

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Detection of Antimicrobial Residues in Chicken Muscle and Liver Sold at Retail Outlets in Trinidad

Nkechi V. Offiah¹ and Abiodun A. Adesiyun²

¹School of Veterinary Medicine, Faculty of Medical Sciences,
University of the West Indies, St. Augustine, Trinidad and Tobago

²Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Pretoria, South Africa

Abstract: Antimicrobial agents are used for the prevention or treatment of diseases in animals but concerns have been raised that tissues of food animals contaminated with antimicrobial residues may cause adverse side effects in consumers. In Trinidad and Tobago, neither are withdrawal periods following treatment of food animals with antimicrobial agents enforced nor the practice of routine testing of meats for antimicrobial residues. This study determined the frequency of detection of three antimicrobial agents commonly used in the poultry industry in Trinidad and Tobago with the Charm II test and related it to processing plants and sale outlets. For chicken muscles, the prevalence of antimicrobial agents was 18.1, 1.7 and 0.0% for tetracycline, β -lactams and macrolides, respectively and the differences were statistically significantly different ($p < 0.05$; X^2) while the corresponding prevalence for chicken liver samples was 52.9, 0.0 and 14.9% ($p < 0.05$; X^2). Overall, the frequency of detection of antimicrobial agents in chicken liver (17.8%) was significantly ($p < 0.05$; X^2) higher than was found in chicken muscles (6.4%). Although the frequency of detection of the three antimicrobial agents in both chicken muscle and liver tissues was higher in supermarket samples (11.3%) than in those from poultry depot and other sources (6.3%), the difference was not significant ($p > 0.05$; X^2). For the 48 tetracycline-positive (muscle and liver) samples, 41 (85.4%) originated from supermarkets while only 14.6% were collected from other sources ($p < 0.05$; X^2). The frequency of detection of antimicrobial agents was not significantly ($p > 0.05$; X^2) affected by the processing plants from which the samples originated. Tetracycline residues appear to pose the highest health risk (allergic reactions and development of antimicrobial resistance), a finding in agreement with the fact that of the three antimicrobial agents tested, tetracycline was the most commonly used by broiler chicken producers in the country.

Key words: Antimicrobial residues, chicken, muscle, liver, charm test, Trinidad

INTRODUCTION

The production of high quality poultry meat for consumption is very essential and may require the use of antimicrobial agents for prophylaxis or chemotherapy (Aamer *et al.*, 2000; Agunos *et al.*, 2012; Gilchrist *et al.*, 2007). Antimicrobials in poultry have been established to have their importance in enhancing growth, feeding efficiency and reducing the incidence of disease in birds (Donoghue, 2003; Doyle, 2006). However, overuse or incorrect application of antimicrobials in animal production has led to the development of antimicrobial resistance (Gilchrist *et al.*, 2007) and antibiotic residue persistence in animal tissues such as muscle, heart, liver and kidney after processing (Donoghue, 2003). Antimicrobial residues are trace amounts of an antimicrobial remaining in animal food products after processing (Poultry Industry Council Research and Education Feed Medications, 2008). The use of antimicrobials for the treatment or prevention of disease in animals closely follows their uses in human (Pavlov *et al.*, 2008); hence the health hazards of

antimicrobial residues in edible poultry meat to consumers have drawn criticisms of their use.

There are concerns that tissues are contaminated with harmful concentrations of residues and these will possibly cause adverse effects to consumers (Donoghue, 2003; Doyle, 2006). Possible health effects include allergic and/or toxic reactions to residue; chronic toxic effects occurring with prolonged exposure to low levels of antibiotics and development of antibiotic resistance which may complicate successful treatment of human infections (Donoghue, 2003; Gilchrist *et al.*, 2007; Reig and Toldra, 2008). Drug residues that persist in edible tissues may become introduced into the human diet as a consequence of the farmer's negligence to observe the withdrawal period for the drug (s) and/or and applying antibiotics incorrectly to livestock (Kabir *et al.*, 2004; Tajick and Shohreh, 2006). Residue monitoring ensures better protection levels for consumers and as such, is enforced in the European Union (EU), a requirement laid down in the EU Directive 96/23/EC and Commission Decision 97/747/EC (Reig and Toldra, 2008; Weiss *et al.*, 2007).

