



Evaluate antibiotic residues in beef and effect of cooking and freezing on it

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ABSTRACT

This study was performed to determine the occurrence of antibiotic residues in 50 random samples of fresh beef marketed at Giza governorate, Egypt. It was found that 16 samples were positive and 34 samples were negative. 13 samples from positive samples were contain more than one antibiotic as follow, 2% of samples contain Sulphonamides and Ciprofloxacin, (B-Lactam + Oxytetracyclines), (Sulphonamides + Oxytetracycline), (Sulphonamides plus B-Lactam), (Aminoglycosides plus Ciprofloxacin), (Macrolides plus Ciprofloxacin) (Aminoglycosides plus B-Lactam), (Macrolides plus B-Lactams), (Macrolides plus Oxytetracycline, 4% of the samples were regarded for Aminoglycosides plus Oxytetracyclines, Macrolides plus Ciprofloxacin.

The study was extended to include an experimental trial to reduce the load of oxytetracycline and ciprofloxacin in rabbit using boiling, roasting, microwaving and freezing, the obtained result indicated that freezing was the effective methods to degrade ciprofloxacin residues followed by microwaving. Microwaving and boiling and roasting were the effective heat treatment methods on degrading oxytetracycline residues to safe level. Therefore, efficient heat treatment of meat is highly recommended before serving to human to reduce the risk of antibiotic residues in meat.

Keywords: Beef, Residues, Antibiotic.

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1. INTRODUCTION

The veterinary drugs used in food producing animals has led to residues occurring in animal products as (meat, milk, eggs and honey), poses a health hazard to the consumer. Due to drug's properties and their pharmacokinetic characteristics, physicochemical or biological processes of animals and their products are subsidiary factors for occurring of residues of veterinary drugs and Improper drug usage and disrespect the withdrawal period. The major public health significances of drug residue are development of antimicrobial drug resistance,

hypersensitivity reaction, carcinogenicity, mutagenicity, teratogenicity, and disruption of intestinal normal flora. (Beyene *et al.* 2016). A study was carried out to detect the antibiotic residues in raw beef meat sold for human consumption at the vicinities of Hyderabad, Mirpurkhas, Sukkur, Larkana and Karachi divisions of Sindh. A total 300 samples collected 60 samples from the vicinity of each Division i.e. Hyderabad, Mirpurkhas, Sukkur, Larkana, and Karachi of Sind province were examined for the occurrence of antibiotic residues. It was observed that the presence of

antibiotic residues in beef samples was found to be higher at Karachi (48.33%) followed by Sukkur (41.67%), Hyderabad (36.67%), Mirpurkhas beef samples was found to be higher at Karachi (48.33%) followed by Sukkur (41.67%), Hyderabad (36.67%), Mirpurkhas beef samples was found to be higher at Karachi (48.33%) followed by Sukkur (41.67%), Hyderabad (36.67%), Mirpurkhas beef samples was found to be higher at Karachi (48.33%) followed by Sukkur (41.67%), Hyderabad (36.67%), Mirpurkhas (33.34%) and Larkana (31.67%) divisions. Out of total beef samples obtained from study area overall 38.33 percent were observed as contaminated with the antibiotic residues. The average zone size in diameter of beef samples of Karachi division was found to be significantly ($P < 0.05$) high followed by Sukkur, Hyderabad, Larkana and Mirpurkhas. The results of present study showed that a considerable contamination of antibiotic residues in beef meat was found at all divisions of Sind province. It could be concluded that withdrawal periods of antibiotics were seems to be not followed by livestock farmers or butchers Mangsi, et al. (2014). Antibiotics used in food animals can affect the public health because of their secretion in edible animal tissues in trace amounts usually called residues. The ongoing threat of antibiotic contamination is one of the biggest challenges to public health that is faced not only by Egypt or Africa, but also by the human population worldwide Carset *et al.* (2008). Accumulated tetracyclines residues in food have public health risks as microbial resistance, bone and teeth staining and teratogenic effects. Morshdy *et al.* (2013) Ciprofloxacin is a fluoroquinolone used in veterinary medicine. It has high activity against gram-positive and gram-negative bacteria. Its mechanism of action through it inhibit DNA-gyrase enzyme in a bacterial cell. Hassouan *et al.* (2007). Ordinary cooking

