



DETERMINATION OF TETRACYCLINE RESIDUES IN EGGS OF LAYING BIRDS REARED IN MAKURDI METROPOLIS

*Bosha Joel Aonduhulugh, Jat Kim Bitrus and Agbo Joseph Odeh

Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

*Corresponding Author: Bosha Joel Aonduhulugh

Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

Article Received on 21/12/2022

Article Revised on 11/01/2023

Article Accepted on 31/01/2023

ABSTRACT

The occurrence of veterinary drug residues in eggs remains a global problem that poses health hazard to consumers especially when the withdrawal period is not adhered to. This study was carried out to determine the presence of tetracycline (TC) residues in eggs produced in Makurdi metropolis and also determine if the residues are above the maximum residue limits (MRL) set by international regulatory agencies. Tetracycline residues in egg were assayed using microbiology assay method. A total of 50 egg samples were analyzed. The results showed 98% incidence of TC residues in eggs. Albumen had the highest incidence of 20%. Seventy eight (78%) incidence occurred in both albumen/yolk. The level of tetracycline residues in eggs produced in Makurdi metropolis is far below the recommended MRL stipulated by regulatory agencies and is within the acceptable daily intake.

KEYWORDS: Tetracycline, Eggs, Residue, Layers, Makurdi.

INTRODUCTION

Poultry farming is a fast means of bridging the protein deficiency gap common in many developing countries of the world and efficient means of supplying the fast-growing human population with high quality protein.^[1] However, in most developing countries of the world including Nigeria, indiscriminate administration of antibiotics to food animals is a common practice due to unregulated distribution and access of farmers to veterinary drugs in the open markets or over the counter without veterinary prescription and supervision.^[2] The indiscriminate use of antibiotics in food animals could be incriminated as a critical factor leading to antimicrobial resistance as well as posing health risks to consumers due to the presence of antimicrobial residues in animal products.^[3,4]

Antibiotics have been used extensively since the 1950s for a better treatment of microbial disease in humans, and also improve food quality production. Antibiotics have been used in animal health, prophylaxis and growth promotion especially in poultry.^[5]

The most commonly used antibiotics in poultry farms is tetracycline.^[6] Tetracyclines are a class of antibiotics discovered more than 50 years ago. They are relatively inexpensive and less toxic drugs with a broad spectrum of activity. Consequently, they have been extensively used in the prophylaxis and therapy of human and animal

infections and as animal growth promoters.^[7] They are used in small dosages to increase protein and decrease fat components.^[6] Besides the microbial treatment, tetracyclines are used as anti-inflammatory and tumour growth inhibitor.^[8] Even though there are advantages in the use of these antibiotics, there are also concerns in the use of these antibiotics.^[9] Concern has been expressed about possible harmful effects on humans through the use of these drugs, as follows: (1) increased microbial drug resistance, (2) drug residues in food, (3) allergic reactions and sensitization to antimicrobials, and (4) drug toxicity.^[10] Further spread of resistance to the environment occurs as a result of agricultural runoff and contaminated effluents which would affect the surrounding water.^[5]

Failure to adhere to the withdrawal period or the Maximum Residual Limits of the antibiotics in poultry production is reported to be the primary cause of antibiotic residues in edible poultry tissues and products like eggs.^[2] Withdrawal periods ranging from a few days to a few weeks are recommended for approved Veterinary drugs.^[2] The health status of the animal, such as the presence of any infection or inflammation, may increase the potential of residues formation through affecting the pharmacokinetics and drug metabolism.^[12]

The use of antibiotics in animals has recently become an important public health issue. The increased use of such

compounds has shown many harmful effects on consumer such as, stimulation of microbial resistance and thus increase the risk of food borne infections when such antibiotic resistant pathogenic bacteria enters the food chain.^[13] Other harmful effects may include; hypersensitivity reactions and alteration of the intestinal microflora.^[14] Human consumption of toxic levels of antibiotic residues in food of animal origins (meat, milk or egg) had caused several pathological defects in man that are of public health importance.^[2, 11, 15, 16, 17]

