Studies on Antibiotic Residues in Imported and Locally Frozen Chicken Meat
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Abstract
A total of 120 random frozen chicken samples as 70 locally and 50 imported frozen chicken meat samples were collected from chicken shops and supermarkets at Dakhalia Governorate, Egypt. All samples were examined for presence of antibiotic residues using Microbiological Inhibition Assay Technique. The results revealed that the incidence of antibiotic residues could only be detected in local frozen chicken meat as follow 18(15%), 15(12.5%) and 18(15%) in breast muscles, thigh muscles and liver, respectively. Oxytetracycline in positive samples was detected and quantified using HPLC. The results revealed that 10 out of 18(55.55%) muscle samples contains oxytetracycline residues which ranged from 0.240444 to 1.13906 µg/gm with mean 0.5311 µg/gm. And also about 10 out of 18(55.55%) liver samples contains oxytetracycline residues which ranged from 0.573166 to 3.91728 µg/gm with mean 1.3444 µg/gm and found that 19 out of 20 samples contain oxytetracycline residues above the maximum residue limits (MRLs) while one sample had level below (MRLs) according to the FAO (1999) the maximum residue limits (MRLs) for oxytetracycline residues as follow: 0.2 and 0.6 µg/g for muscles, liver, respectively. then make heat treatment to positive samples as boiling at 100°C for 30 minutes, roasting at 200°C for 15 minutes and frying in cotton seed oil at 200°C for 10 minutes to positive samples. So, No residues could be detected after heat treatment as heat treatment has destructive effect on antibiotic residues.

Introduction
Antibiotics are substances produced by living organisms that are able to kill or inhibit the growth of microorganisms but the definition of antibiotics has also been used to include chemically derived synthetic antibacterial drugs.
Antibiotics are double edge weapon. They are widely used in veterinary field as growth promoters, prophylaxis and therapy. Antibiotic residues in meat derived from treated animal could pose health threats to consumers (El-Kahky and Allam 2005)
Residues of antibiotics are pharmacological active substances either active principle or their metabolites which remain in food stuffs above Maximum Residue Level. WHO and FAO establish tolerances (Maximum Residue Level) for drugs in the relevant tissues of food producing animals. The tolerance is the tissue concentration below which a marker residue for the drug or chemical must fall in the target tissue before that animal edible tissues are considered safe for human consumption (Nisha, 2008).
added food. The withdrawal time is the time required for the residue of toxicological concern to reach safe concentration as defined by tolerance. It is the interval from the time an animal is removed from medication until permitted time of slaughter.

Meat is not consumed raw but required preparation such as boiling, frying and other ways of cooking before consumption. These handlings may lead to decrease the amount of drug residues that might present in these edible tissues, and also freezing lead to decrease amount of residues in chicken meat. The heat stability of antibiotics is evaluated either by quantitative reduction in drug concentrations or by decreasing antimicrobial activity against susceptible microbes after heat treatments. Thermal treatments may reduce the concentration of veterinary drug residues in foods and thereby might decrease possible pharmacological and/or toxic effects of these compounds. Higher percentage reductions were apparent at higher temperatures showing up to 99% reduction at 121 °C in contrast to 54.4% at 100 °C for TC and most other antibiotics indicating that the degree of reduction was associated with heating temperature. (Hsieh et al, 2011)

The methods used to detect antibiotic residues in food of animal origin are microbiological, immunes-enzymatic and chemical. A screening method is the first-hand analysis of the sample to establish the presence or absence of residues and microbiological methods are suitable for large scale screening because of their convenience and broad spectrum characteristics (Aerts et al., 1995).

High performance liquid chromatography procedures are widely used to quantify various antibiotic residues in food products with good sensitivity and specificity (Muriuki et al. 2001).

Potential adverse effects from antibiotic residues in meat could include acute toxicity, carcinogenicity, reproductive effects, and allergic reactions. The presence of antibiotic residues represents serious problems for human beings consuming such tissues as hypersensitivity or even toxicity and development of bacterial resistant strains. (Hassan et al. (2009))

MATERIAL AND METHODS

Sampling:
Seventy locally and 50 imported frozen chicken meats represented as breast muscles, thigh muscles and liver were randomly collected from chicken shops and supermarkets at Dakahlyia Governorate. Each sample weighted 50-100 g was analyzed for determination of antibiotic residues.

Detection of antibiotic residues in chicken meat samples:
1) Microbial inhibition assay technique:
The technique of Levetzow and Weise (1979) is adopted for the detection of antibiotic drugs in meat samples.

