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Screening of Antimicrobial Residues and Confirmation of Doxycycline in Samples Collected from Chicken Farms and Processing Units Located Around Mumbai, India

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ABSTRACT

The presence of antimicrobial residues in meat has several impacts on health aspects to the consumer. Strategic and scientific study on risk assessment of antibiotic residue in poultry production chain with different set of practices has not been conducted in order to protect the health of common consumers. The present study was planned to screen antibiotic residue in chicken meat production chain managed under different set of practices of farming and processing. Chicken farms managed under non-integrated, partially integrated and completely integrated sector and three different individual chicken processing units identified as retail shops, semi-automatic processing unit and automated processing unit along with one product processing unit were randomly selected for the collection of samples. A total of 271 samples (meat, serum, water and feed) comprising of 180 samples from poultry farms, 81 samples from chicken processing units and 10 muscle samples from poultry product processing units were collected and subjected for qualitative antimicrobial screening by Microbial Inhibition Assay Test Kit and confirmation by High Performance Liquid Chromatography /Liquid Chromatography Mass Spectrometry was done. On screening by Microbial Inhibition Assay Test Kit, Muscle (2), Liver (2) and Feed (7) samples (6.11%) were positive from non-integrated (3.88%) and partially integrated farming systems (2.22%). Significant difference ($P<0.05$) was observed for antibiotic residue in feed samples of non-integrated (55.55%) and partially integrated (22.22%) farms. On screening of 91 samples, collected from chicken processing and product processing units, 08 (8.79%) samples were -positive. The HPLC analysis indicated that 9.5% and 5.26% muscle and liver samples, respectively were positive for doxycycline residue. Feed samples (57.14%) found positive to Chlortetracycline and 4-Epichlorotetracycline residues when analyzed by LCMS, with mean concentration of 2722.12 and 3720.87µg/kg, respectively. The presence of antibiotic residues in muscle and liver samples of unorganized farms and retail processing unit was higher in comparison. Therefore, the poultry farming and processing systems should be kept under continuous monitoring for preventing the occurrence of antibiotic residues in meat and meat products.

Key words: Chicken, Antibiotic residue, HPLC, Doxycyline.

INTRODUCTION

India is the world's second largest emerging economy with rapidly expanding poultry sector at annual growth rate of 10-12 per cent for broiler production (Kotaiah, 2016). The consumer demand for processed poultry products like chilled or frozen chicken has also been increased. Out of total meat production in India (8.11 million tons), poultry alone contributes 50% share amongst all categories of meat (GOI, 2019). Indian broiler production for year 2016 estimated to be 4.2 million tons with 3.1 kg per capita consumption of poultry meat per year (USDA, 2015). Shift in the process of traditional poultry supply chain to vertical integration has developed way for poultry meat availability and better value creation of poultry meat products. Still there are two important food born risk factors from poultry to human health which includes food pathogen contamination and residues from veterinary medication (Kiilholma, 2007).

Amongst veterinary residues, antibiotic residues in meat have been a rising issue in the recent years in India. Antibiotics have been used in poultry for treatment of infections and also to counteract the adverse consequences of stress responses (Refsdal, 2000; Oyekunle *et al.,* 2003; Apata, 2009) however, the indiscriminate 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 antibiotic residues is a relatively recent preoccupation.

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The presence of antimicrobial residues in meat has several impacts on health aspects to the consumer like possible hypersensitivity reaction and contribution to the development of antibiotic resistance bacteria (Dewdney *et al*., 1991; Al-Ghamdi *et al*., 2000; Bogialli and Corcia, 2009). The extensive use of antimicrobials in human and animals has led to an increase in bacterial multidrug resistance which includes several bacterial strains *viz*; Methicillin-Resistant *Staphylococcus aureus*, *E. coli*, *Pasteurella* spp. and *Campylobacter* spp. etc. (Gilchrist *et al*., 2007; Nikaido, 2009). National Antimicrobial Resistance Monitoring System of USA reported decline in antimicrobial resistance levels of *Salmonella* isolated from retail chicken, from 38 per cent in 2009 to 20 per cent in 2013 (FDA, 2016). The situation in the developing countries like India is however different, where antimicrobial agents including unapproved fixed dose combination are readily available to people at local drug stores without prescription (Laxminarayan and Chaudhary, 2016; McGettigan *et al*., 2018) and national level surveillance network is not available.