The occurrence of veterinary drug residues in edible animal tissues remains a global problem.^[18] However, in some developing countries including Nigeria it was reported that food standards and regulations are inadequate and in some case, does not even exist.^[2,18] This is an indication that there are no adequate veterinary public health regulatory control and national program for monitoring drug residues in foods of animal origin in Nigeria.^[18, 19, 20] Currently, a large population of Nigeria is at high risk of the several diseases caused by the consumption of meats or eggs from exotic breeds of chickens with toxic levels of antibiotic residues.

The increase in production and consumption of poultry and its products in Makurdi means that there is more danger of exposure to drugs that may be present in the eggs and other tissues. Consumers are thus at risk. A study of the residues of tetracyclines in poultry will thus provide basis for control of this drug and further prevention of antibiotic resistance.

MATERIALS AND METHODS

Materials

The materials used in this work include petri dishes, sterile swabs sticks, autoclaves, syringes and needle,

forceps, absorbable paper discs, incubator, refrigerator, weighing balance, glass bottles, eggs, gloves.

Chemicals

Mueller Hinton agar, water for injection, tetracycline standard (NAFDAC)

Microorganism

Bacillus subtilis ATCC 3366, obtained from National Veterinary Research Institute (NVRI) Vom, Plateau state, Nigeria.

Study Area

Makurdi is the capital of Benue state in the middle belt of Nigeria. It's located on longitude $8^{\circ} 32' 10''$ E & latitude $7^{\circ} 43' 50''$ N. The River Benue where the state derived its name from divides the town in to two unequal parts. The Southern part is the main town where the seat of government and most commercial and public institutions are concentrated. It has seven wards out of the eleven wards in the town. The northern part is called north bank and comprise of four (4) wards. The University of Agriculture is located in the north bank. Five poultry farms that kept layers were identified from within Makurdi. The farms were selected based on flock size, location within Makurdi, history of antibiotic usage and consistency of production. The inclusion criteria was farms with flock size one thousand and above with continuous production of up to five years, while the exclusion criteria was flock size of less than one thousand and continuous production of less than five years.

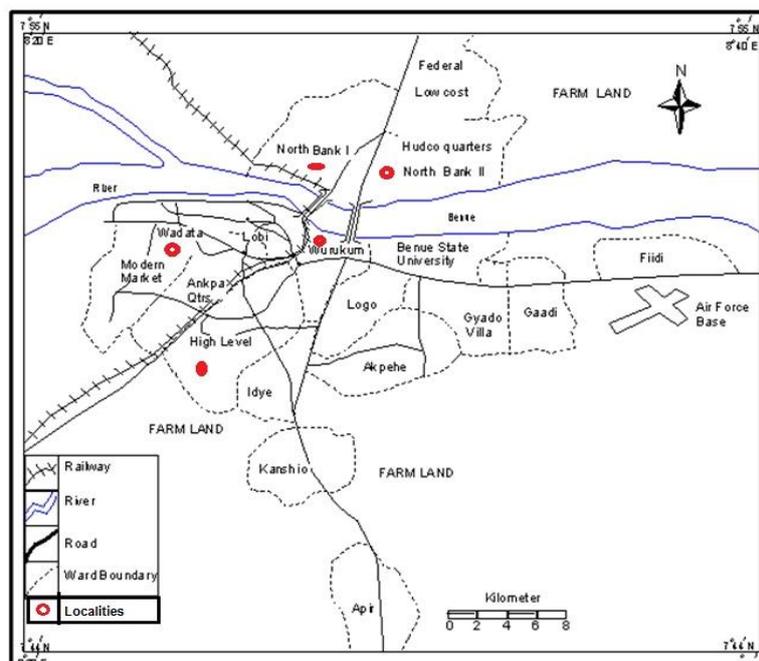


Figure 1: Administrative Map of Makurdi Local Government Area.