Interpretation of the results:
The result would be indicated by measuring the diameter of inhibition zones of the growth of the Bacillus subtilis around meat samples. The zone which is more than or equal to 2 mm would be recorded as positive result. The zone which is from 1 to 2 mm would be recorded as suspicious result. The zone which is less than 1 mm would be considered as negative result.

2) High performance liquid chromatography (HPLC) method) (Heitzman, 1994):
The meat samples determined as positive by microbiological assay would be further analyzed by HPLC for identification and quantification of antibiotic residues. (Senyuva et al. 2000).

a) Calibration curve:
It was prepared by using concentration of 0.01, 0.1, 0.5, 1.25, 2.5, 5, 10, 20 and 50 mg/L of OTC in eluent. These standards were prepared from the daily prepared stock solution and treated as 100 mg of oxytetracycline standard was accurately weighed and put in a 100 ml volumetric flask, the powder was dissolved in 100 ml of methanol to make a stock solution of 1000 ppm. Several serial dilution of stock solution was carried out. The detection limit for oxytetracycline was 0.01 ppm while the retention time was 3.7 min.

3) Different cooking procedure for positive samples:
Boiling:
A five gram samples (for breast muscles, thigh muscles and liver) which showed positive result in the Microbial inhibition assay technique were placed into a pan filled with cotton seed oil at 200°C and fried for 10 minutes. (Lolo et al., 2006)

Results and Discussion:

It is documented that the extensive use of antibiotic in poultry farms for increasing growth rates, prophylaxis and therapy gives rise to problem of drug residues. Drug residues may be attributed to lack of adherence to withdrawal time, improper use of drug, extra label use and failure to observe withdrawal time for each antibiotic. The achieved result in table (1) showed that examined chicken carcasses were 120 samples, classified according to the type of frozen chicken samples present in market into 70 locally frozen chicken samples and 50 imported frozen chicken ones. Antibiotic residues were concentrated in local frozen chicken samples but no residues found in imported frozen chicken samples. This may be attributed to lack of adherence to withdrawal time, improper use of drug and extralable use.

Davidson and Branan (1993) and Basyoni and Brr (2009). Antibiotic residues present in frozen chicken samples were 18(15%), 15(12.5%) and 18(15%) in breast muscles, thigh muscles and liver, respectively. Nearly result obtained by Sultan (1995) who record that antibiotic residues were 18%, 12% and 24% for breast muscles, thigh muscles and liver, respectively from 50 poultry carcasses.

Higher result were obtained by Shareef et al. (2009) they found that 14(56%), 11(44%) and 14(56%) which contained antibiotic residues in breast muscles, thigh muscles and liver, respectively. While Shahid et al. (2007) recorded 13(39.4%) in liver samples and 7(20.4%) in muscle samples were positive for presence of residues. And also, Mahmoud and Mohsen (2008) as 50%, 52.5% and 67.5% in breast muscles, thigh muscles and liver, respectively. Lower result reported by Salehzadah et al. (2007) they found that 8% in muscles and 13.3% in liver contain antibiotic residues in chicken samples. While Khodary and Shorbagy (2002) showed that the presence of antibacterial residues in breast muscles, thigh muscles and liver were 8%, 6% and 20%, respectively.

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administrations increased the potential effect for antibiotic residues at the injection sites. These results were in disagreement with Abd El-Hamid and Amin (2004) who recorded the highest mean values of inhibition zones of antibiotic residues was in kidney followed by liver then in thigh muscles as 5.04, 3.84 and 2.66, respectively. And also, Mahmoud and Mohsen (2008) found that the highest mean of inhibition zone of residues were detected in liver followed by breast muscles then thigh muscles as 3.84, 2.8 and 2.66, respectively. The composition and properties of the medium used in microbiological inhibition test influence the detection limits of antibiotics as the PH of media and tissue components. Okerman et al. (1998). Data presented in table (3) showed the Boiling of positive frozen chicken meat samples (breast muscles, thigh muscles and liver) at 100°C for 30 minutes, result declared that no antibiotic residues were detected after boiling.