procedures boiling decreased the initial concentrations of tetracycline residues by 56 to 82%. Also, microwaving degrades the initial concentrations of tetracycline residues by 56 to 82% Gratacós-Cubarsí *et al.* (2007). The effect of freezing time on OTC concentration in muscle and liver tissues at both pH, at pH 6.0, muscle sample with initial mean inhibition zone of 5.81 changed to 5.81, 5.72, and 5.71; at pH 7.2, the initial mean inhibition zone change from 6.0 mm to 6.0, 6.2, and 6.0 at the 3rd, 6th, and 9th days of freezing, respectively. The same pattern was followed for liver samples. There was little or no reduction in the mean concentrations of freezing in both tissues at different time intervals, the effect of the cooking methods on OTC residue in muscle at pH 7.2. There was no statistical differences ($p > 0.05$) in the mean values of raw (7 mm) and microwaved (3.94 mm) with 34.33% reduction. Statistical differences were observed between raw versus roasted (2.81 mm) and raw versus boiled (1.94 mm) muscle samples with 53.7% and 67.99%, respectively. Vivienne EE., *et al.* (2018).

2. Materials and methods

2.1. Collection of samples:

A total of 50 fresh raw beef from different cuts of carcasses were collected from Giza Governorates shops. The average weight of each sample 250g; each sample was put in sterile polyethylene bag then sealed and labeled. The samples were transferred to the laboratory in ice box without delay. The samples were frozen at (-18°C) until examined within few days.

2.2. Method:

2.2.1. Samples Preparation

Beef Samples Extraction were carried out using 0.1M (pH 6.0) for penicillin, 0.1M NaOH for sulphonamides, 0.1M (pH 8.0) for

aminoglycosides (Gentamicin), 0.2M (pH 8.0) for Macrolides (Erythromycin).

2.2.2. Agar Diffusion Technique

The wells in agar plates at different (PH 6, 7.2, 8, 8) containing sensitive organisms; *Bacillus subtilis*, *Bacillus Subtilis* and *Micrococcus luteus* respectively; were dig. Accurately, 200ul of sample extract was dispensed in 2 wells in each plate and in the center well of different plates standard Penicillin, Sulfadimidine; Gentamicin, and Erythromycin were dispensed respectively. The *Bacillus Subtilis* containing plates were incubated at 30⁰C for 18h while the plate containing *Micrococcus Luteus* incubated at 37⁰C for 24h. The diameter of inhibition zones was recorded for samples and standards in different plates of the define groups of antibacterial and antibiotics then calculate the concentrations from the standard solutions.

2.2.3. Determination of ciprofloxacin :(Agar diffusion)

Microbiological Determination of ciprofloxacin residues in beef meat was done using the One Plate Test (OPT). The test organism was Escherichia coli (ATCC 10536) and the agar media pH (6) seeded with *E. coli*. Just 200ul of sample were dispensed in 2 wells in agar plate and the standard solution in center well in the plate. The plates of the samples were incubated at 37⁰C for 24 hrs. Zones of inhibition was measured with mm graduated ruler for samples and standard and calculate the concentrations of ciprofloxacin. The positive samples were confirmed by HPLC (high performance liquid chromatography) by pooling all positive samples for ciprofloxacin in only two samples (Verdon, *et al.*, 2004).

2.2.4. Determination of (Tetracyclines) Oxytetracycline Residues:

The single plate microbiological assay for determination of all tetracyclines including oxytetracyclines by using antibiotic No 8 (PH 6) seeded with *B.cereus var mycoides* (ATCC

11778) and supplemented with penicillinase enzymes. The samples of beef were extracted for tetracyclines by using buffer (0.1 M PH 4.5). Just 200ul of sample extract was dispensed into the test wells. Standard reference of Oxytetracyclines was also dispensed in the plate. The plates were incubated at 29⁰C for 16-18h. The inhibition zones were recorded and calculated from the standard curve. The positive samples were confirmed by HPLC (high performance liquid chromatography) by pooling all positive samples for ciprofloxacin in only two samples (Hamide *et al.*, 2000).