In Trinidad and Tobago, 96.6% of all the broiler chicken produced is consumed in the local market (Singh and Seepersad, 2001). The local poultry industry, like any other in the world, utilizes antimicrobials for efficient production. The Caribbean Poultry Association (CPA) in its CPA Standards on veterinary drug use for poultry recommends compliance with the Codex Recommended International Code of Practice in the use of veterinary drug (Harnarine, 2007). The standards also refer to Codex Maximum Residual Limits, such as those established by Codex Alimentarius Commission, may be useful in determining minimum safety standards for feed (Harnarine, 2007). To date, there is no existing monitoring system to detect antimicrobial agents in meat samples in the country despite the fact that residues have been detected in raw milk from dairy animals (Adesiyun *et al.*, 1997) and in table eggs (Adesiyun *et al.*, 2005).

The study was therefore conducted to determine the frequency of detection of three commonly used antimicrobial agents (tetracycline, β -lactam and macrolides) in the broiler industry in Trinidad and Tobago and to relate the frequency of detection to the processing plants and sale outlets.

MATERIALS AND METHODS

Poultry industry in Trinidad and Tobago: The broiler system in Trinidad and Tobago is based mainly on contract farming where the processing plants contract farmers to raise broiler chicks to slaughtering age after which they are slaughtered and processed at the respective plants. The dressed broilers are thereafter packaged in bags with the company's labels and distributed to sale outlets primarily supermarkets where they are kept frozen or chilled for purchase by consumers. In addition, there are small poultry operators who purchase live broilers from farmers and slaughter and sell the dressed chickens at cottage poultry processors also called 'pluck shops' across the country. A majority of the chickens sold to consumers at the 'pluck shops' are fresh (unrefrigerated) while a few outlets sell their products chilled, primarily because slaughter is carried out on the request of the prospective buyer.

Study design: The study design was to randomly collect samples of chicken liver and muscle from sale outlets across the country, primarily supermarkets and poultry depots. At each outlet, a minimum of hundred grams (100 g) each of liver and muscle was obtained. The liver and muscle samples were not related to each bird. During the study, samples were bought from a total of 24 poultry depots and 65 supermarkets. Each outlet was sampled once for either muscle or liver or both. Each branch of a supermarket chain was treated as independent outlets.

Source and collection of samples: Eight (8) processing plants (A to H) which were in operation at the time of the study and distributed their dressed chickens to sale outlets across the country were included in the study. Samples were randomly collected from brands which originated from the processing plants. For each sample collected from the outlets, the type of sample (chicken muscle or liver), the identity of the outlets and processing plants were recorded. A total of 125 liver samples and 125 muscle samples were purchased but on a few occasions, the exact sample size could not be achieved for each of the sale outlets used in the study. Samples collected from the supermarkets were stored either refrigerated or frozen but those purchased from poultry depots were kept at room, refrigerated or frozen temperatures. All samples collected from the sale outlets were transported on ice to the laboratory where the fat on the carcasses was trimmed off before being stored at -20°C until analyzed.

Reagents and equipment: Charm II Test kit purchased from (Charm Sciences Inc. 659 Andover, Lawrence, MA, USA) comprised MSU Multi-Antimicrobial Concentrate Standards, MSU Extraction buffer, M2 buffer, Tissue Performance Negative Concentrate, Tablet reagents and Scintillation fluid.