This result was in an agreement with Gehan (1991) and Haagsma (1993) who didn't detect the antibiotic residues in tissues (muscles) and organs after boiling at cooking temperature. This result was in disagreement with those obtained by Moats (1999) who mentioned that ordinary cooking procedures for meat, even to "well done", cannot be relied on to inactivate even the more sensitive compounds such as penicillin and tetracycline but, more sever heating as for canning or prolonged cooking with moist heat can in activate the more heat sensitive compounds. There was a significant decrease of residues after boiling. Javadi et al., (2011), daoud (2004), Abd El-Hamid and Amin (2004) Hassan et al., (2009) and Abo El-Enean (2008). Rose et al., (1996) discussed that the heat stability of oxytetracycline (OTC in water and vegetable oil), Result showed that the drug was unstable in water at 100°C with a half- life of about 2 min, but more stable in oil at 180°C where the half- life was about 8 min. Frying of positive samples (breast muscles, thigh muscles and liver) at 200°C for 10 minutes in cotton seed oils, the result declared that no antibiotic residues were detected after frying.

This result was in an agreement with El-Kahky and Allam (2005), Daoud and El-Mossalami (2002) and Hassan et al. (2009) who revealed that frying had destructive effect on antibiotic residues.

Boiling of meat contained antibiotic at 100°C for 30 minutes could reduce the amount of residues upto 42.85%, while in frying process was 85.71%. They concluded that the effect of frying oil direct heat was more effective than boiling water, and these results with agreement with the obtained results. Marouf and Bazalou (2005).

Roasting of positive samples (breast muscles, thigh muscles and liver) at 200°C for 15 minutes, the result declared that no antibiotic residues were detected after roasting. This result was in an agreement with Hassan et al. (2009) and Javadi et al., (2011) revealed that significant decrease of residues after roasting. Gergis-Aida (1998) who studied the effect of the heat treatment as boiling, frying and roasting and showed that heat treatment decreased the concentration or leads to complete inactivation according to degree of temperature and time of exposure.

Table (4) indicated that the positive samples had analyzed by high performance liquid chromatography (HPLC) technique for estimation of oxytetracycline residues. High performance liquid chromatography (HPLC) procedures are widely used to quantify various antibiotic residues in food products with a good sensitivity and specificity. One of the major advantages over microbiological method is that the lower detection limit of 0.01μg/ml makes it a highly precise instrument. (Muriuki et al. (2001) and Senyuva et al. (2000)).

So, it was found that twenty samples had oxytetracycline residues as 10 in liver samples and 10 in muscles samples. From the twenty samples which contains oxytetracycline residues, there were 19 samples had exceeded the maximum residue limits (MRLs) than those tolerated by FAO (1999) for oxytetracycline residues which were
recorded as follow: 0.2, 0.6 and 1.2 μg/g for muscles, liver and kidney respectively. 10 Out of 18 (55.55%) samples of muscles had detectable oxytetracycline residues which their concentration ranged from 0.240444-1.3444 μg/g with a mean of 0.5311μg/g. While liver samples showed 10 Out of 18 (55.55%) samples had detectable oxytetracycline residues which their concentration ranged from 0.573166-3.917284 μg/g with a mean of 1.3444μg/g. The mean level of oxytetracycline residues was higher in liver samples than muscles samples; this might due to that the liver is the organ which is responsible for metabolism and detoxication of drug by its microsomal enzymes Mohmoud and Mohsen (2008), Abd-ElHamided and Amin (2004), Hassan (1998), Cheong et al. (2010) and Daoud (2004).

Similarly similar result obtained by Shahid et al. (2007) who recorded 57.1% in muscles samples and 23.1% in liver samples contained oxytetracycline residues, while Abd El-Monem et al., (2002) declared that muscles and liver contain 40% and 60% respectively oxytetracycline residues. Higher result obtained by Iqbal (2000) who showed that the incidence of oxytetracycline was 100% in samples. And also, Van Wambeke (1999) recorded oxytetracycline present in muscles as 76%.

Salehzadeh et al., (2006) record that 95.55% oxytetracycline residues were above MRL in the examined samples, while muscles and liver contain 27.77% and 95.85%, respectively contained oxytetracycline residues. Lower results detected by Shareef et al., (2009) as muscles and liver contained oxytetracycline residues as 28% and 16% respectively. And Basyoni and Brr (2009) declared that 10% from muscles samples and 30% from liver samples.

References:


الحيوية. ووجدت هذه البقايا في عيّنات الدجاج المحلي المجمد بنسبة 15% و12.5% و15% في عيّنات عضلات الصدر.

