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Detection of antimicrobial residues among chicken meat by simple, reliable, and highly specific techniques

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Abstract

This study aimed to investigate the incidence of antimicrobial residues among chicken meat samples including thigh muscle, breast muscle, and wings. A total of 390 samples were collected randomly from different retail outlets at Erbil Governorate from January 1st to June 30th, 2019. The residues were detected by using qualitative field disc assay and disc diffusion assay against *Bacillus subtilis* bacteria on agar plates. The incidence rate was (13.3%) and (10.8%) according to qualitative field disc assay and disc diffusion assay, respectively. The highest rates were found in breast and thigh samples by both assays. However, no significant differences were found between the meat cuts or the site of sample collection (urban or suburban). Regarding the monthly variations, the progress of winter-spring months was found to be associated with a decrease in antibiotic residue levels among chicken meat. Further evaluation of the one-year monitoring and antimicrobial stability period in chicken meat is recommended.

Keywords: Antibiotic resistance, incidence, Kurdistan region, Qualitative field disc assay, Disc diffusion assay

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INTRODUCTION

Chicken meat is a cheap, easily available, and nutritive source of important diet constituents, especially in low-income countries. It contains a significant amount of the essential polyunsaturated fatty acids (PUFAs), especially the omega (n)-3 fatty acids, B-complex vitamins, minerals, and essential fatty acids. Chicken meat is also one of the recommended constituents of Dietary Approaches to Stop Hypertension (DASH) diet (Donma and Donma, 2017; Esnaola-Gonzalez et al., 2020). However, as other food of animal origin, chicken meat is frequently reported to be contaminated with antibiotic residues.

Chemically, antimicrobials are heterogeneous groups of bioactive small organic molecules naturally produced by secondary metabolism of microorganisms like fungi and bacteria, at low concentrations. This group of drugs comprises antifungals, antibiotics, antiprotozoal that either kill pathogens or stop their growth (Savarino et al., 2020; Singh et al., 2019). The massive animal production in current decades has been aided by the use of veterinary medicinal products; especially, anti-infective drugs. Antimicrobials in food animals and poultry are used for three main purposes; therapeutic, prophylactic, and growth promotion. Prophylactic administration of antimicrobials is achieved by sub-therapeutic doses to healthy animals considered to be at risk but before the expected onset of disease. On the other hand, administration at very low doses of antimicrobials, usually as a feed additive, may be practiced to suppress gut bacteria leaving more nutrients for animals or birds to be absorbed (Jammoul and El Darra, 2019; Maharjan et al., 2020).

After the administration of antimicrobials, such drugs leave residues in the tissues of these animals and the foods derived from them like chicken meat, red meat, milk, table eggs (Al-mashhadany, 2019). The presence of antibiotic residues in foods of animal origin, combined with a failure to comply with the instructions for their use, particularly dose and withdrawal period, as well as poor livestock production practices, can have serious consequences for consumer health (Aidara-Kane et al., 2018; Stella et al., 2020). The presence of antibiotic residues in food of animal origin above the maximum residue limits (MRLs) is recognized globally by several public health authorities as being illegal (Beyene, 2016; Polveiro et al., 2020). Other factors are known to contribute to the problem of antibiotic residues in meat include the availability of antimicrobials to a nonprofessional, prevalent usage or unnecessary dosages of antibiotics, and lack or absence of implementation of limiting legislation to use antibiotics (Almashhadany, 2009; FSA, 2020).

Persistence of antibiotic residues is known to cause direct toxicity, allergic reactions, disturbance of the normal GIT microbiota, and bone marrow disorders. Various congenital anomalies may be seen in the newborn children due to long term exposure of antibiotic residues during the gestation period (Chen et al., 2019; Menkem et al., 2019). Carcinogenic impacts, the mutagenic effect are another dangerous impact of antibiotic residues. Additionally, the emergence of antibiotic-resistant bacteria is also linked to antibiotic residues (Bacanlı and Başaran, 2019; Stella et al., 2020). There are insufficient data at the Erbil governorate regarding the scale of antibiotic residues among chicken meat. Hence, this work was conducted to determine the degree of residues prevalence in chicken meat sold in the Erbil governorate using standard techniques. The association between months and occurrence of antibiotic residues was also investigated.

MATERIALS AND METHODS

Study design and sampling

The current study was conducted to detect antibiotic residues in chicken meat and evaluate its public health significance at the Erbil governorate. A total of 390 thigh muscle, breast muscle, and wing (130 for each) were randomly collected from different retail markets in Erbil city, (Kurdistan region, Iraq) during the period from January to June 2019. Approximately 50 to 100 gm of each sample was wrapped in polythene bags and put in cool boxes with dry ice or freezer packs at 4°C and transported to the Department of Medical Lab Science (DMLS), College of Science (CSCN), Knowledge University (KNU). Samples were stored at -20° C until analyzed.