Antibiotic test principle: Antimicrobial agents in the samples were analyzed using the Charm II test kit (Charm Sciences Inc. 659 Andover, Lawrence, MA, USA) which is a radioimmunoassay as described by the manufacturer's instructions and also by earlier reports (Al-Mazeedi *et al.*, 2010; Reig and Toldra, 2008). For the detection of tetracycline, H^3 -labelled tetracycline was used; for macrolides $-\text{C}^{14}$ labelled erythromycin tracer and β -Lactams $-\text{C}^{14}$ labelled penicillin tracers were used. The amount of tracer on the binding agent was measured using the scintillating counter. Antibiotics in a sample compete with the tracer for receptor sites on the binding agents. The centrifugation step separates the unbound tracer with bound tracer-binder complex. The pellet (containing tracer-binder complex) collected after centrifugation was analyzed in the counter for one (1) minute to determine the count. High count results measured as counts per minute (cpm) reflect low antibiotic levels and the samples were considered negative for antimicrobial agents and low counts were considered positive for antimicrobial agents.

Processing of samples: The frozen chicken muscle and liver samples were allowed to thaw at room temperature (25°C) before processing. Forty ml of MSU extraction buffer, provided in the test kit, was added to 10 g of liver in a 50 ml centrifuge tube. The mixture was poured into a food processor and homogenized for 30-60 sec. The homogenate was poured back into the 50 ml centrifuge

tube and incubated at 80°C for 45 min. The tube containing the incubated homogenate was placed in an ice water bath for 10 min then centrifuged at 1750G for 10 min. The resulting supernatant was decanted into a clean 50 ml centrifuge tube and used for testing and the tissue pellets were discarded.

Detection of antimicrobial residues in samples: The supernatant obtained after the liver processing was diluted in a ratio of 1:4 with negative control. The pH was then observed using a pH strip and adjusted to pH 7.5 by using M2 buffer obtained from the kit. The same procedure used for the liver samples was applied to process muscle tissue. However, the supernatant was not diluted. The final extract was tested for β-lactams, macrolides and tetracyclines using appropriate test kits and the Charm II protocol provided by the manufacturer. The control point for members of the three groups of antimicrobial agent residues in liver assay by Charm II were: β-lactams (1085 ppb), macrolides (1202 ppb) and tetracycline (1085 ppb). All antimicrobial agents were detected qualitatively. Samples with counts per minute (cpm) less than or equal to the control point were considered “suspect”. The suspect samples were retested with the negative control and the positive control as prescribed by the manufacturer. If the retested sample counts were still less than or equal to the control point and the control test results were in the expected range, the sample was considered positive.

Statistical analysis: The frequency of detection and type of antimicrobial agent was compared to sources and outlets (supermarkets and poultry depots), by the Chi-square test using the statistical Package for Social Sciences (SPSS) version 15. All statistical analyses were two-sided and were interpreted at the 5% level of significance.

RESULTS AND DISCUSSION

Frequency of detection of antimicrobial residues in chicken tissues: The frequency of detection of the three antimicrobial agents in both chicken muscle and liver when classified by the sale outlets from which they were obtained is shown in Table 1. Overall, the frequency of detection of tetracycline was 18.1% (21 of 116) for muscle tissue and 52.9% (27 of 51) for liver tissues and the difference was statistically significant ($p < 0.05$; X^2). β-lactams was detected at a frequency of 1.7% (2 of 121) and 0.0% (0 of 87) for chicken muscle and chicken liver respectively but the difference was not statistically significant ($p > 0.05$; X^2). For macrolides, the residue was detected at a statistically significantly ($p < 0.05$; X^2) higher frequency in chicken liver, 14.9% (13 of 87) compared with 0.0% (0 of 123) in chicken muscle. Overall, the frequency of detection of antimicrobial agents (tetracycline, β-lactam and macrolides) in chicken liver

Table 1: Frequency of detection of antibiotic residues in chicken muscle and liver by sale outlet