Experimental procedure

The manufacturer instruction was followed in preparing the media. The media was allowed to cool and 20 ml was poured out into the petri dishes and was allowed to settle at room temperature. From the stock culture, a suspension of *Bacillus subtilis* ATCC 3366 was prepared and standardized using McFarland standard to obtain a solution of 10^8 cell/ml. A drop of 0.1 ml of the suspension was uniformly spread on the Mueller Hinton agar surface using swap stick. One create of egg was collected from each farm for three consecutive days. Ten eggs were selected from each farm taking three eggs from each create and one at random. The eggs were labeled according to farms and the yolk and albumen aspirated using sterile syringe and needle and poured in separate bottles. The bottles were labeled as yolk (Y) and albumen (A). The absorbable paper discs were placed into the bottles to absorb the egg contents. Both the bottles and discs were autoclaved before use. At least four (4) discs were put into each bottle. The discs were placed on the agar surface in the petri dish with the help of sterile forceps. This was done in quadruplicate on each petri dish.

The agar diffusion method was used to access the inhibitory activity of the antibiotic tetracycline. Two (2) mm Whatman's filter paper was impregnated with tetracycline (0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156, and 0.0078) $\mu\text{g/ml}$. Egg albumen and not distilled water was used as medium for the dilution. The filter papers were placed on seeded agar plates and both were incubated at 37°C overnight. A metre rule was used to measure the zones of inhibition around the tissues. The average zones of inhibitions for the egg and the tetracycline were determined and a graph of zones of inhibition against concentration was drawn and the curve determined with an equation derived. This was used to determine the amount of tetracycline in the eggs by comparing the zones of inhibition of the various eggs to those of tetracycline.

Data Analysis

The data was presented as means \pm standard error of mean (SEM) in tables, graphs and percentages.

RESULTS

From the 50 eggs samples obtained from the poultry farms, there was 98% incidence of tetracycline residues in the eggs. The breakdown based on the egg part

showed that the albumen had the highest incidence (20%). Seventy eight (78%) percent incidence occurred in both albumen and yolk (table 2).

The farm by farm analysis of the various egg parts is presented in tables 3-7.

Farm 1

It had a total of fifteen (15) positive cases representing 100%, with the albumen alone having 4 cases (26.7%). Eleven (11) of the eggs had residues in both albumen and yolk, representing 73.3%. The highest level of tetracycline quantity ($-0.1727 \mu\text{g/ml}$) was found in the albumen with the lowest ($-0.3122 \mu\text{g/ml}$) found in the yolk (table 3).

Farm 2

It showed that the total number of positive cases was 9 representing 90%. The albumen alone had 1 case (9.99%) with both the yolk and albumen having 8 cases (80%). The highest residue of $-0.2192 \mu\text{g/ml}$ was seen in the albumen (table 4).

Farm 3

It also showed that the total positive cases were 10, representing 100% with the albumen alone having 20 % (2 cases) with both the albumen and yolk having 80% (8 cases). The highest residue ($-0.1355 \mu\text{g/ml}$) was found in the albumen (table 5).

Farm 4

The total number of positive cases was 15 (100 %) with the albumen alone having 3 (20 %). Eggs containing residues in both albumen and yolk were 12 (80 %). The highest residue of $-0.1912 \mu\text{g/ml}$ was found in the albumen while the lowest of $-0.3029 \mu\text{g/ml}$ was found in the yolk (table 6).

Farm 5

Total number of positive cases was 10 (100%). Albumin alone had 5 (50%) cases while albumin and yolk also had 5(50 %) cases yolk alone had no case. The highest residue was $0.3029 \mu\text{g/ml}$ while the lowest was $0 \mu\text{g/ml}$ (table 7).

The result of the standard tetracycline in which the inhibition zones from the tissues were compared to establish their quantities in mg is presented in table 1.