2.2.5. Experimental Study

This study was planned to determine the residues of ciprofloxacin and Oxytetracyclines in experimentally treated rabbits' samples after cooking treatments (Boiling; Microwave; Roasting) and freezing of the rabbit samples. The method of determination of the residues using HPLC. The experiment was planned as follow:

2.2.5.1 *Rabbits*: fifty rabbits (Newzeland species each weighed 3kg) were divided into 3 groups (each 5 five rabbits) free from any antibiotics.

2.2.5.2 *Drugs*: Ciprofloxacin 10% ALNASR (Injection I/V) and Oxytetracyclines (20%, S/C, ADWIA).

2.2.5.3 *Procedures*: The first group was injected single dose (10mg/kg BWT (I/V) of ciprofloxacin. The second group was injected single dose oxytetracyclines S/C 5mg/kg Bwt. The third group was kept as control. The groups of rabbits were kept in cages for 24 hrs then slaughtered.

2.2.5.4 *Samples*: From each rabbit group (3 thighs and breast) was treated as follow:

2.2.5.5 *Cooking*: Boiling for 30min, Microwave for 20min, Roasting for 30minat 100⁰C. Each cooked group was three of each (thigh and breast).

2.2.5.6 *Freezing*: Three thigh and breast from each group were kept deep freeze at -20°C for 6 and 12 months.

All for mentioned treated groups were either cooking or freezing were examined for the presence of ciprofloxacin and oxytetracyclines residues using HPLC according to (Verdon, *et al.*, 2004).

3. RESULTS

Table 1 showed that the positive samples of beef for antibiotic residues were 16 with percent of 32%. The Concentrations (µg/kg) of different antibiotics residues in each individual raw beef samples were illustrated in Table 2 and Figure 1, the result showed 3 samples of amino glycosides, 2 samples of β-lactam (penicillin), one sample of Ciprofloxacin were rejected. The Frequency Distribution of different types of antibiotics Residues in raw beef samples was shown in

table 3 and Figure 2, the frequency range of Aminoglycosides (Gentamicin), B-Lactam (Penicillin), Ciprofloxacin, Macrolides (Erythromycin), Oxytetracyclines, Sulphonamides were 4, 6, 6, 4, 5 and 3 respectively. 56% of samples were contained antibiotic residues. Figure 3 was viewed the effect of boiling on reduction for both ciprofloxacin and Oxytetracycline residues in Rabbit muscle were 20.74% and 87.97%, respectively. The effect of microwave on reduction for both ciprofloxacin and Oxytetracycline residues in Rabbit muscle were 38.14% and 86.95%, respectively. The roasting effect on reduction for both ciprofloxacin and Oxytetracycline residues in Rabbit muscle were 12.84% and 73.98%, respectively. After 6 months of freezing at -18°C the ciprofloxacin reduced to 65.73% whereas after 12 months the same residues were not detected which viewed in Figure 4.

Table 1: Percent of antibiotic residues positive beef samples.

Total beef samples	Positive	Percent	Negative	Percent
50	16	32	34	68

Table 2: Concentrations (ug/kg) of different antibiotics residues in each positive raw beef samples.

Antibiotics Types	Antibiotics Concentrations (ug/kg) of different samples					
Aminoglycoside(Gentamicin)	46	90.67*	112.7*	76*		
B-Lactams(Penicillin)	20	51*	31.36	42.73	55	67.73*
Ciprofloxacin	56.45	44.63	36.45	91	81.91	111
Macrolides (Erythromycin)	41.38	45.54	34.29	29.29		
Oxytetracyclines	27.23	86.7	119*	40.9	19.9	
Sulphonamides	18	12.67	19.33			

* Rejected samples according to EEC 1990

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Table 3: Frequency distribution of beef samples contain more than one antibiotic (n= 13 contain more than one antibiotic).

