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RESEARCH ARTICLE
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Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh

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Abstract

Aim: The present study was described thin layer chromatography (TLC) and ultra-high performance liquid chromatography (UHPLC) method for the detection of antibacterial substances in poultry muscle (breast and thigh), kidney, and liver.

Materials and Methods: TLC method was used for screening detection of tetracycline, amoxicillin, ciprofloxacin, and enrofloxacin residues in poultry tissues. The samples were extracted with trichloroacetic acid (30%), diethyl ether, followed by detection in pre-coated TLC paper on ultraviolet detector. The UHPLC method was used for the quantification of antimicrobial residues in poultry tissues.

Results: The residues of tetracycline were 48% in livers, 24% in kidneys, 20% in thigh muscles, and 24% in breast muscles. Ciprofloxacin residues were found 44% in liver, 42% in kidneys, 34% in thigh muscles and 30% in breast muscles. Enrofloxacin residues were found 40% in liver, 34% in kidneys, 22% in thigh muscles, and 18% in breast muscles. Amoxicillin residues were found 42% in liver, 30% in kidneys, 26% in thigh muscles and 22% in breast muscles. Most of the cases highest residues were found in liver such as tetracycline (48%), ciprofloxacin (44%), enrofloxacin (40%) and amoxicillin (42%) and almost lowest in breast muscles. In addition, nine positive samples from broiler were selected for amoxicillin residue quantification by UHPLC. It was observed that the concentration of amoxicillin residue in liver was ranging from 16.92 µg/kg to 152.62 µg/kg and in breast muscle was 45.38 µg/kg to 60.55 µg/kg, respectively. The maximum and minimum peak time was 4.7-5.2 min. Among the poultry tissues, liver had the highest level of antibiotic residues in comparison to other samples but the variation was not significant ($p>0.05$).

Conclusions: Evidence suggests that more judicious use of antimicrobials in food animals will reduce the selection of resistant bacteria and help to preserve these valuable drugs for both human and veterinary medicine. The method described in this study is a simple, easy inexpensive which can be readily adopted by any laboratory for the detection of antibiotic residues in tissues of food-producing animals.

Keywords: antibiotic residues, broiler, layer, thin layer chromatography, ultra high-performance liquid chromatography.

Introduction

Antibiotics are natural products of a micro-organism or identical synthetic products or similar semi-synthetic products that inhibit the growth of or destroy microorganisms [1]. In veterinary medicine, antibiotics are widely used as therapeutic, prophylactive and growth promoting agents and nutritive purposes in livestock and poultry production [2]. Various antibiotics take different time periods to be excreted from the body. It becomes a potential hazard to human health [3]. The presence of xenobiotics, especially antibiotic residues in the foodstuffs of animal origin is one of the most important indexes for their safety. Many livestock producers treat their animals themselves. Even if they use the same drugs as veterinarians, they have little understanding of the conditions and quantities to administer or the waiting periods. In addition, there are cases of veterinary products intended for ruminants being administered to other species. Indiscriminate use of antibiotics results noticeable residues in meat, milk, cheese, butter and other livestock products causes microbiological resistance that may cause serious problems at microbial infections [4]. The use of antimicrobials for the treatment or prevention of disease in animals closely followed their uses in humans and today antimicrobial drugs are used to control, prevent and treat infection, and to enhance animal growth and feed efficiency [5]. Currently, approximately 80% of all food-producing animals receive medication for most their lives [6]. The prevalence of pathogens on farms depends on many factors, not least the type of husbandry, the environmental pressure on a farm, and the standard of the stockman ship [7]. Factors related to farm