Sale outlet	Type of tissue processed											
	Chicken muscle tissue						Chicken liver					
	Tetracycline		β-lactam		Macrolide		Tetracycline		β-lactam		Macrolide	
	No. of samples tested	No. (%) positive	No. (%) positive	No. (%) positive	No. (%) positive	No. (%) positive	No. of samples tested	No. (%) positive	No. of samples tested	No. (%) positive	No. of samples tested	No. (%) positive
Supermarkets	101	18 (17.8)	2 (1.9)	107	23 (62.2)	0 (0.0)	37	0 (0.0)	45	0 (0.0)	45	7 (15.6)
Poultry depot	15	3 (20.0)	0 (0.0)	16	2 (28.6)	0 (0.0)	7	0 (0.0)	13	0 (0.0)	13	0 (0.0)
Others ^a	0	0 (0.0)	0 (0.0)	0	2 (28.6)	0 (0.0)	7	0 (0.0)	29	0 (0.0)	29	2 (6.9)
Total	116	21 (18.1)	2 (1.7)	123	27 (52.9)	0 (0.0)	51	0 (0.0)	87	0 (0.0)	87	13 (14.9)

^aComprising 7, 27 and 27 samples from local markets tested for tetracycline, beta-lactam and macrolide respectively, and 2 samples each from the University Field Station tested for beta-lactam and macrolides

(17.8%) was statistically significantly ($p < 0.05$; X^2) higher than found in chicken muscles (6.4%). The overall prevalence of 6.4 and 17.8%, respectively for chicken muscle and liver for antimicrobial residues detected in the current study is considerably lower than the 87 and 100% reported for chicken muscle and liver in Saudi Arabia (Al-Ghamdi *et al.*, 2000) and also lower than 50% detected in chickens in Iran (Tajick and Shohreh, 2006) and the 33.1% broiler chickens at slaughter houses in Nigeria (Kabir *et al.*, 2004). It was hardly a surprise that tetracycline was the predominant residue detected at a frequency of 28.7% (48 of 167) for all chicken liver tissue and muscle samples amongst the three antimicrobial agents tested. This is because the antimicrobial agent is the most frequently used in the broiler chicken production system in Trinidad and Tobago. Detection of antimicrobial agents in chicken tissues (liver and muscles) in the current study is an indication that the withdrawal period was not observed by the farmers prior to slaughter for human consumption. The frequency of detection of tetracycline in this study (28.7%) is lower than the 90.4% reported for chickens in Belgium (De Wasch, 1998). In 2012, Olusola *et al.* (2012) reported that a high percentage of frozen chickens offered for sale in Nigeria exceeded the maximum level of tetracycline set by international food safety agencies. The widespread use of tetracycline in the poultry industry in France was also attributed to the presence of antimicrobial residues in poultry products (Chauvin *et al.*, 2005). However, the prevalence of tetracycline reported in the current study is slightly higher than the 25.71% prevalence documented for the antimicrobial agents in chickens sampled in southern Benin (Mensah *et al.*, 2011). It is however pertinent to consider the detection methods for reported frequency of detection of antimicrobial agents in foods, including chickens, because of the differences in sensitivity and specificity of such tests (Dong *et al.*, 2012; Jiang *et al.*, 2013; Macarov *et al.*, 2012; Olusola *et al.*, 2012). The high frequency of tetracycline in both chicken muscle and liver samples in the current study may also be attributed in part to the broiler production system in Trinidad and Tobago. The commercial broiler processing plants operate a system where small contracted farmers are supplied day-old chicks, feeds, medication and veterinary services but are not compensated for losses of birds during production. To reduce losses, the farmers therefore self-administer antimicrobial agents, predominantly tetracycline because it is comparatively inexpensive, purchased from feed stores without the supervision of veterinarians and most importantly, failed to observe withdrawal periods. It has also been documented that tetracyclines are the most prescribed antibiotic in Africa and of all antibiotic-associated residues, they account for 41% of cases (Darwish *et al.*, 2013). The choice of local broiler farmers to use tetracyclines is supported by the fact that the