Table 1: Inhibition zones of Tetracycline reference standard.

Concentration($\mu\text{g/ml}$)	Inhibition zone (cm) mean \pm sem (cm)
0.5	4.87 \pm 0.27
0.25	3.85 \pm 0.10
0.125	2.93 \pm 0.48
0.0625	2.50 \pm 0.00
0.03125	2.47 \pm 0.07
0.0156	2.33 \pm 0.07
0.0078	2.33 \pm 0.17

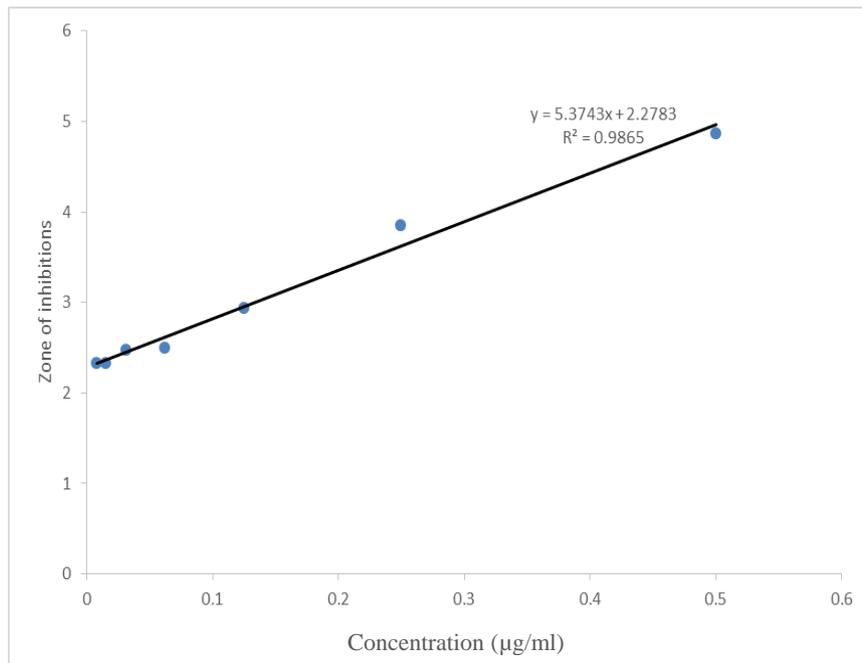


Figure 1: Graph of inhibition of tetracycline reference standards against concentration.

Table 2: Tetracycline Residues in the albumen and yolk of eggs sampled.

Egg content	No present	Percentage +ve cases	No absent	% -ve cases
Total residue	49	98%	11	22%
Albumen only	10	20%	0	0%
Yolk only	0	0%	10	20%
Albumen/yolk	39	78%	1	2%
Highest residue	-0.1355			
Lowest residue	-0.3215			

Table 3: Results of Farm 1.

Egg Content	Incidence	Percentage
Total positive	15	100%
Albumen only	4	26.7%
Yolk only	0	0%
Albumen/yolk	11	73.3%
Total negative (yolk)	4	26.7%
Highest residue	-0.1727 µg/ml	
Lowest residue	-0.3122 µg/ml	

Table 4: Results of Farm 2.

Egg Content	Incidence	Percentage
Total positive	9	90%
Albumen only	1	9.99%
Yolk only	0	0%
Albumen/yolk	8	80%
Total negative (yolk)	1	9.99%
Negative (albumen/yolk)	1	9.99%
Highest residue	-0.2192 µg/ml	
Lowest residue	-0.3029 µg/ml	

Table 5: Results of Farm 3.

Egg content	Incidence	Percentage
Total positive	10	100%
Albumen only	2	20%
Yolk only	0	0%
Albumen/yolk	8	80%

Total negative (yolk)	2	20%
Highest residue	-0.1355 µg/ml	
Lowest residue	-0.3215 µg/ml	

Table 6: Results of Farm 4.