management help to minimize the use of antimicrobials. However, even if well managed, the increased density of livestock or poultry in intensive rearing operations requires an aggressive approach to disease control that can lead to heavy prophylactic and therapeutic antimicrobial use [5]. The use of antimicrobials in food-producing animals may result in the presence of residues in foodstuffs of animal origin. Protection of public health against possible harmful effects of veterinary drug residues is a relatively recent preoccupation. The most frequently identified substance in urine, feces and tissue is the parent tetracycline. As much as 30% is excreted unchanged in feces. Tetracycline's are reversibly bound to plasma proteins and are widely distributed. Tetracycline's diffuse throughout the body and found in highest concentrations in kidney, liver, spleen, and lungs. They are also deposited at active sites of ossification [8]. Microbial resistance to antibiotics is a worldwide problem in human and veterinary practices [9,10]. It is generally accepted that the main risk factor for the increase in antibiotic resistance is an extensive use of antibiotics. The antimicrobial agents used in animal care are also significant, not only in increasing the resistance in animal pathogen, but also in bacteria transmitted from animals to humans [11]. The earliest screening methods used for detecting antimicrobial residues in foods including milk were based on the detection of growth inhibition of various bacterial strains. Such methods were based on microbial agar diffusion tests or on the inhibition of acid production by starter organisms [12]. Microbiological inhibition tests are considered as multi-residue screening tests for antibiotics in milk, meat and other animal tissues. Microbial tests are commonly used for the screening of antibiotic residues in animal tissues in a residue monitoring programmed, and their role was extended to the identification of antibiotics in animal feeding stuffs [13]. From the above facts, it may be mentioned these antibiotic residues might be potential hazards for human as well as animal health and a great obstacle to export poultry meat. In this context, these research work was undertaken in this country to detect antibiotic residues in broiler and laying hens in Chittagong district with encountered the objectives of detection the presence of residues of tetracycline and quinolones groups of antibacterial in broilers and layers tissues by thin layer chromatography (TLC) test and to determine the level of residues of antibacterial in broiler tissues by ultra high performance liquid chromatography (UHPLC).

Materials and Methods

A total of 200 samples were collected from 35 broiler and 15 layer birds. These samples were collected from different retail outlets in Chittagong metropolitan area (CDA market, Jhawtala Bazar and Wireless Bazar) during the period of July-December, 2012. The pre-prepared questionnaire was filled up properly before sample collection.

Methods used for TLC

Sample preparation and antibiotics extraction

These samples were stored in the deep freeze at −20°C until further advanced procedures were performed. Samples were blended with a food processor properly for 3-5 min. It was done in three steps. Running of blender was continued for 1 min and a pause of 5-8 s followed by running again. This technique was repeated until tissues were blended properly. These blended samples were taken into properly cleaned and sterilized petri dishes with proper care as well as covering. From this 4 g of aliquot sample (2 g for kidney sample) was taken into beaker with the help of electric balance and spatula. The homogenization was done with the addition of 10 ml phosphate buffer (5 ml for kidney sample) (pH-6.5). After proper mixing, protein contents of these samples were precipitated with the addition of 2 ml trichloroacetic acid (1 ml for kidney samples) (30%) maintaining sufficient care and attention. Then these samples were taken into properly cleaned and sterilized centrifuge tubes for centrifugation. The centrifugation was performed at 7000 rpm for 15 min with the help of automatically time regulated centrifuge machine. Then, filtration of the supernatant was performed with the help of Whatman filter paper and funnel. Filtration fluid was collected into beaker with sufficient care. The supernatant was extracted with an equal volume of diethyl ether to perform de-fatation. Then mixture was kept for 10 min to become into a separate layer. Then by using cleaned and sterilized separating funnel, these mixtures were separated from each other, and upper oily layer was discarded but only the bottom layer was collected. This extraction of supernatant was repeated twice with diethyl ether. Then, the extracts were evaporated until dryness. The dried sample was reconstituted within 2 ml of mobile phase made by mixing of methanol and acetone (1:1). Then, the extracts were collected into screw cap vial with proper care and kept into refrigerator for further advanced analysis. Total procedure was performed as the reference cited by Poppelka *et al.* [14].

Preparation of TLC silica plates

TLC silica plates with 0.25 mm thickness (Merck, Germany), were activated in 120°C for 2 h before use [15].

Preparation of standard solution

For comparison of extracted residues with routinely used four raw antibiotics (tetracycline, amoxicillin, ciprofloxacin, and enrofloxacin) were prepared by dissolving of 0.1 g of each powder in 4 ml methanol [15].

Pointing, running and detection

A volume of 50 µl of methanol dissolved deposits was pointed on silica plates. Treated plates transferred to TLC tank containing acetone-methanol (1:1)

as mobile phase. After reaching of solution front to end of plates, chromatograms observed on ultraviolet light at 256 nm.

Determination of amoxicillin residue by UHPLC

A total of 30 mg of amoxicillin trihydrate CRS was dissolved in mobile phase-A and diluted to 50 ml with mobile phase-A.

Extracted antibiotic solutions for TLC were filtered through 0.2 MFS syringe filters (0.2 m, Advantec MFD, Inc., Japan).