Presences of antibiotics such as tetracycline, sulphadimidine and fluoroquinolones etc. was reported in more than 25 per cent of chicken meat samples by various workers in India and abroad by using screening and confirmatory tests (Gaudin *et al.,* 2009 Cetinkaya *et al*., 2012; Gebre, 2012; Kim *et al.,* 2013a CES, 2014). The poultry meat is now a most common commercially available animal protein and in demand by consumers in India (Singh *et al*., 2016). Strategic and scientific study on risk assessment of antibiotic residue in poultry production chain with different set of practices has not been conducted in order to protect health of common consumers Healthy safe meat must be free of antibiotics residues or within maximum residual limits (MRL) as recommended by the international Codex Alimentarius Commission (CAC) and Food safety ad standards authority of India(FSSAI). Therefore, considering all above facts, the present study was planned to screen antibiotic residue in chicken meat production chain managed under different set of practices.

MATERIALS AND METHODS

Sampling

Study area

Chicken farms managed under non-integrated (N=9), partially integrated (N=9) and completely integrated (N=12) sectors, three different individual chicken processing units (retail shops, semi-automatic processing unit and automated processing unit) and one product processing unit located around Mumbai, India were randomly selected for the collection of samples.

Samples

Samples *viz*. tissue, (muscle, liver and kidney) feed, water and serum for the antibiotic residue detection were collected from broiler poultry farms and poultry processing units. A total of 271 samples comprising of 180 samples from poultry farms (30 each of muscle, liver, kidney, feed, water and serum) and 81 samples from chicken processing units (27 each of muscle, liver and kidney) and 10 muscle samples from poultry product processing units were collected and subjected for qualitative antimicrobial screening by Microbial Inhibition Assay Test Kit (Premi® Test Kit). Samples from farm live birds were collected by slaughtering the birds at 6th week of age. Samples were collected in sterile bags and placed in an insulated box at 4-5°C before being transported to the laboratory and stored at -18°C till further analysis. Feed samples were also stored in refrigeration temperature to avoid fungal growth.

Screening of antimicrobial residue

The frozen tissue samples were thawed and the liquid therein was recovered from tissue used for analysis. The samples were processed as per the manufacturers' instruction. Water and serum samples were directly subjected for analysis as they do not require any extraction process. Feed sample weighing 10 gm was mixed with 30 ml deionized water in the glass beaker. The mixture was then stirred for 30 minutes at room temperature using open platform shaker and suspension was used for analysis. The samples subjected for an initial screening with the Microbial Inhibition Assay Test Kit (DSM Premi® Test, Netherlands), a qualitative test helps to detect the family of beta-lactam antibiotics, sulfonamides, amino-glycosides, quinolones, macrolides and tetracyclines. Premi® test kits ampoules containing agar with spores of *Bacillus stearothermophilusvarcalidolactis* were inoculated with 100 µl extract of tissue (muscle, liver, kidney) feed suspension, water and serum. The ampoules were heated at 64°C, for the germination of spores. The germination and multiplication of spores lead to formation of an acid, if no inhibitory substances are present, leading to a colour change from purple to yellow of the indicator. When antimicrobial molecules are present above the detection levels, spore will not germinate and the colour remains purple (Kilinc *et al*., 2007).

Confirmation of doxycycline residue

A total of 59 samples (positive by Microbial Inhibition Assay Test and randomly selected negative samples) comprising of muscle (21), liver (19), kidney (19) were subjected for confirmation of Doxycycline residue by High Performance liquid Chromatography. (HPLC).

