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Effect of cooking methods on some antibiotic residues in chicken meat

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Abstract

The great invasion of antibiotics in our food has become a crude problem due to the non-codified use of them in our farms for treatment of infections or as a food supplement to improve growth and animal output that inducing sever harmful health problems on human. This study aimed to evaluate the residues of three of the most commonly used antibiotics in poultry farms and effect of heat treatment on these residues. A total of 300 chicken meats, giblets and chicken meat products were examined using a microbiological method. The positive samples were subjected to high performance liquid chromatography (HPLC) for determination of oxytetracycline, gentamicin and tilmicosin residues. The antibiotics were validated according to the guidelines laid down by the joint FAO/WHO Expert Committee on Food Additives. The incidence of antibiotic residues by microbiological method were 22%, 32%, 54%, 24% and 6% in breast, thigh, liver, gizzard, and luncheon, respectively. The presence of oxytetracycline, gentamicin and tilmicosin residues above the maximum residue limits (MRLs) in 24, 5 and 14 samples, respectively. Although calculated estimated daily intakes (EDIs) for antibiotics presented showed lower exposure levels than the fixed values of acceptable daily intakes (ADIs), the presence of these residues even in lower ADIs on long run predisposes consumers to drug resistance and allergic reactions. Cooking methods reduce antibiotic residues in experimentally administered chicken meat with varying percentages from 35.17 to 74.27%.

Keywords: Chicken meat, Gentamicin, HPLC, Oxytetracycline, Tilmicosin

Introduction

Antibiotics are widely used in veterinary field as growth promoters in low doses in poultry feed for increasing growth rates, reduce mortality of growing chickens and increasing body weight gain with improving feed conversion, while for prophylaxis or therapy much higher dose levels were administrated. Approximately 80% of all food producing animals receive medication for a part or most their lives[17]. Antibiotics are still used in a large scale in veterinary field and poultry farms with a little amount of control[12] with ignorance of withdrawal periods in slaughtered poultry resulting in a residue deposition in meat tissues and other animal derived products inducing toxicological, microbiological or immunological effects[25]. Tetracyclines, over the years, have remained one of the most commonly used groups of antibiotics in poultry production as feed additives. Various human health conditions have been associated with consumption of food contaminated with tetracycline residues especially at sub-chronic levels which include gastrointestinal disturbances, a teratogenic risk to the fetus, allergic reactions, bone and teething problems due to binding with calcium ions and the emergence of resistance bacteria strains in humans and animals[19]. The illicit use of antibiotics could increase the risk of food-borne infection with antibiotic resistant pathogenic bacteria and other harmful effects related to antibiotic residues in food which including immunopathological effects, autoimmunity, carcinogenicity (oxytetracycline), mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity and allergy (penicillin)[11].Toxic effects are not probable because residue contents in food are in very low concentrations. Allergic reactions may also be produced in sensitive or sensitized individuals. Due to the importance of chicken meat and its products and the fact that many chicken products in the market are not treated with any analytical procedure for antibiotic residues. Therefore, the present study was undertaken to investigate the prevalence of antibiotic residues in random marketed chicken meat and their products with the effect of different cooking procedures on antibiotic residues in experimentally administered chickens.

Material and Methods

Three hundred chicken meats, giblets and chicken meat products were collected from markets in Sharkia Governorate, Egypt. The samples were represented by chicken breast, thigh, liver, gizzard, luncheon and frankfurter fifty samples of each.

Detection of antibiotic residues:

Microbiological inhibition assay technique using Bacillus subtilis (ATCC-6633) as an indicator organism. The level of antibiotics can be evaluated by measuring the diameter of inhibition zone observed on an agar layer seeded with a test organism (with a caliper) according to Levetzow and Weise[16]. The indicator organism was obtained from Department of Bacteriology, Animal Health Research Institute in Doki, Giza, Egypt.

Quantitative evaluation:

HPLC analysis was used for determination of antibiotic residue levels in positive samples resulted from the microbiological inhibition assay. Oxytetracycline residues were detected according to Heitzman[10].

Gentamicin residues were detected according to USDA[29].Tilmicosin residues were analyzed according to Patel *et al.* [21].

Estimated daily intake (EDI) was calculated based on the integration of data from analysis of antibiotics, chicken consumption rates, and body weight of Egyptian adults. EDI (μg/kg/day) for oxytetracycline, gentamicin and tilmicosin was obtained using the following equation EDI=(Cm xFIR)/BW described by the Human Health Evaluation Manual, where Cm is the concentration of the antibiotic in the sample (mg/kg wet weight); FIR is the food (chicken meat) ingestion rate in Egypt, which was estimated at 27.4 g/day[7]; BW is the body weight of Egyptian adults, which was estimated at 70 kg.

Experimental work:

A total of 60 one day old chicks were fed on free antibiotic diets till reached 1.8 ± 0.2 kg body weight then they were classified into three groups 20 bird /group. The first group was received oxytetracycline at a dose of 10 mg/kg b.wt for 5 consecutive days in drinking water, the second group was received a daily dose of 4mg/kg b.wt for 5 consecutive days subcutaneously of gentamicin sulfate and the third group provided with tilmicosin at a dose of 30 mg/kg b.wt daily for 5 consecutive days in drinking water. Chicken were slaughtered following the complete antibiotic dosing regardless to withdrawal period.