class of antibiotic is one of the most commonly used antimicrobial families in veterinary therapy, especially in poultry farming and medicine (Chopra and Roberts, 2001). The favourable properties of tetracyclines such as their broad range of activity (against gram-positive and gram-negative bacteria), the possibility of oral administration and the absence of major adverse side effects have led to their extensive use in the therapy of human and animal infections. In addition, tetracyclines are one of the cheapest classes of antibiotic available and their cost in real terms is further declining due to improved manufacturing technology (Miranda *et al.*, 2009). These facts make these agents particularly attractive for use in developing nations. However, the widespread use of these drugs has become a serious problem since they are known to leave residues in animal products intended for human consumption that can be directly toxic or cause allergic reactions in some hypersensitive individuals (Berrada *et al.*, 2008), as well as promote the occurrence of antimicrobial-resistant bacteria (Kowalski, 2008). It is however pertinent to mention that in the current study, it was not possible to match chicken liver and muscle tissues to individual broilers because the samples were obtained as packs sold at sale outlets. Antimicrobial residues in meat have been reported to cause adverse effects in consumers (Er *et al.*, 2013). These effects include allergic reactions, development of antimicrobial resistance of bacterial agents which could reduce effectiveness of therapeutic interventions and induction of toxicity in consumers (Berrada *et al.*, 2008; Doyle, 2006; Kowalski, 2008; Reig and Toldra, 2008). Although the chicken samples tested in the current study were raw and expected to be subjected to heat treatment prior to consumption, it has been reported that some antimicrobial residues such as enrofloxacin are not affected by the cooking process while the heating process which results in water loss actually increase the concentrations of the residues (Lolo *et al.*, 2006). There is therefore a need to enforce the observation of the withdrawal period through routine assay for antimicrobial residues in meats and applying appropriate penalties for non-compliance. It is also not unexpected to have a statistically significantly higher frequency of detection for tetracycline residues in liver tissues (52.9%) than in chicken muscles (18.1%). This is due to the fact that tetracyclines are distributed widely into body tissues and are found in high concentrations in the excretory organs, especially the liver and in the bile (Al-Ghamdi *et al.*, 2000). It is also known that tetracyclines undergo extensive enterohepatic circulation which leads to prolongation of their elimination half-lives; thus persisting in the body, especially the liver for a long time after cessation of drug administration. The findings in the current study agree with a published report where liver tissues are more contaminated by antimicrobial residues and even in

higher concentrations than found in chicken muscles (McCracken *et al.*, 2005). These have resulted in long withholding/withdrawal periods for oral use of tetracyclines up to 21 day depending on indications and type of tetracycline in the USA (Al-Ghamdi *et al.*, 2000). Although our survey of the broiler industry in the country demonstrates that the farmers use the three antimicrobial agents studied (tetracycline, β -lactams and macrolides), tetracyclines are by far preferably used as was reflected by the comparatively lower prevalence of β -lactams (1.0%) and macrolides (6.2%) detected in both tissues (muscle and liver) studied. It is noteworthy that although the frequency of the three antimicrobial agents in both chicken muscle and liver tissues was higher in samples collected from supermarkets (11.3%) than in those from poultry depot and other sources (6.3%), the difference was however not statistically significant ($p > 0.05$; X^2). However, of all the 63 samples positive for any of the three antimicrobial residues 50 (79.4%) originated from supermarkets and of the 48 tetracycline-positive (muscle and liver) samples, 41 (85.4%) originated from supermarkets while only 14.6% were collected from poultry depots/other sources ($p < 0.05$; X^2). Failure to detect significantly higher frequency of the three antimicrobial agents in chickens sampled at the supermarkets compared to other outlets in the current study, may be explained in part, by differences in the use of antimicrobial agents on the farms from where the broilers originated. This is because, although the chickens may have been supplied to the sale outlets by the processing plants or individual farmers, the present study was not designed to determine the practice regarding the use of antimicrobial agents on the respective farms. The comparatively lower prevalence of antimicrobial agents in chickens sampled at the 'pluck shops' may have also been due to the practice where the live birds taken to these outlets were not necessarily slaughtered the same day they arrive which may allow more time for the metabolism of antimicrobial agents that may be present in the chicken tissues compared to the supermarkets that receive most of their dressed chickens directly from the processing plants.