Egg content	Incidence	Percentage
Total positive	10	100%
Albumen only	2	20%
Yolk only	0	0%
Albumen/yolk	8	80%
Total negative (yolk)	2	20%
Highest residue	-0.1912 µg/ml	
Lowest residue	-0.3122 µg/ml	

Table 7: Result of Farm 5.

Egg Content	Incidence	Percentage
Total positive	10	100%
Albumin only	5	50%
Yolk only	0	0%
Albumin/yolk	5	50%
Total Negative(Yolk)	0	0%
Highest	0.3029 µg/ml	
Lowest	0.00 µg/ml	

DISCUSSION AND CONCLUSION

Discussion

Antibiotics are the most widely used veterinary drugs in poultry.^[21] They are used for various purposes including combating bacterial infections^[20], in low doses to aid feed efficiency and prophylaxis and probiotics^[15,19], growth promoters^[22, 23] and enhance egg production.^[19]

Tetracycline was chosen as an antibiotic to access its residue because it is one of the most common antibiotics used in poultry.^[5,22,24] In addition to the benefits of antibiotics generally in animal production, tetracycline has an anti-inflammatory^[25] and antitumor effect.^[8] This may also be a contributory factor to the popularity of tetracyclines.

Because of the importance of antibiotics in poultry production, as well as their undesirable effects, regulatory agencies have set out tolerance or maximum residue levels that are not supposed to be exceeded in all tissues of animal origin for consumption including poultry products (meat and eggs). For tetracycline, it is 200 µg/kg for eggs.^[26]

The result of the experiment shows that tetracycline residues are present in egg yolk and albumen. The use of antibiotics in layers as egg boosters by farmers results in higher residues in layers and their eggs.^[20] In the present study, tetracycline residue was found to be 98%. The mean recovery value of tetracycline in eggs was found to be 76%.^[27] The mean oxytetracycline residue value in the eggs was already lower than the Maximum Residue Limit (MRL) level and reached 118 ng/g. The between-day precision was 4.8% and 5.0% respectively for oxytetracycline. Tetracycline positive sample of local

egg, commercial egg, boiled local egg, and boiled commercial egg had residue level of 9%, 45%, 7%, and 44%, respectively.^[28] This showed that commercial eggs may contain more antibiotics than local eggs and heat treatment may affect the residue. The variation in the amount of antibiotics in eggs can be explained by so many factors. It has been demonstrated that most veterinary drugs have varying degree of stability during cooking and that cooking influences the level of risk posed by drug residues.^[29] However, the effect of boiling on antibiotic residue was not significant.^[28] Refrigeration also causes disappearance of penicillin and through pasteurization, most of antibiotics losses activity. The use of activated charcoal, resin and UV irradiation also helps in lowering the level of antibiotic residues in animal tissues.^[15] In case of eggs, the pattern of appearance of drug residues will be influenced by the formation of yolk and white.^[30] The behavior of the producers in a particular region and their personal experiences with disease and drugs can influence their use of drugs.^[31]

The highest residue that was realized was -0.1355 µg/ml which was seen in the albumen, meanwhile the MRL for eggs is 200µg/kg. This value is far below the standard MRL and so there is significantly no threat posed here.

There are various methods that are used in screening these antibiotics. Some of the most common ones include microbial assays, Thin Layer chromatography(TLC), High performance liquid chromatography(HPLC), Gas Chromatography-mass spectrometry (GC-MS) as well as immunoassays.^[32, 33]