Sample extraction

Sample was extracted as per the method described by Şenyuva *et al*., (2000) with slight modification in centrifugation of sample. Chicken meat sample (breast muscle and/or leg muscle) was minced and used for analysis. About 2 g of minced sample (muscle/tissue) was homogenized using blender for 2 min. To this blended mixture, 0.1 g citric acid monohydrate, 1 ml nitric acid (30%), 4 ml methanol and 1 ml deionized water was added. This suspension with solid particles was vortexed followed by ultrasonication for 15 min and centrifuged at 4000 rpm for

10 minutes. Supernatant was filtered through Whatman filter paper number 02. The supernatant liquid was mixed well by shaking and centrifuged at 5000 rpm for 10 minutes. Filtrate obtained was dried under vacuum (30 psi/45 min) and then the residue was re-suspended in 1 ml Mobile phase (0.01 M oxalic acid and acetonitrile). Finally the re-suspended filtrate residue was filtered through 0.22 µm syringe filter (MDI Pvt. Ltd) and used for analysis.

HPLC System and chromatographic conditions

Analyses of samples were carried out on Agilent Technologies Model 1260 Infinity HPLC system. Separation was carried out by C18 column by Supelco (Sigma Aldrich) (C18 - 250 x 4.0 mm -5 µm) coupled with a Diode Array Detector (DAD). Centrifugation was performed with a refrigerated centrifuge model Remi PR-24 (Jouan). Drying of sample was done in Eppendorf vacuum concentrator plus. Chromatographic condition were adjusted as, Flow rate: 0.5 mL/min, Column temperature: 20°C, Injection volume: 25 µL, Detector wavelength: 360 nm, Mobile phase: Distilled water (0.01M oxalic acid) / acetonitrile (85:15 v/v) and Run Time: 10.0 minutes for sample analysis. Quantification was carried out by comparison of the analyte peak areas of sample versus calibration curve.

Confirmation of doxycycline, chlortetracycline and its metabolite 4-Epichlorotetracyclin residue in feed Samples

To determine the residuals level of Doxycycline, Chlortetracycline and its metabolite 4-Epichlorotetracyclin by liquid Chromatography Mass Spectrometry (LCMS), feed samples (positive by Microbial Inhibition Assay Test and few negative) were submitted to Microchem Silliker Pvt. ltd. Laboratory (TTC Industrial Area, MIDC, Mahape, Navi Mumbai, India). Standard protocol of AOAC 995.09/1999 and Agilent 6460 LCMS/MS were used to analyze these samples.

RESULTS AND DISCUSSION

Screening of 180 samples with Premi® test kit indicated that, 11 samples (6.11%) were positive with per cent occurrence, corresponding to 3.88% and 2.22% for non-integrated and partially integrated farming systems, respectively. Amongst various farming systems antibiotic residue per cent occurrence of 12.96% and 7.40% was observed in non-integrated and partially integrated farms respectively, whereas antibiotic residue was not identified in completely integrated farms. On screening of farm, muscle (6.6%), liver 6.6% and feed (23.33%) samples were positive. None of the kidney, serum and water samples from various farming systems was positive. Significant difference ($P<0.05$) was observed for antibiotic residue in feed samples of non-integrated (55.55%) and partially integrated (22.22%) farms (Table 1).

Eight samples (8.79%) were positive from chicken processing and product processing units, in screening test. Muscle (8.10%) and liver (18.51%) samples were positive, while kidney samples were negative. Amongst various processing units retail shops (18.51%) showed highest

Table 1: Antibiotic residue in samples of various farming systems screened by Microbial Inhibition Assay.

Types of farming system	Number of samples	No. of positive samples						Total occurrence
		Muscle (n=30)	Liver (n=30)	Kidney (n=30)	Serum (n=30)	Water (n=30)	Feed (n=30)	
Non-integrated farms (n=9/samples type)	54	01(11.11)	01(11.11)	00	00	00	05[a] (55.55)	07 (12.96) [3.88]
Partially integrated farms (n=9/samples type)	54	01(11.11)	01(11.11)	00	00	00	02[b] (22.22)	04 (7.40) [2.22]
Completely integrated farms (n=12/samples type)	72	00	00	00	00	00	00[b]	00
Total	180	02[6.66]	02[6.66]	00	00	00	07[23.33]	11[6.11]

Note:

1. Figs in the parenthesis indicate percent occurrence.
2. () = Occurrence within farming system and [ ] = Overall occurrence amongst sources/system.
3. Values in the same column with the different superscripts letters are significantly different at 1% and 5% level.

