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Original article

Utilizing Microbiological Techniques: The Residual Microorganisms in Poultry Flesh Raised at Random Between 2024 and 2025

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Abstract
Veterinary biomass is an effective resource used throughout the rural areas of the study region. However, animal use is unclear and can lead to unhealthy debt management, thus contributing to animal consumption, and even to the environment. This study aimed to evaluate the presence of biomass in poultry meat using microbiological methods based on traditional methods recommended and approved by the French Food Safety Authority. For this purpose, 150 poultry meat samples were taken from different farms and directly examined using a standardized agar diffusion technique, cultured with two strains: Bacillus licheniformis and Staphylococcus aureus. After 110 original analytical results were generated from 150 poultry meat samples, the diagnostic result was 73% for antibiotic residues. However, most of them contained 36.36% oxytetracycline, 22.72% penicillin, and 9% sulfadimidine. In contrast, gentamicin yielded a positive rate of 31.8%. This suggests to physicians that these medications are being used indiscriminately and without specific treatment, without a prescription, or following the protocol and health requirements for the use of vital types of insurance, and adhering to the drug withdrawal period. This leads to the permanent presence of these meats, and farmers are also failing to adhere to the 45-day marketing period, all of which will harm consumers.
Keywords. Poultry Meat, Antibiotic Residues, Oxytetracycline, Penicillin, Sulfadimidine, Gentamicin.

Introduction

Poultry meat is considered the main source of protein in Libya, especially in the city of Al-Bayda. It has increased, especially since 1994, when the poultry industry began and became a very popular industry in this city [1]. Many inexperienced breeders use large amounts of medications, enzymes and hormones for treatment and growth stimulation, and they are used randomly without proper care for the withdrawal period and for the slaughter age [2].

Antibiotic residues have a very negative effect on human health because they are transmitted through foods such as meat, eggs, and milk [3]. Investigative tests must be conducted to determine the maximum levels of these residues in food, and strict penalties and measures must be implemented to prevent excessive use, especially in light of the absence of previous studies on this topic [4]. This study aimed to evaluate the presence of biomass in poultry meat using microbiological methods based on traditional methods recommended and approved by the French Food Safety Authority.

Methods

This study was conducted in Al-Bayda, Libya, which is considered one of the important regions in Libya known for poultry farming, whether on randomly raised farms or farms that follow health requirements. 150 samples of poultry meat prepared for slaughter were collected from the chest area of chickens and prepared for antibiotic evaluation using standard methods. All samples were placed in transport containers and sterilized. 120 grams of poultry meat targeted by the study were taken under sterile conditions. The samples were placed in sterile bags labeled with the sample code, farm name, and collection date. All samples were quickly transported on the same day to the veterinary laboratory at Omar Al-Mukhtar University. Several thin slices of 10 mm in diameter and 4 mm thick were taken from the breast, distributed in Petri dishes, and frozen until microbial testing was conducted.

Microbiological tests were performed according to the standard method of the French Food Safety Agency (AFSA), and the four-plate method was adopted to detect veterinary antibiotic residues in meat [5]. Positive samples were confirmed by antibiotic receptors. Thawed meat samples were placed on the surface of Conda pronadisa agar plates. These plates were inoculated with reference strains of Staphylococcus aureus. All these reference strains were obtained from the Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda, Libya. All meat slices were placed on the agar surface with the following dimensions (10 mm diameter x 4 mm thickness).

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Table 1. Detection of veterinary antibiotic residues based on microbial testing and pH

Agar	Microbe testing	PH	Incubator temperature	Types of antibiotics
1	Bacillus. licheniformis	7.2	30	Oxytetracycline
2	Bacillus. licheniformis	8.0	30	sulfadimidine
3	Bacillus. licheniformis	8.0	30	Penicillin
4	Staphylococcus. Aureus	6.0	37	Gentamicin

All Petri dishes were incubated at 30°C for Bacillus licheniformis (pH 6.0, 7.2, and 8.0) and 37°C for Staphylococcus aureus (pH 6.0). After 24 hours of incubation, the diameter of the inhibition zone was measured using a caliper. In parallel, standard solutions of penicillin and sulfadimidine were prepared for susceptibility and diffusion tests. The interpretation of the results aimed to find out which poultry meat samples were targeted for the study and which inhibited the growth of the tested microorganisms with an annular zone of at least 4 mm in diameter. Table 1 shows the different veterinary antibiotic classes that were evaluated and detected by inhibition of each microorganism.

