prevent illnesses in animals. Extensive usage of these medications can result in residues in animal products including meat, milk, and eggs, despite their apparent advantages(1).

Animal products tainted with veterinary medication residues have the potential to harm humans' immune systems. As germs start to develop resistance to the most widely used antibiotics, the most significant worry regarding the excessive use of veterinary herbs is how it may affect the efficacy of human medications(2). The usage of veterinary



medications is rigorously controlled to address this issue and safeguard human customers(3).

Similar to other meats, poultry flesh has a high proportion of proteins and lipids; it is a very complex matrix. The most difficult aspect of the multi-residue approach is sample preparation in terms of extraction and cleanliness since there are so many target compounds from many chemical classes with various chemical characteristics. To assess veterinary medication residues in meat, three primary sample preparation techniques were employed. As a clean-up technique, liquid extraction mixed with solid-phase extraction (SPE) was utilized to determine the antimicrobials in meat, tylosin in chicken flesh, and tetracycline in calves' tissues(4).

The European Union (EU) has established maximum levels for certain medication residues in meals derived from animals(5).

Any drug that has medicinal properties administered to an animal that produces food will unavoidably leave traces in the food. Legislation at the national and international levels aims to protect food consumers from exposure to residues in potentially dangerous quantities. To limit consumer exposure, several expert groups, notably the Joint Expert Committee on Food Additives (JECFA), the Codex Alimentarius Commission, and the European Union, have defined a series of maximum residue limits in edible tissues. to drug residues from authorized pharmaceuticals at levels safe for human health(6).

Drug withdrawal times must be followed before the animal is put to death. Food for humans shouldn't include any dangerous medication residues(7).

## Material and Methods



(120) chickens that were marketed at points of sale in the city of Al-Bayda Al-Kabda, which were ready for consumption, were purchased and used in this study. After performing the slaughtering process and preparing the samples (liver, kidneys, chest muscles), they were transferred to the central laboratory at the Veterinary Teaching Hospital and preserved at a temperature of 4%. All samples were analyzed using the HPLC method to discover and identify 4 types of antibiotics that were targeted in the study.

### Chemicals used:

The four antibiotic reagents (erythromycin, colistin, and enrofloxacin), methanol, (EDTA)(Sigma-Aldrich), picric acid, and acetonitrile (all chemicals used in the study were HPLC grade). 0.1 mg/mL standard stock solutions

Methanol was used to produce the standards (Tylosin, Erythromycin, Colistin, and Enrofloxacin), while acetonitrile was used to prepare the Tylosin standard. The approach suggested by (\$\$\$\$) was modified and used in the study in a lab setting. Before doing the test, muscle tissue has to be isolated from fat and skin tissue and finely chopped using an electric blender. Pieces weighing 3.4–5 g each were put into 50 ml polypropylene centrifuge tubes, and the mixture was then mixed with 10 ml of ultra-distilled water: methanol (70:30, v/v) and 200 µl of 0.1 M EDTA. The mixture was vortexed for 15 minutes after being combined for 30 seconds, then it was centrifuged for 5 minutes at  $2507 \times g$ .

A 0.45 µm filter membrane (Sartorius; Göttingen) was used to filter 500 µl of the extract diluted with 2 ml of distilled water. Lastly, 20µL of the filtrate—which included acetonitrile and 0.1% formic acid—was added to the HPLC apparatus. A calibration plot was created using study standards that were diluted at different concentrations of 5, 10, 20, 50, and 100 µg/kg from the standard stock solution. To create calibration graphs and determine the LOD (limit of detection) and LOQ (limit of quantification), various concentrations of these solutions were examined three times. By introducing standard solutions to samples with known antibiotic contents at three different concentrations, recovery rates were determined. Analysis of regression was done(8).

The standard calibration formula, which may be found in the table Tylosin, Erythromycin, Colistin, and Enrofloxacin, is applied to the NCFE statistics program findings. 1 together

with their price recovery, R<sup>2</sup> (R squares), LOD, and LOQ values. Every outcome was visible(9).