وعضل شباء الكبد على التوالي. وقد تم التعرف على نوع المضادات الحيوية المعزول باستخدام جهاز الفصل الكروماتوغرافي لتحليل العيّنات التي تحتوي على بقايا المضادات الحيوية. ووجدنا 10 عيّنات من العضلات بنسبة 55.55% بمتوسط 1.25 ميكروجرام/جرام. و كذلك 10 عيّنات من الباكيك بنسبة 55.55% بمتوسط 3.44 ميكروجرام/جرام تحتوي على بقايا عقار الأوكسي تيتراسيكلين. وقد وجد أن 19 عيّنة من 20 عيّنة تحتوي على بقايا عقار الأوكسي تيتراسيكلين بنسبة أعلى من الحد الأعلى المسموح به لبقايا الأوكسي تيتراسيكلين من قبل منظمة الصحة العالمية للغذاء لعام (1999) والتي حددتها كالآتي: بالنسبة للعضالات 0.2 ميكروجرام/جرام. بالنسبة للاكباك 0.6 ميكروجرام/جرام.

وعند معالجة العيّنات بالخلايا التي تحتوي على بقايا المضادات الحيوية لمدة تصف ساعة عند درجة حرارة 100 درجة مئوية وكذلك عند استخدام لقلي باستخدام زيت شجرة التقطين عند درجة حرارة 200 درجة مئوية لمدة عشر دقائق وأيضا عند وضع العيّنات في الفرن عند درجة حرارة 200 درجة مئوية لمدة خمس عشرة دقيقة. وجد اختفاء بقايا المضادات الحيوية من العيّنات. لذلك فإن المعاملات الحرارية لها تأثير مباشر على بقايا المضادات الحيوية. وتم مناقشة الأهمية الصحية لوجود بقايا المضادات الحيوية على صحة الإنسان.

20

10 Figure (1): Standard curve of oxytetracycline.

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Area</th>
<th>Area</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6158727</td>
<td>1250263</td>
<td>637437</td>
</tr>
<tr>
<td>2.5</td>
<td>274372</td>
<td>148574</td>
<td>64408</td>
</tr>
<tr>
<td>1.25</td>
<td>18294</td>
<td>1000</td>
<td>0.1</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01</td>
<td>0</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Table (1): detection of antibiotic residues in the examined frozen chicken meat by Microbial inhibition assay technique four plate test (N=120 for each).

<table>
<thead>
<tr>
<th>Frozen chicken samples</th>
<th>No.</th>
<th>Positive samples</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Breast</td>
<td></td>
<td></td>
<td>Thigh</td>
<td></td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>70</td>
<td>18</td>
<td>15</td>
<td>25.71</td>
<td>18</td>
<td>25.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>imported</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>18</td>
<td>15</td>
<td>15%</td>
<td>15</td>
<td>12.5%</td>
<td>18</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table (2) Inhibition zones in mm in examined samples:

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>Mean</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Muscles</td>
<td>18</td>
<td>7.0833</td>
<td>±0.8709</td>
</tr>
<tr>
<td>Thigh Muscles</td>
<td>15</td>
<td>7.4333</td>
<td>±0.7759</td>
</tr>
<tr>
<td>Liver</td>
<td>18</td>
<td>5.2777</td>
<td>±0.4904</td>
</tr>
</tbody>
</table>

N= Number  SE= Standard Error

Table (3) Effect of cooking procedures on antibiotic residues by Microbial inhibition assay technique:

<table>
<thead>
<tr>
<th>Samples</th>
<th>Boiling</th>
<th>Frying</th>
<th>Roasting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Breast muscles</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Thigh muscles</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Liver</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

Table (4) oxytetracycline concentration in chicken meat samples

<table>
<thead>
<tr>
<th>sample</th>
<th>Positive samples/ total number</th>
<th>% of OTC positive samples</th>
<th>Concentration of OTC(µg/gm)</th>
<th>MRL (µg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Muscles</td>
<td>10/18</td>
<td>55.55%</td>
<td>0.240444-1.13906</td>
<td>0.5311</td>
</tr>
<tr>
<td>Liver</td>
<td>10/18</td>
<td>55.55%</td>
<td>0.573166-3.917284</td>
<td>1.3444</td>
</tr>
<tr>
<td>Sig.</td>
<td>0.0195 (P&gt; 0.05)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OTC= oxytetracycline  MRL=Maximum Residual Limit

Dept. of Food Hygiene, http://vet.scuegypt.edu.eg/
Figure (2): HPLC chromatogram of oxytetracycline residue in liver

Figure (3): HPLC chromatogram of oxytetracycline residue in liver

Figure (4): HPLC chromatogram of oxytetracycline residues in muscle

Figure (5): HPLC chromatogram of oxytetracycline residues in muscle