Sulphonamides + Ciprofloxacin	1	2
B-Lactam + Oxytetracyclines	1	2
Sulphonamides + Oxytetracycline	1	2
Aminoglycosides + Oxytetracyclines	2	4
Sulphonamides + B-Lactam	1	2
Aminoglycosides +Ciprofloxacin	1	2
Macrolides +Ciprofloxacin	2	4
Aminoglycosides + B-Lactam	1	2
Macrolides +Ciprofloxacin	1	2
Macrolides +B-Lactams	1	2
Macrolides + Oxytetracycline	1	2
Total	13	26

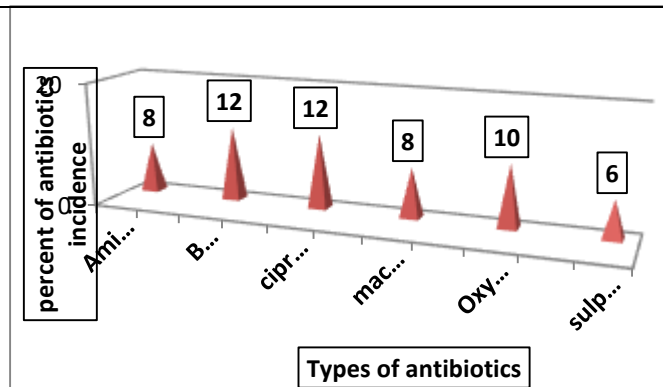


Fig.1. Incidence of different antibiotics residues in raw beef samples.

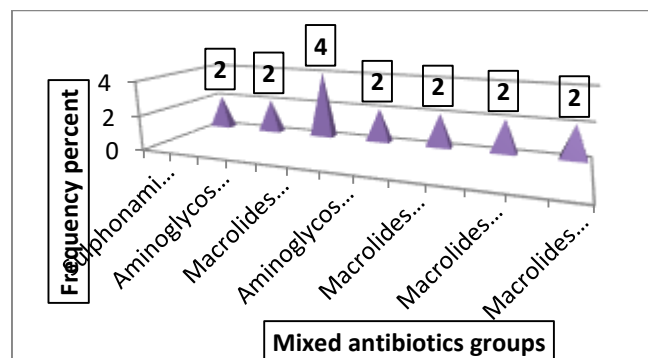


Fig.2. Frequency incidence of presence of more than antibiotics residues groups in examined raw beef samples.

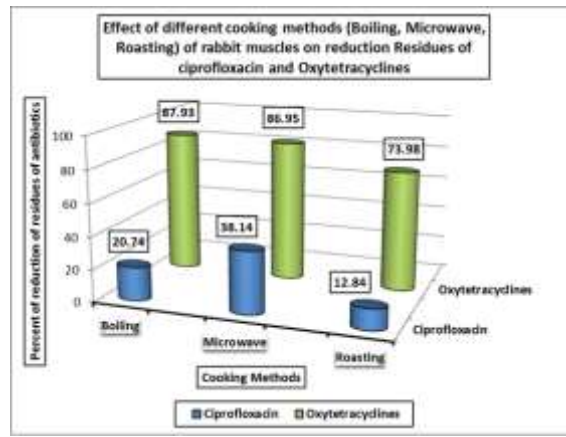


Fig.3. Effect of different cooking methods (Boiling, Microwave, Roasting) of rabbit muscles on reduction Residues of ciprofloxacin and Oxytetracyclines.

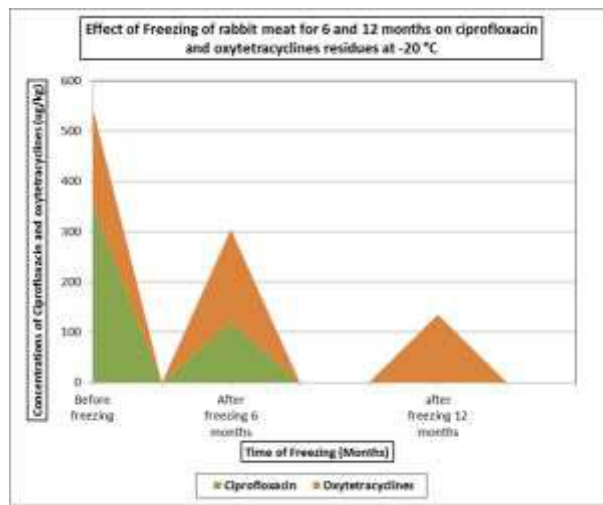


Fig.4. Effect of freezing of rabbit meat for 6 and 12 months on ciprofloxacin and oxytetracyclines residues at -20 °C.