## Results

Using the high-performance liquid chromatography (HPLC) method, tests were conducted on four types of antibiotic residues and for each type of poultry sample (liver, kidney, chest muscle). 120 samples were examined, of which 70 (58%) sample results were positive. Details of the results Positivity, number, percentage of positives The type of antibiotic and the type of sample are given in Table 1. Of the 40 liver samples tested, 17 (24%) were positive for the antibiotic (tylosin) and 11 (15%) were positive for the antibiotic erythromycin. When kidney samples were tested, 19 (27%) were positive for tylosin 7 (%10) were positive for the antibiotic erythromycin, and 14 (20%) of the chest muscle samples were positive for the antibiotic tylosin and 2 (2.8%) were positive for the antibiotic erythromycin. No results were detected. Colistin or Enrofloxacin residues were detected by high-performance liquid chromatography (HPLC) in all samples tested. Tylosin was the predominant antibiotic detected (71.4%), among the four antibiotics studied. It was followed by erythromycin (28.5%). The liver had the highest percentage of residuals, at 28 (40%), followed by the kidneys, at 26 (37%), then the chest muscles, at 16 (22.8%).

**Table 1: Number (%) of positive samples for four antibiotics.**

Antibiotic	chest muscle (N=40)	kidney (N=40)	Liver (N=40)	Total samples (N=120)
Tylosin	(%20)14	(%27)19	(%24)17	(%41.6)50
Erythromycin	(%2.8)2	(%10)7	(%15)11	(%16.6)20
Enrofloxacin	0	0	0	0
Colistin	0	0	0	0
Total	(%40)16	(%65)26	(%70)28	(%58.3)70

## Discussion

It is known that the residues of antibiotics used during poultry farming remain on animal products (meat) and are considered a threat to human health, as they cause many health problems(10).

The high-performance liquid chromatography (HPLC) method used in our study was very expensive to measure the concentration of the four antibiotic residues targeted in the study but to detect their presence, the high-performance liquid chromatography (HPLC) method must be used because it is more accurate in giving results in most laboratories(11). However, our results showed that more than half of the samples screened were positive (58%). This result may be difficult to interpret, because we have no information about the exact time it was prepared and offered for sale, nor the length of time it was marketed, which may affect its presence in poultry carcasses. Anyway, these were the results

Similar to those obtained by(12), who found that more than 40% of poultry meat samples contained noticeable antibiotic . The percentage was higher (70%) than (13) reported from examining 46 broiler farms, with 60% and 96% positive for the antibiotic oxytetracycline in the liver and kidneys, respectively. A higher percentage (97%) was recorded in America during the period 1990-1994 until 1208 samples of poultry were examined (14) Tylosin sample The predominant antibiotic residue detected in our study, followed by erythromycin, then colistin and enrofloxacin were not detected. However, erythromycin and the antibiotic tylosin (15) were recovered by examining 6,800 samples of meat, including poultry.



The high rate of detection of erythromycin may be due largely to its use in Poultry sector to reduce deaths resulting from respiratory infections in poultry(16). Detection of erythromycin and tylosin may have been scheduled in our study as they are considered among the most important antibiotics that are absorbed from the intestine in large amounts(15), while colistin and enrofloxacin were not detected due to their poor absorption. The presence of erythromycin at a higher concentration may pose a health risk due to excess toxicity, which requires continued monitoring of residues. The antibiotic erythromycin has been widely used in treatment, but there is increasing concern about its causative agents causing tumors, allergies and disturbances in homeostasis. Longer withdrawal times and stricter monitoring of residues by responsible authorities must be adhered to(17).

Excessive use of antibiotics without consulting doctors, especially in the veterinary field, and the absence of oversight has led to what is known as microbial resistance to antibiotics. Breeders and farmers must be educated about the potential dangers of these residues, especially in foods of animal origin(18)

## Conclusions

In this research, the results indicate the presence of antibiotic residues in samples of poultry meat sold in stores in the city of Al-Bayda. The presence of antibiotic residues is due to the lack of knowledge of most breeders about the drug withdrawal period and adherence to health conditions. The high levels of antibiotic residues are cause for concern. Also, long-term exposure to doses of antibiotic residues in the body can lead to acute or chronic toxicity to organs and the entire body. Their presence may also cause allergic reactions or produce drug-tolerant bacteria in humans after prolonged exposure.

Hence, there is a need to respect antimicrobial withdrawal periods in order to minimize the level of antimicrobial residues in meat samples as well as strengthen controls through regular sampling and analysis. It is recommended to conduct further studies on the different types of antibiotics used in poultry, sheep and cow farms within the city of Al Bayda.



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