The chromatographic procedure was carried out by the following ways:

1. A stainless steel column C18 (2 µm) P/N 891-5002, 2 mm ID×100 mmL No. 22G2C-001 was used for chromatography.

2. Mobile phase was run at a flow rate of 0.2 ml/min.

Dilute sodium hydrogen was added to 250 ml of 0.2 M potassium di-hydrogen phosphate R up to pH 5.0 and diluted to 1000 ml with water R. A spectrophotometer detector was set at 254 nm to measure the wavelength. Injection volume was 20 µl.

Regression equation:

$Y=ax$

Here, Y=Component area or height
a=Slope of the calibration line
x=Uncorrected amount
b=Intercept.

Statistical analysis

Obtained data were imported and stored in Microsoft-Excel-2007 and results were analyzed statistically using STATA/IC-11 (College Statin, Texas, USA). Descriptive analysis was performed using mean and standard error for each outcome variable.

Results

TLC

In TLC study, a total of 200 samples of different poultry tissues (140 for broiler and 60 for layer) were tested to detect antibiotic residue.

The TLC results (Table-1) revealed that the highest prevalence of antibiotic residue in liver tissue is tetracycline (48%) followed by ciprofloxacin (44%), amoxicillin (42%) and enrofloxacin (40%), respectively. In kidney highest prevalence was ciprofloxacin (42%), followed by enrofloxacin (34%), amoxicillin (30%) and tetracycline (24%), respectively. The highest percentage detected in breast muscle was ciprofloxacin (30%), followed by tetracycline (24%), amoxicillin (22%) and enrofloxacin (18%), respectively. The thigh muscles were showed the highest prevalence of ciprofloxacin (34%), followed by amoxicillin (26%), enrofloxacin (22%) and tetracycline (20%). Among the poultry tissues, liver contained the highest level of antibiotic residues in comparison to other samples. The order of sequences from the present study was highest in liver followed by kidney, thigh muscles and breast muscle, respectively.

In the broiler liver contains the highest percentage of residue for tetracycline (45.71%) followed by ciprofloxacin (42.85%), amoxicillin (37.14%) and enrofloxacin (37.14%), respectively. In broiler kidney the highest percentage was detected for ciprofloxacin (48.57%), followed by enrofloxacin (31.43%), amoxicillin (28.57%) and tetracycline (25.71%) respectively. In broiler breast muscle, the highest prevalence recorded for ciprofloxacin (31.43%) followed by tetracycline (25.71%), amoxicillin (22.85%) and enrofloxacin (20%), respectively. The highest percentage in broiler thigh muscle was found for ciprofloxacin (40%), followed by amoxicillin (25.71%), tetracycline (22.85%), and enrofloxacin (22.85%), respectively.

In the case of layer in all the selected tissue contain the highest percentage of ciprofloxacin (66.67% in liver, kidney and thigh muscle, respectively) except breast muscle where ciprofloxacin, tetracycline and amoxicillin contribute equally (26.67% for each). The lowest percentage in layer breast muscle is for enrofloxacin (20%). It was revealed that 26.67% of layer liver contained enrofloxacin, 53.33% contained tetracycline and 46.67% contained amoxicillin residues. Result also showed that 26.67% layer kidney samples were positive for enrofloxacin, 13.34% for tetracycline and 20% for amoxicillin residues. The thigh muscles were showed 20% with enrofloxacin, 20% with tetracycline and 26.67% with amoxicillin residues. Among the poultry tissues, liver contained the highest level of antibiotic residues in comparison to other samples such as kidney, thigh muscles, and breast muscles. The order of sequences from the present study was highest in liver followed by kidney, thigh muscles and breast muscle (Table-2).

From the Table-2, it was revealed that liver contained the highest level of antibiotic residues in comparison to other samples like kidney, thigh muscles and breast muscle in both broiler meat and layer meat. The variation of antibiotic residue in different organ between broiler and layer meat was not significant ($p>0.05$).

Table-1: Overall percentages of different antibiotics residue in different tissue of broiler and layer.

Organ samples	Positive (%)			
	Ciprofloxacin	Enrofloxacin	Tetracycline	Amoxicillin
Liver (N=50)	22 (44)	20 (40)	24 (48)	21 (42)
Kidney (N=50)	21 (42)	17 (34)	12 (24)	15 (30)
Breast muscle (N=50)	15 (30)	9 (18)	12 (24)	11 (22)
Thigh muscle (N=50)	17 (34)	11 (22)	10 (20)	13 (26)

Table-2: Comparison of antibiotics residue between different organ of broiler and layer.