Preparation of test bacteria

Spores suspension of *Bacillus subtilis* was prepared at desired concentration according to method previously described by Al-mashhadany et al., 2018. Briefly, heavy inoculums of *Bacillus subtilis* were introduced to the surface of nutrient agar plates (HiMedia, India) and incubated at 30°C for 10 days to induce sporulation. After the incubation period, growth was harvested into 10 ml of sterile normal saline and heated at 70°C for 10 minutes to kill the vegetative cells. After sterilization of the newly prepared nutrient agar medium, an inoculum of 0.1 ml of spore suspension (≈1.5×108 CFU/ml) was mixed with 100 ml of the medium before solidification. The molten agar was poured into Petri dishes and allowed to solidify at room temperature. Plates were used on the same day of preparation or held at the refrigerator and used within one week.

Detection of antibiotic residues

Qualitative field disc assay

Qualitative field disc assay as described by the AOAC was followed according to previously described (Mangsi et al., 2014). A disc-shaped meat sample of 2 mm thick and 8 mm in diameter was made by sterile cork borer and placed on an agar plate containing test bacteria (B. subtilis). The plates were incubated aerobically at 37ºC for 24 hours. After incubation, the presence of transparent zones of 1 cm or more around the disc-shaped sample was considered as a positive result indicating the presence of antibiotic residues. Absence of inhibition zone around samples considered negative.

Disc Diffusion Assay

Incisions were made in the meat samples using clean sterile forceps. A sterile paper disc was placed into the incision and left until it was saturated and then the disc was transferred and placed on the agar surface. The plates were then inverted and incubated at 37°C for 24 hours. The existence of antibiotic residues in the sample was indicated by the appearance of an inhibition zone with a diameter equal to or more than 2 mm. The absence of residues was indicated by the absence of the inhibition zone around the disc (Al-mashhadany et al., 2018; Elnasri et al., 2014).

Statistical analysis

Data were analyzed using the SPSS software version 25. Confidence intervals of prevalence were calculated using “exact” Clopper-Pearson method at alpha level of 0.05. Chi square test was applied to test the difference between groups.

RESULTS AND DISCUSSION

Occurrence of antibiotic residues

Out of 390 chicken meat samples, 52 samples (13.3%) were positive for the presence of antibiotic residues detected by qualitative field disc assay and 42 samples (10.8%) by disc diffusion assay (Table 1).

Table 1. Occurrence of antibiotic residues among chicken meat samples.

Type of meat	No. Examined	Positive samples n (%)	95% CI	*P* value
Qualitative field disc assay				
Thigh	130	17 (13.1)	7.81 – 20.11	0.243
Breast	130	22 (16,9)	10.92 – 24.49	
Wing	130	13 (10.0)	5.43 – 16.49	
Total	**390**	**52 (13.3)**	**10.12 – 17.11**	
Disc Diffusion Assay				
Thigh	130	15 (11.5)	6.60 – 18.32	0.134
Breast	130	18 (13.8)	8.42 – 21.00	
Wing	130	9 (6.9)	3.21 – 12.74	
Total	**390**	**42 (10.8)**	**7.87 – 14.28**	

Statistically, up to 17.11% of chicken meat sold in Erbil is expected to contain antibiotic residues. There is no significant difference between meat types in terms of antibiotic residue level. The qualitative field disc assay method detected high proportions of antibiotic residues among chicken meat samples (13.3%) than the disc diffusion assay method (10.8%), but this difference is not statistically significant (P = 0.284).

The current findings are consistent with the study results previously conducted in Erbil city (Al-mashhadany et al., 2018). The total occurrence of antibiotic residues in poultry meat samples was 11.1 % where 8.9% of thigh muscle and 6.7% of breast muscle harbored detectable levels of residues. Additionally, other similar studies from Nigeria (Ezenduka et al., 2014), and Egypt (Amroa et al., 2013; Morshdy et al., 2015) reported occurrence rates ranging from 10% to 15% in breast and thigh muscles. A worth mentioning point is that these studies reported liver samples to contain higher detectable levels than muscle samples obtained from the same chicken. Yet, the lower rate was reported from Bulgaria (Pavlov et al., 2008) where the incidence of antibiotic residues was as low as 4% in breast muscle. In contrast, higher prevalence ranges (22% to 29%) were reported from Sudan (Elnasri et al., 2014), India (Sahu and Saxena, 2014), Palestinian Gaza strip (Albayoumi, 2015), Turkey (Er et al., 2013), Nepal (Maharjan and Neupane, 2020), and China (Wang et al., 2017) in muscle tissue by different methods. Moreover, much higher rates were documented from numerous countries in different regions around the globe (Tajick and Shohreh, 2006; Chaiba et al., 2017; Islam et al., 2016; Lee et al., 2018). The rates ranged from 36% in Morocco (Chaiba et al., 2017) to 56% in Bangladesh (Islam et al., 2016). Such rates mostly result from irrational use of antibiotics and violations of withdrawal period schedules (Chen et al., 2019; Menkem et al., 2019).