All the various method has their merits and demerits. In the present studies we used microbial assays.^[31,33] The microbial assays rely on the residues of the antibiotics to inhibit the growth of the test organism, and the pH to promote or inhibit the activities of some antibiotics. Tetracycline activities increase in acidic pH.^[32,33] It is generally used for screening purposes where large samples are involved.^[19,32,33] Other merits of the method include the fact that it is inexpensive and simple and can reveal some information on the nature or type of residue.^[32,33] In addition, this method can be improved upon by the derivation of an equation. This helps in quantifying the residues in the test medium through the equation $Y = -X + C$. Its major demerit is that some lack specificity and are generally qualitative. The others have high specificity but are expensive.^[32,33] It is clear from the result that antibiotics already exist in our food chain, especially in eggs. The awareness regarding to residue testing should be increased to determine the exact level of antibiotic residue in food for ensuring public health safety. It is therefore important that their use in food animals be done with utmost care; antibiotics should be given at recommended doses and with appropriate supervision. Adequate withdrawal period should be observed in all laying hens following therapeutic use of antibiotics.

CONCLUSION

In conclusion, the assessment of tetracycline residues using the microbiological assay as carried out in the study revealed that the level of tetracycline residues in eggs produced in Makurdi is far below the recommended MRL stipulated by the regulatory agencies and is within the acceptable daily intake.

REFERENCES

1. Heinke H, Alexandra C, Ludwig, T. The Poultry Market in Nigeria: Structures and potentials for Investment in the Market. IFAMA, 2015; 8(1): 197-221.
2. Dipeolu MA. Residues of tetracycline antibiotics in market goats and pigs in Lagos and Ogun States, Nigeria. Trop J Ani Sci., 2000; 5(2): 47-51.
3. Agbo JO, Saganuwan SA, Onyeyili PA. Tissue distribution of sulphadimidine sodium in non-starved and starved grower turkeys (*Meleagris gallopavo*). International Journal of Pharmacology and Toxicology, 2016; 4(2): 154-158.
4. Akogwu EI, Saganuwan SA, Onyeyili PA. Effects of piroxicam on tissue distribution of sulfadimidine in West African Dwarf male and female goats. Hum Exp Toxicol, 2018; 37(1): 61-68.
5. Gustafson RH, Bowen, R.E. Antimicrobial use in animal agriculture. J. Appl Microbiol, 1997; 83: 531-541.
6. Jahantigh M, Samadi K, Dizaji RE. *et al.* Antimicrobial resistance and prevalence of tetracycline resistance genes in *Escherichia coli* isolated from lesions of colibacillosis in broiler chickens in Sistan, Iran. BMC Vet Res., 2020; 16: 267.
7. Fabio GC, César R. Tetracyclines in Food and Feeding stuffs: From Regulation to Analytical Methods, Bacterial Resistance, and Environmental and Health Implications. J Anal Meth Chem., 2017; 2017: 24.
8. Wang S, Zhang HY, Wang L et al. Analysis of sulphonamide residues in edible animal products. *Food Addit Con*, 2006; 23: 362-384.
9. Manuel GR, Juan C. Angeles H. Antibiotic and synthetic growth promoters in animal diets: Review of impact and analytical methods. Food Ctrl., 2017; 72(B): 255-267.
10. Black WD. The use of antimicrobial drugs in agriculture. Can. J. Physiol. Pharmacol, 1984; 623: 1044-1048.
11. Doyle ME. Veterinary drug residues in processed meat: Potential health risk? Food Research Institute Briefings. University of Wisconsin-Madison, March 2006.
12. Kaneene JB, Miller R. Problems associated with drug residues in beef from feeds and therapy. Rev Sci Tech., 1997; 16: 694-708.
13. Donoghue DJ. Antibiotic residues in poultry tissues and eggs: Human health concerns? Poultry Sci. J., 2003; 82: 618-621.
14. Adebawale OO, Adeyemo OK, Awoyomi O. *et al.* Antibiotic use and practices in commercial poultry laying hens in Ogun State Nigeria. Rev. Elev. Med. Vet. Pays Trop., 2016; 69(1): 41-45.
15. Nisha AR. Validation of HPLC method of analysis of tetracycline residues in eggs and broiler meat and its application to a feeding trial. Antibiotic residues: A global health hazard. Vet. World, 2008; 1(12).
16. Shareef AM, Jamel ZT, Yonis KM. Detection of antibiotic residues in stored poultry products. Iraqi J Vet Sci., 2009; 23: 45-48.
17. Muhammed F, Aktar M, Rahman ZU. *et al.* Role of veterinarians in providing Residue-free veterinary food. Pakistan Vet J., 2009; 29(1): 42-46.
18. Geidam YA, Usman H, Musa HI. *et al.* Ox tetracycline and Procain Penicillin residues in tissues of slaughtered cattle in Maiduguri, Borno state, Nigeria. Terrestrial Agua Environ Toxicol, 2009; 3(2): 68-70.
19. Kabir J, Umoh, VJ, Audu E. *et al.* Veterinary drug use in poultry farms and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State. Nig Food Ctrl., 2004; 15: 99-105.
20. Olatoye IO, Ehinwomo AA. Oxytetracycline Residues in Edible tissues of cattle slaughtered in Akure, Nigeria. Internet J. Food Safety, 2009; 11: 62-66.
21. Simon AH, Baxter GA. Biosensor screening for veterinary drug residues in food stuffs. J. AOAC Int., 2006; 89(3): 862-867.
22. Alaboudi A, Basha E.A. and Musallam I. Chlortetracycline Sulfanilamide residues in table