Sample	Antibiotics	Broiler (%) (N=35)	Layer (%) (N=15)	p value
Liver	Ciprofloxacin	15 (42.85)	7 (46.67)	0.8
	Enrofloxacin	13 (37.14)	7 (46.67)	0.52
	Tetracycline	16 (45.71)	8 (53.33)	0.62
	Amoxicillin	14 (40)	7 (46.67)	0.66
Kidney	Ciprofloxacin	17 (48.57)	4 (26.66)	0.15
	Enrofloxacin	11 (31.42)	6 (40)	0.55
	Tetracycline	9 (25.71)	3 (20)	0.66
	Amoxicillin	25 (71.42)	10 (66.66)	0.73
Breast muscle	Ciprofloxacin	11 (31.42)	4 (26.66)	0.73
	Enrofloxacin	7 (20)	2 (13.33)	0.57
	Tetracycline	9 (25.71)	3 (20)	0.66
	Amoxicillin	8 (22.85)	3 (20)	0.82
Thigh muscle	Ciprofloxacin	14 (40)	3 (20)	0.17
	Enrofloxacin	8 (22.85)	3 (20)	0.82
	Tetracycline	8 (22.85)	2 (13.33)	0.44
	Amoxicillin	9 (25.71)	4 (26.66)	0.94

Amoxicillin residues determined by UHPLC

The column was equilibrated with a mobile phase with ratio A:B of 98.8. The assay was validated until the resolution between the two principal peaks was at least 2.0. Peak area was used for quantification. The calibration curves were used to calculate the amoxicillin concentration of the quality control samples and unknown samples. The spiked samples were processed and analyzed with the developed procedure. Therefore, the extraction recovery was obtained by comparing the observed peak areas obtained from the processed standard samples to direct injections of standard aqueous solutions prepared at concentrations with represented 100% recovery.

The concentration of amoxicillin residues in organ samples of liver and muscles of broiler was estimated. Both the highest (152.62 μg/kg) and the lowest (16.92 μg/kg) concentrations of amoxicillin were found in liver. In breast muscle, the amoxicillin concentration ranging from 45.38 μg/kg to 60.55 μg/kg. Retention times of peaks of different samples were between 4.7 and 5.2 min and peak areas of these samples were in between 2,315,569 and 6,319,022.

Discussion

The TLC method was used to separate and identify the ciprofloxacin, enrofloxacin, tetracycline and amoxicillin from poultry meat. A similar type of study was conducted by Amjad *et al.* [16], separated and identified the ciprofloxacin and enrofloxacin residues from chicken liver, kidney and muscles using TLC. Chicken liver contained the highest of proportion of antibiotic residues than the rest of three samples. This finding has similarities with the report of Naeem *et al.* [17] and Islam, [18] that chicken liver contained the highest level of enrofloxacin as well as ciprofloxacin residues than kidney and muscles. Among the antibiotics investigated in the present study, tetracycline found higher in liver which is agree with Kirbis [1], who reported that tetracycline residues were most commonly detected residues in meat. In the case of ciprofloxacin, it was observed that liver and kidney was the major harboring site showing the highest proportion of residues. A similar finding was reported by Naeem *et al.* [17] and Islam, [18]. Enrofloxacin residues were found in different percentages in liver, kidney, breast muscles and thigh muscles and this finding has close similarities with Islam, [18].

Amoxicillin residues were detected in various percentages in liver, kidney, breast muscles and thigh muscles. Poppelka *et al*. [14] detected amoxicillin residues in poultry meat. Hussain *et al*. [15] identified amoxicillin residues in poultry meat by using TLC. Tetracycline residues were found in livers, kidney, thigh muscles, and breast muscles samples. Salehzadeh *et al.* [19] detected that tetracycline residue above maximum residual limits (MRLs), which were 27.77%, 95.55%, and 18.88% in muscles, liver and kidney samples respectively. They also reported that liver contained the highest percentage of antibiotic residues. Here, we used nine TLC positive samples from broiler for quantification of amoxicillin residue by UHPLC. The average concentration of amoxicillin residue in liver tissues and breast muscles were crossed the MRL of liver and muscle tissues of cattle, sheep and pigs which was 50 μg/kg [20]. The current findings also agree with the research work of Hossain, [21] in poultry. Lemus *et al.* [22] detected the antibiotics in plasma at high mean concentrations (ciprofloxacin=0.15±0.066 μg/ml, n=6, enrofloxacin=0.089±0.049 μg/ml, n=8, amoxicillin=0.09 μg/ml, n=1, oxytetracycline=0.005 μg/ml, n=1) in adult cinereous vultures and enrofloxacin and ciprofloxacin were found in all samples of liver tissue from nine dead cinereous vultures at mean concentrations of 0.18±0.11 μg/g (range 0.08-0.21 μg/g) and 0.09±0.04 μg/g (range 0.03-0.07 μg/g), respectively. Bousova *et al.* [23] reported most of the detected antibiotic was within MRL. Cheong *et al.* [24] reported the concentration of sulfonamides detected in samples from 11 states in Peninsular Malaysia ranged from 0.006 to 0.062 μg/g in breast meat samples and 0.08-0.193 μg/g in liver samples. Hussein and Khalil, [25] detected the residual of tetracycline ranged from 0.156 μg/g to 0.900 μg/g with a mean value of 0.394±0.111 μg/g and regarding to enrofloxacin residues, ranges from 0.04 μg/g to 0.757 μg/g. Tajik *et al.* [26] detected chloramphenicol level as minimum and maximum levels of 0.54 and 155.2 ng/g in the kidney and liver, respectively. But it's clear from the result that, antibiotic residue already exist in our food chain, especially in broiler. However, a comprehensive study require in Bangladesh to detect and estimate all the antibiotics used in broiler and layer chicken to take potential steps to protect the mankind and environment from antibiotics residue hazards.