Frequency of detection of residues by processing plants: Table 2 shows the frequency of detection of antimicrobial residues in chicken muscle and liver by the processing plants from which they originated. For the residues detected in chicken muscle tissues, both tetracycline (31.8%) and β -lactams (8.7%) were detected at the highest frequency in Plant A while for chicken liver samples, Plants D and E had the highest frequency of tetracycline residue (100.0%) but macrolides (50.0%) were highest in frequency in Plant D. The differences were however not statistically significant ($p > 0.05$; X^2). For both tissues (muscle and liver) tested, the frequency of detection of tetracycline

Table 2: Frequency of detection of antibiotic residues in chicken muscle and liver by processors

Processor	Type of tissue processed											
	Chicken muscle tissue				Chicken liver				Total			
	Tetracycline		β -lactams		Macrolides		Tetracycline		β -lactams		Macrolides	
No. of samples tested	No. (%) positive	No. of samples tested	No. (%) positive	No. of samples tested	No. (%) positive	No. of samples tested	No. (%) positive	No. of samples tested	No. (%) positive	No. of samples tested	No. (%) positive	
A	22	7 (31.8)	23	2 (8.7)	25	0 (0.0)	9	6 (66.7)	11	0 (0.0)	11	2 (1.8)
B	35	3 (8.6)	36	0 (0.0)	35	0 (0.0)	13	6 (46.2)	15	0 (0.0)	15	1 (6.7)
C	33	6 (18.2)	36	0 (0.0)	37	0 (0.0)	16	9 (56.3)	24	0 (0.0)	24	3 (12.5)
D	10	3 (30.0)	12	0 (0.0)	12	0 (0.0)	1	1 (100.0)	2	0 (0.0)	2	1 (50.0)
E	5	0 (0.0)	5	0 (0.0)	5	0 (0.0)	2	2 (100)	2	0 (0.0)	2	0 (0.0)
F	1	0 (0.0)	1	0 (0.0)	1	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
G	10	2 (20.0)	8	0 (0.0)	8	0 (0.0)	10	3 (30.0)	33	0 (0.0)	31	6 (18.2)
H	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)	2	0 (0.0)	2	0 (0.0)
Total	116	21 (18.1)	121	2 (1.7)	123	0 (0.0)	51	27 (52.9)	87	0 (0.0)	87	13 (14.9)

ranged from 0.0% (0 of 1) in Plant F to 41.9% (13 of 31) in Plant A; for β -lactams, the range was from 0.0% (Plants B to H) to 5.9% (2 of 34) in Plant A and for macrolides, the lowest frequency of detection, 0.0% was detected in Plants E, F and H and the highest, 15.4% (6 of 39) in Plant G. The differences were not statistically significant ($p>0.05$; X^2). The finding that the range of prevalence of the three antimicrobial agents was wide, albeit with the difference not statistically significant, reflects varying practices at the broiler farm level which may be affected by the frequency of use of antimicrobial agents and possible voluntary compliance with withdrawal periods recommended by the manufacturers. Again, the absence of a significant difference may have been compounded by the fact that the current study was product-based and not farm-based. In conclusion, the need to institute measures to enforce the compliance with the stipulated withdrawal periods for antimicrobial agents used in the country cannot be over-emphasized. Routine random testing of poultry products incorporated into a trace back system to identify the origins of residue-positive chickens is also necessary. It is only when these interventions are implemented that consumers will be exposed to antimicrobial residue-free chicken and chicken products.

ACKNOWLEDGEMENTS

The study was funded by the University of the West Indies, St. Augustine Campus Research and Publication Fund Committee (CRP.4.FEB07.16). The technical assistance of Alva Stewart-Johnson and Roshawn Pierre-Alfred is appreciated. The authors are grateful to Mr. Anthony Bastaldo for his assistance in the sample collection.