- eggs; Prevalence, distribution between yolk and white and effect of refrigeration and heat treatment. *Food Ctrl.*, 2013; (33): 281-286.
23. Jajere SM, Lawal JR, Geidam YA. Antibiotic Residues in edible poultry Tissues and Products in Nigeria; A potential public Health Hazard. *International Journal of animal and veterinary advancement*, 2015; 7(3): 55-61.
 24. Adetunji VO, Bello ED, Odetokun LA. Assessment of Tetracycline, lead and cadmium residues in frozen chicken vended in Lagos and Ibadan, Nigeria. *Pakistan j Biol Sci.*, 2013; 15(170): 839-844.
 25. Fuoco D. Classification Framework and Chemical Biology of Tetracycline-Structure-Based Drugs. *Antibiotics (Basel)*, 2012; 12(1): 1-13.
 26. Muriuki FK, Ogara WO, Njeruh FM. *et al.* Tetracycline residue levels in cattle meat from Nairobi slaughter house in Kenya *J. Vet. Sci.*, 2001; 2(2): 97-101.
 27. De Ruyck H, De Ridder H, Van R.R. *Food Addit Contam*, 1999; 16(2): 47-56.
 28. Suchaya C, Mohammad, M.H, Mahabub A. *et al.* Antibiotic residues in milk and eggs of commercial and local farms at Chittagong, Bangladesh. *Vet World*, 2015; 8(4): 467-471.
 29. Javadi A, Mirzaei H, Khatibi S.A. Effect of roasting process on antibiotic residues in edible tissues of poultry by FPT plate. *Journal of Animal Veterinary Adv*, 2009; 8(12): 2468-2472.
 30. Mishra A, Singh S.K, Sahni *et al.* HPLC determination of cloxacillin residue in milk and effect of pasteurization. *Res. J. Pharm. Bio. Chem. Sci.*, 2011; 3: 11-16.
 31. Hakeem A, Titouche Y, Houali K, *et al.* Screening of Antibiotics Residues in Poultry Meat by Microbiological methods. *Bulletin UAVSM, Veterinary Medicine*, 2013; 70(1): 77-82.
 32. Khan MC. (2010). Chemical residues in food and fibre. *The merck veterinary manual 10th edition*. Merck and co. N.J. USA pp. 1962-1966.
 33. Ibrahim IG, Sabiel AY, Thoria O.O. *et al.* Microbial and HPLC assays for detection of Tetracycline Residues in chicken meat. *Asian J Sci Tech.*, 2015; 6(2): 1020-1022.