Results and discussion

The results showed the presence of contamination in most of the poultry meat samples analyzed. About 110 of the 150 samples tested positive for veterinary antibiotic residues (73%). In contrast, no positive antibiotic residues were observed in 40 samples (26.26%).

Table 2. Number and percentage of veterinary antibiotic residues in chicken breast meat samples

Total number of meat samples targeted by the study	Examination results	
	Number of positive samples that appeared	Number of negative samples that appeared
150	110 (73%)	40 (26.26%)

However, most of the positive samples were found to contain a residue concentration of 36.36%. In contrast, penicillin and sulfadimidine were found to contain 22.72% and 9%, respectively. Gentamicin was found to be present in 31.8% of the positive samples for antibiotic residue testing.

Table 3. Percentage and number of veterinary antibiotic families present in the poultry meat samples targeted by the study.

Total number of meat samples targeted by the study	Examination results			
	Oxytetracycline	Sulfadimidine	Penicillin	Gentamicin
150	(36.36%)	(9%)	(22.72%)	(31.8%)

This method is considered one of the easiest and simplest methods, and the equipment required to implement this (four-box) method is relatively easy to obtain [6]. Unlike other tests used in the detection and evaluation of antibiotics, the four-plate method relies on a combination of pH conditions, which enhance or inhibit the activity of veterinary antibiotics. The pH of the medium always influences the activity of antimicrobial substances. Oxytetracycline and gentamicin are most effective at acidic pH, while penicillin is most effective at alkaline PH [7]. There is difficulty in interpreting the effect of pH on antimicrobial activity as it depends on the sensitivity and resistance of organisms to veterinary antibiotic molecules [8]. The type of culture medium used affects the inhibition zone, which affects the rate of diffusion of antimicrobial agents, as well as the growth of the microorganism on the Conda pronadisa agar used in this study Mueller Hinton agar can be used [9].

Contamination of these samples was found in 73% of cases. These results are consistent with an earlier study [1] that reported that more than 61% of poultry meat samples tested positive for antibiotic residues. However, recorded fewer positive cases (32%) [10]. Previous study [11] reported the same results regarding the estimation of oxytetracycline residues in poultry products in rural areas of the Arab Republic of Egypt, and another study [12] reported 62% and 60%, respectively. These percentages varied according to studies, and 21% and 10%, respectively, were recorded in Sudan by a previous study [13].

The high contamination of meat samples in general and poultry in particular is a result of the unjustified and repeated use of antibiotics for long periods in poultry farms that do not adhere to health requirements and without a treatment protocol, along with failure to observe the withdrawal period and early slaughter. This is due to the increased demand for poultry products [14] and according to several studies conducted to investigate the presence of antibiotic residues due to the lack of doctor consultation or doctor's

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prescription [15].

These results show that the application of biosecurity is necessary to reduce the presence of veterinary bio residues [16]. All results showed that the analyzed poultry meat samples from the chest area were contaminated with oxytetracycline (36.36%), followed by gentamicin at 31.8%, and then penicillin and sulfadimidine at 22.72% and 9%, respectively. These results are consistent with those reported by a previous study [17] and also conducted by another researcher [9], indicating that all farms focus on this type of veterinary antibiotic in their random poultry farms. Most of these farms use antibiotics excessively and frequently in their drinking water and feed. However, many private farms raise poultry, rabbits, and sheep, where oral administration of veterinary antibiotics is the preferred and most widely used option, followed by injection, which is less commonly used [18,19]. All previous studies have shown the use of excessively large quantities of antibiotics in respiratory and digestive diseases and eye infections. HPLC can be used to determine maximum residue limits for antibiotics [1].

Conclusion

These results, which are considered worrying to the health of consumers due to the presence of these quantities of antibiotic residues, are attributed to the excessive use of antibiotics randomly and without adhering to the preventive program and withdrawal period, as well as the health requirements for slaughter, as random breeding often leads to the breeder's lack of knowledge of the conditions for using these drugs, as well as the age of slaughtering poultry, and the absence of periodic monitoring by the competent authorities. Good practices, such as cleanliness and vaccination dates, are necessary.

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