REFERENCES

Aamer, M., A.A. Javaid and A. Muhammad, 2000. Rational use of drugs in broiler meat Production. *Int. J. Agri. Biol.*, 2: 269-272.

Adesiyun, A.A., L.A. Webb and H.T. Romain, 1997. Relatedness of *Staphylococcus aureus* strains isolated from milk and human handlers in dairy farms in Trinidad. *Zentralbl. Veterinar. B.*, 449: 551-560.

Adesiyun, A.A., V.N. Offiah, N. Seepersadsingh, V. Lashley, S. Rodrigo, L. Musai and K. Georges, 2005. Microbial health risk posed by table eggs in Trinidad. *Epidemiol. Infect.*, 133: 1049-1056.

Agunos, A., D. Leger and C. Carson, 2012. Review of antimicrobial therapy of selected bacterial disease in broiler chickens in Canada. *Can. Vet. J.*, 53: 1289-1300.

Al-Ghamdi, M.S., Z.H. Al-Mustafa, F. El-Morsy, A. Al-Faky, I. Haider and H. Essa, 2000. Residues of tetracycline compound in poultry products in the eastern province of Saudi Arabia. *Public Hlth.*, 114: 300-304.

Al-Mazeedi, H.M., A.B. Abbas, H.F. Alomirah, W.Y. Al-Jouhar, S.A. Al-Muftly, M.M. Ezzelregal and R.A. Al-Owaish, 2010. Screening for tetracycline residues in food products of animal origin in the State of Kuwait using Charm II radio-immunoassay and LC/MS/MS methods. *Food Addit. Contam.*, 27: 291-301.

Berrada, H., F. Borrull, G. Font and R.M. Marce, 2008. Determination of macrolide antibiotics in meat and fish using pressurized liquid extraction and liquid chromatography-mass spectrometry. *J. Chromatogr. A.*, 1208: 83-89.

Chauvin, C., S. Le Bouquin-Leneveu, A. Hardy, D. Haguët, J.P. Orand and P. Sanders, 2005. An original system for the continuous monitoring of antimicrobial use in poultry production in France. *J. Vet. Pharmacol. Ther.*, 28: 515-523.

Chopra, I. and M. Roberts, 2001. Tetracycline antibiotics: mode of action, applications, molecular biology and epidemiology of bacterial resistance. *Microbiol. Mol. Biol. Rev.*, 65: 232-326.

Darwish, W.S., E.A. Eldaly, M.T. El-Abbasy, Y. Ikenaka, S. Nakayama and M. Ishizuka, 2013. Antibiotic residues in food: the African scenario. *Jpn. J. Vet. Res.*, 61: 13-22.

De Wasch, K., L. Okerman, S. Croubels, H. De Brabander, J.D. Van Hoof and P. De Backer, 1998. Detection of residues of tetracycline antibiotics in pork and chicken meat: correlation between results of screening and confirmatory test. *Analyst.*, 123: 2737-2741.

Dong, C.Y., Y.Y. Liu and Y. Liu, 2012. Determination of sparfloxacin concentrations in chicken serums and residues in chicken tissues and manures using self-ordered ring fluorescence microscopic imaging technique. *Guang. Pu Xue. Yu Guang. Pu Fen Xi.*, 32: 2759-2764.

Donoghue, D.J., 2003. Antibiotic residues in poultry tissues and eggs: Human health concerns? *Poult. Sci.*, 82: 618-621.

Doyle, M., 2006. Veterinary Drug Residues in Processed Meats-Potential Risks. A Review of the Scientific Literature, pp: 1-5.

Er, B., F.K. Onurdag, B. Demirhan, S.O. Ozgacar, A.B. Oktem and U. Abbasoglu, 2013. Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey. *Poult. Sci.*, 92: 2212-2215.

Gilchrist, M.J., C. Greko, D.B. Wallinga, G.W. Beran, D.G. Riley and P.S. Thorne, 2007. The potential of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environ. Hlth. Perspect.*, 115: 313-316.