Incidence of antibiotic residues according to sampling location

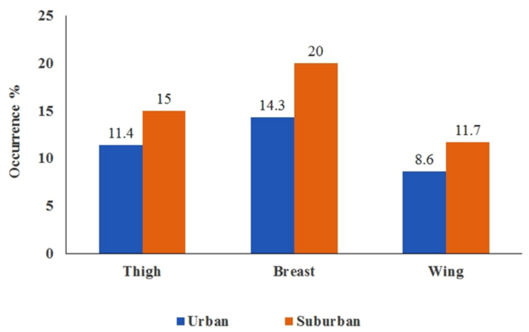

Figure 1. Incidences of antibiotic residues positive samples in urban and suburban areas according to qualitative field disc assay.

One-hundred thirty meat samples of each type were collected from chickens originated from urban and suburban areas. The total occurrence rates of antibiotic residues in samples from suburban areas were higher than urban areas according to qualitative field disc assay (11.4% vs 15.6%) (Figure 1).

Nonetheless, no significant difference was found between locations in terms of antibiotic residues prevalence (P = 0.224). Location-based variations in terms of antibiotic residue occurrence were reported from Nepal (Raut et al., 2017; Pantha et al., 2019), and Indonesia (Haruni et al., 2019) where antibiotic residues were detected in some locales and were absent in others. Such variations are mostly influenced by farmer's awareness and implementation of food safety regulations by local authority.

Monthly variations in occurrence rate of antibiotic residues

The change in the incidence rate of antibiotic residues was observed in a time scale manner. Based on both assays, the highest frequency of antibiotic residues was detected in February (19.7% and 16.7%), while the lowest rate was found in June (6.9% and 5.6%) (Figure 2).

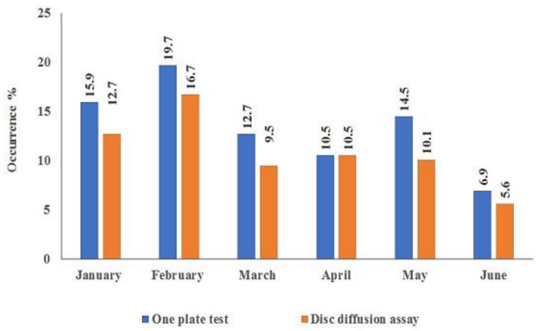

Figure 2. Monthly variations in occurrence rate of antibiotic residues in chicken meat samples.

There is a minor association (r^2 = 0.622) between the progress of winter-spring months and the decrease in the incidence of antibiotic residues in chicken meat.

These findings are in good agreement with previously reported patterns in which winter season progress was associated with a decrease in residue occurrence in meats (Aalipour et al., 2013; Pavlov et al., 2008). Indeed, the decrease of antibiotics residues frequency was associated with an increase in temperature and the concomitant decrease in rain levels and humidity in Erbil city. The wet conditions during winter are most likely associated with higher incidences of poultry diseases that encourage antimicrobial drug misuse (Movassagh, 2012). However, this observation contradicts the previously reported pattern in Nigeria (Omeiza and Nafarnda, 2015). The later study reported that a higher concentration of ARs in chicken eggs during dry windy and harsh weather.

There are several steps that can be taken to control and avoid antibiotic residues in chicken meat (Almashhadany, 2020; Vishnuraj et al., 2016). First, the highest priority of veterinary care should be given to food-producing animals. Reduction of antimicrobial usage in livestock production and enforcement of appropriate withdrawal periods of antimicrobial drugs by government authorities or regulatory bodies are also effective strategies. Additionally, the creation of mass awareness regarding risks of antibiotic residues in meat and meat products along with appropriate hygiene practices will all mitigate the contamination prevalence. Lastly, simple rapid, accurate, and economic methods should be developed to examine food before being introduced to markets.

CONCLUSION

The findings of this work indicated that the presence of antibiotic residues in chicken meat is moderately high. The occurrence rate of residues decreases as wet seasons (winter and spring) progress. Microbiological methods, particularly qualitative field disc assay and disc diffusion assay are quite suitable for the detection of antimicrobial residues especially as they are less expensive than immunochemical and chromatographic methods. Indeed, a large number of samples can be screened at a minimal cost. The withdrawal period should be observed carefully before the birds are slaughtered. Besides, the public should be educated on the appropriate cooking preparation of poultry meat. In addition, we must emphasize the proper use of antimicrobial agents in the treatment, prevention, and control of diseases as well as ensuring effective implementation of regulatory measures.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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