Table 2: Antibiotic residue in samples collected from different poultry Processing units and screened by Microbial Inhibition Assay.

Types of poultry processing unit	Total number of samples	No. of positive samples			Total
		Muscle	Liver	Kidney	
Retail shop (n = 9 /sample type)	27	02	03	00	05(18.51)
Semi -automated (n = 9 /sample type)	27	01	02	00	03(11.11)
Automated (n = 9 /sample type)	27	00	00	00	00
Product processing plant	10	00	NA	NA	00
Total	91	03(8.10)[3.29]	05 (18.51)[5.49]	00	08[8.79]

occurrence followed by semi-automated (11.11%). None of the sample from automated processing unit was positive (Table 2).

The screening results indicated the occurrence of antibiotic residue in poultry tissue samples are in agreement with previous antimicrobial residue screening studies in broiler muscle (Wasch *et al*., 1998; Okerman *et al.*, 2001). Similarly, higher percentage ranging from 13.00 -24.30 was reported in earlier studies (Kim *et al*., 2013a, Hussein and Khalil, 2013; Ezenduka *et al.,* 2014). Interestingly, Bute (2008) in Mumbai, India recorded zero occurrence of antibiotic residue in poultry meat sample by Premi® test kit method. Studies have showed that Premi® test kit was suitable and sensitive for screening studies (Marcinčák *et al,* 2006; Maria *et al.,* 2007).

Results of doxycycline residue by chromatographic analysis (HPLC) (Table 3) indicated that out of 21 muscle and 19 liver samples subjected for confirmation, two muscle (9.5%) and one liver (5.26%) samples were positive. One each muscle sample belonging to retail shop and semi-automated processing plant showed concentration of 186.29 ppb and 89.28 ppb, respectively. Whereas, liver samples belonging to retail shop showed concentration of 125.22 ppb (Fig 1). None of the sample of kidney and meat products were positive for doxycycline residue. One sample out of two muscle samples was found to contain doxycycline above MRL level whereas, liver sample was found below MRL level. The muscle sample which was below MRL found negative by Premi® test kit due to its detection limit of 100 µg/kg of sample.

Detection of doxycycline residue occurrence of 9.5% and 5.26%, in muscle and liver, respectively is in agreement with previous study carried out by Singh *et al.* (2016), who reported 3.3% in muscle and 16.67% in liver samples, but observed 21.73% samples were above MRL recommended by EU. Similarly, Salama *et al*. (2011) and Okerman *et al.* (2001) recorded incidence of 12.67% and 7.01%, respectively in poultry meat. Sattar *et al*. (2014) reported tetracycline residue in samples of liver (48%), kidney (24%), thigh muscle (20%) and breast muscle (24%). Lower concentration of doxycycline was detected by Cetinkaya *et al*. (2012) in the range of 19.9 to 35.6 µg/kg. In the present study one out of two muscle sample was found to contain doxycycline above MRL level whereas, liver sample was found below MRL level. The occurrence of doxycycline in samples might be due to administration of doxycycline as a therapeutic dose at the farm. The results demonstrated that doxycycline used in poultry and may appear at age of slaughter which may pose serious public health hazard.

Eight samples (57.14%) amongst 14 samples subjected to analysis of doxycycline, chlortetracycline and 4-Epichlorotetracycline residues by LCMS were positive. None of the feed sample was positive for the doxycycline residue. Mean concentration observed for 4-epichlortetracycline and chlortetracycline were 2722.12 and 3720.87µg/kg respectively (Table 4a and 4b). Similar results were reported by Siwczyñska *et al.* (2015) with concentrations ranging from 0.32 to 48.98 ppm in 33.3% of feed samples. Contrast results were reported by Sakdinun *et al*. (2006) with average concentration of 77.17 ppm of chlortetracycline residue in only 2.81% poultry feed samples.