Conclusion

The great number of samples was found with antimicrobial residues. It was stated that probably in some cases chicken meat producers do not respect the regulation about withdrawal periods of the centenary products. The use and sometimes misuse of antimicrobials in food animal production have resulted in the emergence and dissemination of resistant pathogens and resistance genes. Antimicrobial resistant bacteria in food animals can affect not only animal health, but also public health when they enter the food chain. Evidence suggests that more judicious use of antimicrobials in food animals will reduce the selection of resistant bacteria and help to preserve these valuable drugs for both human and veterinary medicine. National authorities should adopt a proactive approach that promotes programs aimed at reducing the need for antimicrobials in food animals and ensuring their prudent use. The method described in this study is a simple, easy inexpensive which can be readily adopted by any laboratory for the detection antibiotic residues in tissues of food-producing animals. Further investigation is required for the quantitative determination of antibiotic residues in food products.

Authors' Contribution

S Sattar, MM Hassan and AKM Saifuddin planed and implemented the study design and carried out the laboratory experimentation. S Sattar, MM Hassan, M Alam, AKM Saifuddin, S Chowdhury, SKMA Islam and MSA Faruk drafted and revised the manuscript. All authors read and approved the final version of the manuscript.

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Competing Interests

Authors declare that they have no competing interests.