Harnarine, R., 2007. CPA Standards for Poultry Feeds, Trinidad and Tobago Bureau of Standards. Bureau of Standard withdraw the publication because it is under review. http://www.caribbeanpoultry.org/docs/2005/egg_2005/CPAPoultry&EggSch2005-Poultry_Poultry_RHarnarine_CRO.pdf. pp: 16-17.

- Jiang, W., R.C Beier, Z. Wang, Y. Wu and J. Shen, 2013. Simultaneous screening analysis of 3-methyl-quinoxaline-2-carboxylic acid and quinoxaline-2-carboxylic acid residues in edible animal tissues by a competitive indirect immunoassay. *J. Agric. Food Chem.*, 61: 10018-10025.
- Kabir, J., V.J. Umoh, E. Audu-Okoh, J.U. Umoh and J.K.P. Kwaga, 2004. Veterinary drug use in poultry farms and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State. Nigeria. *Food Contr.*, 15: 99-105.
- Kowalski, P., 2008. Capillary electrophoretic method for the simultaneous determination of tetracycline residues in fish samples. *J. Pharm. Biomed. Anal.*, 47: 487-493.
- Lolo, M., S. Pedreira, J.M. Miranda, B.I. Vazquez, C.M. Franco, A. Cepeda and C. Fente, 2006. Effect of cooking on enrofloxacin residues in chicken tissue. *Food Addit. Contam.*, 23: 988-993.
- McCracken, R.J., J.A. Van Rhijn and D.G. Kennedy, 2005. The occurrence of nitrofurans metabolites in the tissues of chickens exposed to very low dietary concentrations of nitrofurans. *Food Addit. Contam.*, 22: 567-572.
- Macarov, C.A., L. Tong, M. Martinez-Huelamo, M.P. Hermo, E. Chirila, Y.X. Wang and D.J. Barron, 2012. Multi residue determination of the penicillins regulated by the European Union, in bovine, porcine and chicken muscle by LC-MS/MS. *Food Chem.*, 135: 2612-2621.
- Mensah, S.E.P., H.Y. Ahissou, O.D. Koudande, S. Salifou, G.A. Mensah and F.A. Abiola, 2011. Detection of antibiotics residues in meat of reformed and marketed laying hens in southern Benin. *Int. J. Bio. Chem. Sci.*, 5: 2195-2204.
- Miranda, J.M., J.A. Rodriguez and C.A. Galan-Vidal, 2009. Simultaneous determination of tetracyclines in poultry muscle by capillary zone electrophoresis. *J. Chromatogr. A.*, 1216: 3366-3371.
- Olusola, A.V., B.E. Diana and O.I. Ayoade, 2012. Assessment of tetracycline, lead and cadmium residues in frozen chicken vended in Lagos and Ibadan, Nigeria. *Pak. J. Biol. Sci.*, 15: 839-844.
- Pavlov, A., L. Lashev, I. Vachin and V. Rusev, 2008. Residues of antimicrobial drugs in chicken meat and offals. *Trakia J. Sci.*, 6: 23-25.
- Poultry Industry Council Research and Education Feed Medications, 2008. <http://www.poultryindustry council.ca/compendium-antimicrobial.html>.
- Reig, M. and F. Toldra, 2008. Veterinary drug residues in meat: Concerns and rapid methods for detection. *Meat Sci.*, 78: 60-67.
- Singh, R. and G. Seepersad, 2001. A profile of the broiler industry in Trinidad and Tobago. In: *The Caribbean Poultry Industry: competitiveness trade policy and development strategies*. Publication of Caribbean Poultry Association, pp: 20.
- Tajick, M.A. and B. Shohreh, 2006. Detection of antibiotic residues in chicken meat using TLC. *Int. J. Poult. Sci.*, 5: 611-612.
- Weiss, C., A. Conte, C. Milandri, G. Scortichini, P. Semprini, R. Usberti and G. Migliorati, 2007. Veterinary drugs residue monitoring in Italian poultry: Current strategies and possible developments. *Food Contr.*, 18: 1068-1076.