The extensive use of chlortetracycline agent in chicken production systems observed in survey reports of Kim *et al*., 2013b; Kodimalar *et al*., 2014; Chinchilla and Rodríguez,

Table 3: Concentration of doxycycline residue in tissue samples analyzed by HPLC.

S. No.	Sample type	Sample code	Source	MRL in ppb (EU)	Percent occurrence	Concentration in ppb	Positive by Microbial Inhibition Assay
1	Muscle	RSML1	Retail shop	100	9.5 (n =21)	186.29	Yes
2	Muscle	SMMT4	Semi automated			89.28	No
3	Liver	RSL5	Retail shop	300	5.26 (n = 19)	125.22	Yes

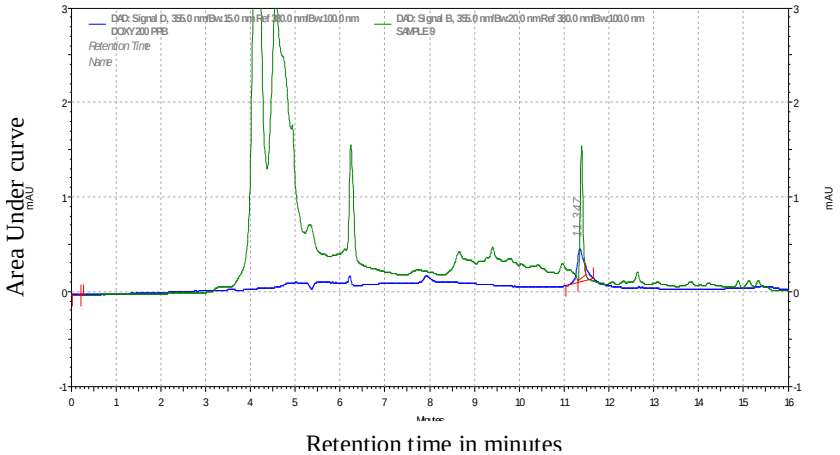

Fig 1: Chromatogram of poultry meat sample collected from retail shop establishment showing positive peak of doxycycline residue.

Table 4(a): Concentration of chlortetracycline residue in feed samples.

Sample Code	Source	Chlortetracycline (µg/kg)	4- epichlortetracycline (µg/kg)
AFR 1	Partially Integrated Farms	4204.00	2807
KBFM		4316.00	3213
LFA1		77.00	54.0
VFM		87.00	44.00
A2F5	Non Integrated Farms	675	407.0
GMFR		4346	3161
BMRF		8745	6492.0
AASFA		7317.00	5599

Table 4(b): Concentration of chlortetracycline residues detected in feed.

Tetracycline	n/N	% positive	Mean (µg/kg)	SD (µg/kg)	Min (µg/kg)	Max (µg/kg)
4- epi chlortetracycline	8/14	57.14	2722.12	2466.35	44	6492
Chlortetracycline	8/14	57.14	3720.87	3271.35	77	8745

N- Total number of samples

n- Number of positive samples

SD- standard Deviation

2017. In Europe, since 1998 cattle and broiler chicken industries voluntarily stoped the utilization of all antibiotic growth promoters, while an EU banned all antibiotics as growth promoters from 2006 recently in 2017 USA [US-FDA part 558] also banned tetracycline for use as growth promoters (Aarestrup, 2003; Jensen and Hayes, 2014).

The presence of antimicrobial residues in meat has several impacts on health aspects to the consumer like possible contribution to the development of antibiotic resistance bacteria (Apata, 2009). Various workers reported presence of drug resistance genes against tetracycline, broad and extended-spectrum β-lactamase antibiotics in food borne pathogens due to selective pressure (Waghamare *et al*., 2018).