4. DISCUSSION

Ab use of antibiotics in food animal production causes wide spreading of resistant pathogens, resistance genes and antimicrobial resistant bacteria in food animals, whereas they enter the food chain so national authorities should put serious laws and programs aimed at reducing the excessive using of antibiotics in food animals and ensure their risky usage. Due to farmers and meat producers don't follow the laws about withdrawal times of the products (Sattar *et al.*, 2014). Spreading of antibiotic-resistant bacteria showed serious problems among public health as mortality rates were increased in cases when resistant infections occurred and increase length of treatments so, require

using more expensive antibiotics or antibiotic cocktails (WHO, 2007). Table 1 showed that the positive samples of beef for antibiotic residues were 16 with percent of 32%. The Concentrations ($\mu\text{g}/\text{kg}$) of different antibiotics residues in each individual raw beef samples were illustrated in Table 2 and Figure1, the result showed 3 samples of amino glycosides, 2 samples of β -lactam (penicillin), one sample of Ciprofloxacin were rejected according to EEC (1990). The Frequency Distribution of different types of antibiotics Residues in raw beef samples was shown in table 3 and Figure 2, the frequency range of Aminoglycosides (Gentamicin), B-Lactam (Penicillin), Ciprofloxacin, Macrolides (Erythromycin), Oxytetracyclines, Sulphonamides were 4, 6,

6, 4, 5 and 3 respectively. 56% of samples were contained antibiotic residues. Ramatla *et al.* (2017). Figure 3 was viewed the effect of boiling on reduction for both ciprofloxacin and Oxytetracycline Residues in Rabbit muscle were 20.74% and 87.97%, respectively. The effect of microwave on reduction for both ciprofloxacin and Oxytetracycline Residues in Rabbit muscle were 38.14% and 86.95%, respectively. The roasting effect on reduction for both ciprofloxacin and Oxytetracycline Residues in Rabbit muscle were 12.84% and 73.98%, respectively. Gehad (2002) mentioned that since the most of foods-producing animals are always cooked before consumption and the variations in oxytetracycline levels in the meat are dependent on type of cooking. More studies about the effect of cooking on oxytetracycline residue are needed to accurately determine consumer exposure to this drug. The boiling for 30 minutes and roasting at 150°C for 30 minutes caused a complete degradation of drug residues. After 6 months of freezing at -18 °C the ciprofloxacin reduced to 65.73% whereas after 12 months the same residues were not detected which viewed in Figure 4. AboulEl (2006) that said Controlling of ciprofloxacin residues by freezing at -18 c had a positive effect on degradation of its residues Morshdy *et al.* (2014) said that Freezing at -20°C caused a lower degradation than that caused by boiling so, neither boiling nor freezing could be used as reliable methods to get rid of oxytetracycline residues in rabbit meats.

5. Conclusion

This study were concluded that High incidence of antibiotic residues in beef due to excess use of antibiotics for treatment without follow of the withdrawal periods of the drugs. Freezing the effective methods to degrade ciprofloxacin residues to safe level

then microwave, as ciprofloxacin is heat stable compound that degrades less by heat treatment process. Microwaving and boiling and roasting are the effective heat treatment methods on degrading oxytetracycline residues to safe level. Microwaving and boiling could degrade oxytetracycline residues higher than roasting but freezing had low degrading effect on oxytetracycline residues. Therefore, efficient heat treatment of meat is highly recommended before serving to human to reduce the risk of antibiotic residues in meat.

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