References

1. Kirbis, A. (2007) Microbiological screening method for detection of aminoglycosides, B-lactames, macrolides, tetracyclines and quinolones in meat samples. *Slovvet. Res.*, 44(1/2): 11-8.
2. Donoghue, D.J. (2003) Antibiotic residues in poultry tissues and eggs: Human health concerns. *Yozrlt. Sci.,* 82: 618-621.
3. Kozarova, L., Mate, D. and Cabadaj, R. (2001) Methods for the determination of sulfonamides residues in milk and animal n products In: Proceeding of Lectures and Posters of the International Conference Hygiena Alimentorium XXIII. Strbske Pleso, High Tatras, SR. p159-163.
4. Lee, W., Li, Z.H., Vakulenko, S. and Mobashery, S. (2000) A light inactivated antibiotic. *J. Med. Chem.,* 43(2): 128-132.
5. Jinap, S., Cheong, C.K., Hajeb, P. and Ismail-Fitry, M.R. (2010) Sulfonamides determination in chicken meat products from Malaysia. *Int. Food Res. J*., 17: 885-892.
6. Lee, H.J., Lee, M.H. and Ruy, P.D. (2001) Public health risks: Chemical and antibiotic residues. *Asian Aust. J. Anirrt. Sci*., 14(1): 402-413.
7. Shareef, A.M., Jamel, Z.T., and Yonis, K.M. (2009) Detection of antibiotic residues in stored poultry products, Iraqi. *J. Vet. Sci.,* 23: 45-48.
8. Riviere, J.E. and Spoo, J.W. (2001) Tetracycline antibiotics. In: Adams, R.H. editor. Veterinary Pharmacology and Therapeutic. 7th ed. Iowa State University Press, Ames, Iowa, USA, p784-796.
9. Hassan, M.M., Amin, K.B., Ahaduzzaman, M., Alam, M., Faruk., M.S.A. and Uddin, I. (2014) Antimicrobial resistance pattern against *E. coli* and *Salmonella* in layer poultry. *Res. J. Vet. Pract.,* 2(2): 30-35.
10. Ahaduzzaman, M., Hassan, M.M., Alam, M., Islam, S.K.M.A. and Uddin, I. (2014) Antimicrobial resistance pattern against *Staphylococcus aureus* in environmental effluents. *Res. J. Vet. Pract.*, 2(1): 13-16.
11. Shahid, M.A., Siddique, M., Abubakar, M., Arshed, M.J., Asif, M. and Ahmad, A. (2007) Status of oxytetracycline residues in chicken meat in Rawalpindi/Islamabad area of Pakistan. *Asian J. Poult. Sci.,* 1(1): 8-15.
12. Mitchell, J.M., Griffiths, M.W., McEven, S.A., McNab, W.B. and Yee, J. (1998) Antimicrobial drug residues in milk and meat: Causes, concerns, prevalence, regulations, tests, and test performance. *Food Prof.,* 61: 742-756.
13. Higgins, H.C. and McEvoy, J.D.G. Accelerated Solvent extraction of animal feeding stuffs for microbial growth inhibition screening for the presence of antimicrobial feed additives. *Food Addit. Contam.,* 19(1): 819-828.
14. Poppelka, P., Nagy, J., Germuska, R., Marcinca, K.S., Jevinova, P. and Derijk, A. (2005) Comparison of various assays used for detection of beta-lactam antibiotics in poultry meat. *Food Addit. Contam.,* 22(6): 557-562.
15. Hussain, S., Khan, S., Ali, J., Sultan, A., Chand, N. and Rafiullah. (2013) Antibiotic residues in commercial poultry meat and egg. International Workshop on Dairy Science Park, Pakistan. [Published on 2013 Nov 29].
16. Amjad, H., Iqbal, J. and Naeem M. (2005) Analysis of some residual antibiotics in muscle, kidney and liver samples of broiler chicken by various methods. *Proc. Pak. Acad. Sci.,* 42(4): 223-231.
17. Naeem, M., Khan, K. and Rafiq, S. (2006) Determination of residues of quinolones in poultry products by high pressure liquid chromatography. *J. Appl. Sci.,* 6(2): 373-379.
18. Islam, A. (2009) Detection of antibiotic residues in laying hens using microbial inhibition test (MIC) and thin layer chromatography (TLC) at Chittagong, Bangladesh. MS Thesis on Pharmacology, Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University, Bangladesh.
19. Salehzadeh, F., Madani, R., Salehzadeh, A., Rokhni, N. and Golchinefar, F. (2006) Oxytetracycline residues in chicken tisuues from Tehran slaughterhouses in Iran. *J. Nutr*., 5(4): 377-381.
20. FAO. (2012) Evaluation of Certain Veterinary Drug Residues in Food. Seventy Fifth Report of the Joint FAO/WHO Expert Committee on Food Additives.
21. Hossain, M.Z. (2011) Antibiotic residue study in meat (cattle, goat and poultry), by modified four plate test (MFPT) and Thin Layer Chromatography (TLC) at Chittagong, Bangladesh. MS Thesis on Pharmacology, Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University, Bangladesh.
22. Lemus, J.A., Blanco-mail, G., Grande, J., Arroyo, B., García-Montijano, M. and Martínez, F. (2008) Antibiotics threaten wildlife: Circulating quinolone residues and disease in avian scavengers. *Plos. One.*, 3(1): 1444.

23. Bousova, K., Senyuva, H. and Mittendorf, K. (2013) Quantitative multi-residue method for determination antibiotics in chicken meat using turbulent flow chromatography coupled to liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A.*, 25: 19-27.
24. Cheong, C.K., Hajeb, P., Jinap, S. and Ismail-Fitry, M.R. (2010) Sulfonamides determination in chicken meat products from Malaysia. *Int. Food Res. J.*, 17: 885-892.
25. Hussein, M.A. and Khalil, S. (2013) Screening of some antibiotics and anabolic steroids residues in broiler fillet marketed in El-Sharkia governorate. *Life Sci. J.*, 10(1): 2111-2118.
26. Tajik, H., Malekinejad, H., Razavi-Rouhani, S.M., Pajouhi, M.R., Mahmoudi, R. and Haghnazari, A. (2010) Chloramphenicol residues in chicken liver, kidney and muscle: A comparison among the antibacterial residues monitoring methods of four plate test, ELISA and HPLC. *Food Chem. Toxicol.,* 2464-2468.

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