Effect of heat treatments:

Forty five quarters of chickens represented by 15 from each antibiotic treated groups were subjected to three different heat treatments (5 quarters/treatment). The first treatment included immersion of quarters into a water bath preheated to 100 °C and boiled for 30 minutes then removed and allowed to cool[14]. The second one involved fried of quarters in a pan contain suitable amount of cotton seed oil at 180 – 200 °C for 10 minutes[17] and Third one through placing samples into microwave at power (900 W) for the specified time (15 min), removed and allowed to cool.[14].

Statistical analysis: Data of the current study was statistically analyzed using SPSS/PC[28] .The statistical method was one way ANOVA test to determine the significance .The values were significant at P≤ 0.05.

Results and Discussion

The antibiotic residues remain in chicken and their edible offals has attracted extensive worldwide attention from national and international public health agencies. Human as a non-target organism of these drugs receives different amount of them [20].

Incidence of antibiotic residues in chicken meat and products:

Antibiotic residues in poultry meat depend on various factors such as drug dosage, type and age of birds, feeding, disease status, poor management, extra-label drug use, withdrawal time, and route of administration[3].

The results of microbiological inhibition assay were recorded in (Fig.1A) which revealed that the highest mean of inhibition zone (I.Z) was recorded in liver, thigh, breast, gizzard and finally luncheon. Liver samples I.Z was significant (P<0.05) all over the examined samples and this may be attributed to the fact that liver is responsible for metabolism and detoxification of antibiotics by its microsomal enzymes. On the contrary, the smallest mean of I.Z detected in luncheon samples seemed to be due to the effect of heat treatment during processing. The positive samples were subjected to HPLC analysis for detection and quantification of antibiotic residues.

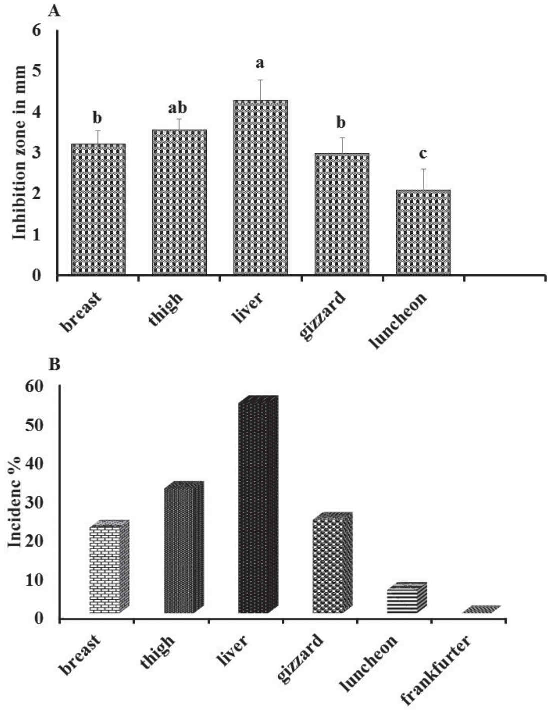

Fig.1 A. Inhibition zone in mm by microbiological inhibition assay technique. B. Incidence of antibiotic residues in examined samples.

The data presented in (Fig.1B) revealed that the antibiotic residues detected in breast, thigh, liver, gizzard and luncheon were 22%, 32%, 54%, 24% and 6% ,respectively. Pervious works illustrate the incidence of antibiotic residues in various chicken organs and meat [12, 18, 19, 25, 27]. Oxytetracycline detected within various percentages and ranged from 6 to 18 % in chicken gizzard and liver with a mean values of 0.67 ± 0.11 and 1.65 ± 0.16 ppm, respectively (Table 1). Maximum residue limits (MRLs) for oxytetracycline were recommended by many agencies where the joint FAO/WHO Expert Committee on Food Additives[8] recommended that the residual level is 0.2 ppm and 0.6 ppm for chicken muscle and liver, respectively. In addition, the EU had set the MRLs for oxytetracycline to be 0.1 ppm and 0.3ppm for chicken muscle and liver, respectively[5].

All means values of oxytetracycline exceed the regulation limits. Oxytetracycline levels above the MRLs had been reported in chicken products in many countries such as Mexico[30], Belgium[4], Bulgaria[22] and Egypt[23].Such residues in the chicken meat may pose a potentially serious health threaten the life of consumer. Gentamicin residues were detected only in chicken thigh and liver with percentages of 2% and 8%, while values were 0.67 and 0.94 ± 0.17 ppm, respectively. The mean values of gentamycin residues exceed the maximum residue limit established [5] as 0.1 and 0.3 ppm in muscle and liver, respectively. The main toxic effects of the gentamycin are nephrotoxicity, hepatotoxicity and ototoxicity. Tilmicosin residues detected between 2% to 12% in chicken luncheon and chicken liver with a concentrations of 0.18 and 1.21 ± 0.29 ppm, respectively. The tilmicosin residues were above the regulation of tilmicosin in chicken muscle 0.15 ppm and lower than established limit 2.4 ppm for liver[3].The incidence of oxytetracycline, gentamicin and tilmicosin was 8%, 1.66 % and 4.6 %, respectively and other antibiotics was 9% (Table 1). This indicates the abuse of antibiotics in chicken farms in addition to wide variety of antibiotics used without attention to the withdrawal time. Calculation of EDI was recorded in (Table 2) also, showed that contribution of chicken and chicken products to dietary intakes were lower than acceptable daily intakes (ADIs) proposed by FAO/WHO[6]

Table 1. Incidence and concentration of different antibiotic residues (ppm) in examined samples

Antibiotics Samples	Oxytetracycline	Gentamicin	Tilmicosin	Other antibiotics
	Incidence Mean ± SD	Incidence Mean ± SD	Incidence Mean ± SD	Incidence
Chicken breast	(5) 10 % 0.94± 0.14	-	(2) 4 % 0.73± 0.56	(4) 8 %
Chicken thigh	(7) 14 % 0.85± 0.12	(1) 2 % 0.67	(3) 6 % 0.83± 0.42	(5) 10 %
Chicken liver	(9) 18 % 1.65± 0.16	(4) 8 % 0.94±0.17	(6) 12 % 1.21± 0.29	(8) 16 %
Chicken gizzard	(3) 6 % 0.67± 0.11	-	(2) 4 % 0.63± 0.44	(7) 14 %
Chicken luncheon	-	-	(1) 2 % 0.18	(2) 4 %
Total	(24) 8%	(5) 1.66%	(14) 4.6 %	(27) 9%

Table 2. Estimated daily intake (μg.kg-1body weight) from consumption of chicken products.

Antibiotic	Oxytetracycline	Gentamicin	Tilmicosin
ADI	30μg.kg-1	20 μg.kg-1	40 μg.kg-1
breast	0.36	-	0.29
thigh	0.33	0.26	0.32
liver	0.65	0.37	0.47
gizzard	0.26	-	0.25
luncheon	-	-	0.07

Effect of cooking methods on antibiotic residues: Most animal-originated food is cooked or underwent various processing such as food additive to increase digestibility, sensory properties, appetizing attribute and shelf-life[4]. In recent years, HPLC was applied to assess changes of veterinary drug residue during processing[9]. In this study, cooking by boiling induce a significant reduction on oxytetracycline, gentamicin and tilmicosin residues with a reduction percentages of 35.17, 35.9 and 36.5%, respectively. Nearly similar reduction percentages obtained In Turkey[24] and Iran11). Comparing the uncooked and the fried chicken meat, a significant reduction was detected in all antibiotics with a percentages of 48.55, 49.6 and 46.4% for oxytetracycline, gentamicin and tilmicosin, respectively. Also, it was shown that during the frying process, the moisture in the chicken meat leached out and replaced by the frying oil. The exchange of moisture and oil could remove antibiotics out from the chicken meat[13].

Table 3. Effect of cooking methods on antibiotic residues in experimentally administered chicken meat.

Cooking method	Oxytetracycline	Gentamicin	Tilmicosin
	Min - Max Mean ± SD	Min - Max Mean ± SD	Min - Max Mean ± SD
Raw	2.48 – 4.23 3.81± 0.24a	2.14 – 3.26 2.56± 0.29a	1.36 – 1.69 1.45 ± 0.06a
Boiled	1.97 – 3.98 2.47± 0.17b	1.52 – 2.13 1.64± 0.23b	0.88 – 1.09 0.92 ± 0.04b
Reduction %	35.17 %	35.9 %	36.5 %
Fried	1.63 – 3.65 1.96 ± 0.19b	1.45 – 2.09 1.29±0.06 b	0.71 – 0.94 0.78 ± 0.09b
Reduction %	48.55%	49.6%	46.4%
Microwaved	0.96 – 1.23 0.98 ± 0.18c	0.76 – 1.34 1.12± 0.09b	0.82 – 0.98 0.86 ± 0.0b
Reduction %	74.27%	56.2%	41%

a,b,cMeans within the same column and bearing different superscripts are significantly different at ($P < 0.05$).

$$R\% = \left(\frac{\text{Concentration in raw-concentration in coocked}}{\text{Concentration in raw}}\right) x100.$$

These results were in parallel to Sharafati-Chaleshtori et al [26] who recorded a significant reduction in oxytetracycline residues in muscle samples of rainbow trout after frying. The microwave cooking method induced a significant reduction with varying percentages 41, 56.2 and 74.27% in tilmicosin, gentamicin and oxytetracycline, respectively (Table 3). The difference in our results may relay to the method of tests, different apparatus or the temperature of cooking method and this confirm the difficulty of comparing antibiotic residual levels in various animal meats after cooking[1].

Conclusion

Sufficient heating temperature and time can reduce nearly fifty percent of some antibiotics residues but it does not generally provide an additional margin of safety for consumers so, veterinary officers should ensure the judicious use of antibiotics in combating bacterial infection.

Furthermore, the observance of the pre-slaughter withdrawal periods after antibiotic usage should be emphasized.

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