The presence of tetracycline antibiotics in feed can occur as a result of the illegal use or as a result of production of medicated feed in mill or extra label administration in farms as growth promoter. Chlortetracycline is available in the market in bulk powder form compared to doxycycline which may be the reason for extensive use of chlortetracycline in feed. The withdrawal period must be appropriately followed before the slaughter of the birds. These results are valid for the samples collected during the study which may change periodically. Adequate withdrawal period and implementation HACCP approach specifically developed to control chemical hazards should be observed in all poultry farms and processing units to check antimicrobial resistance phenomena and maintain their potency for use in human medicine

CONCLUSION

The presence of antibiotic residues percentages in muscle and liver samples of unorganized farms and retail processing unit is higher in comparison. Indiscriminate and irrational use of antibiotics in poultry without following withdrawal period may result in unexpected residues in meat. Occurrence of tetracycline group of antibiotics in poultry tissue and feed samples could pose risk to human and animal health if they are transferred to people via food chain. Therefore, the poultry farming and processing systems should be kept under continuous monitoring for preventing occurrence of residue in meat and meat products.

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REFERENCES

Aarestrup, F.M. (2003). Effects of termination of AGP use on antimicrobials resistance in food animals. Working papers for the WHO international review panels evaluation," Document WHO/CDS/CPE/ZFK/2003.1a, World Health Organization, Geneva, Switzerland.

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

Apata, D.F. (2009). Antibiotic Resistance in Poultry. International Journal of Poultry Science. 8(4): 404-408.

Bogialli, S. and A.D. Corcia (2009). Recent applications of liquid chromatography mass spectrometry to residue analysis of antimicrobials in food of animal origin. Analytical and Bioanalytical Chemistry. 395: 947-966.

Bute, V.S. (2008). Detection of antimicrobial residues in chicken meat. M.V.Sc thesis submitted to Maharashtra animal and Fishery Sciences University Nagpur.

Cetinkayaa, F., A. Yibara, G.E. Soyutemiza, B. Okutanb, A. Ozcanc and M.Y. Karacac (2012). Determination of tetracycline residues

in chicken meat by liquid chromatography-tandem mass spectrometry. Food Additives and Contaminants: Part B: Surveillance. 5(1): 45-49.

Chinchilla, F.B. and C. Rodríguez (2017). Journal of Analytical Methods in Chemistry, Article ID 1315497 in press.

CSE Study (2014). Report on Antibiotics in Chicken Meat, Centre for science and environment 41, Tughlakabad Institutional area, New Delhi.

Dewdney, J.M., L. Maes, J. P. Raynaud, F. Blanc, J.P. Scheid, T. Jackson, S. Lens, C. Verschueren (1991). Risk assessment of antibiotic residues of β-lactams and macrolides in food products with regard to their immunoallergic potential. Food and Chemical Toxicology. 29: 477-483.

Ezenduka, E.V., O.S. Ike and N.J. Anaelom (2014). Rapid detection of antimicrobial residues in poultry: A consequence of non-prudent use of antimicrobials. Health. 6(2): 149-152.

Food Drug Administration (2016). NARMS Now (ed.) Food and Drug Administration. (Rockville, MD: U.S. Department of Health and Human Services). Available from:https://www.fda.gov/animalVeterinary/SafetyHealth/Antimicrobial Resistance/NationalAntimicrobialResistanceMonitoringSystem/ucm416741.htm.

Gaudin, V., Juhel-Gaugain, M., Mortain, J-P., Sanders, P. (2008). AFNOR validation of Premi®Test, a microbiological-based screening tube-test for the detection of antimicrobial residues in animal *muscle* tissue Food Additives and Contaminants. 25:1451-1464.

Gebre, B.A. (2012). Qualitative screening of antibiotic residues and identification of antibiotic resistant *Salmonella* from raw and ready to eat meat in Thailand. International Journal of Advanced Life Sciences. 5(1): 51-64.

Gilchrist, M.J., C. Greko, D.B. Wallinga, G.W. Beran, D.G. Riley and P.S. Thorne (2007). The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. Environmental Health Perspectives. 115: 313-316.

GOI (2019). Government of India (GOI) Basic Animal Husbandry Statistics. Ministry of Agriculture. Department of Animal Husbandry, Dairying and Fisheries. New Delhi.

Hussein, M.A. and S. Khalil (2013). Screening of some antibiotics and anabolic steroids residues in broiler fillet marketed in El-Sharkia governorate. Life Science Journal. 10(1): 2111-2118.

Jensen, H.H. and D.J. Hayes (2014). Impact of Denmark's ban on antimicrobials for growth promotion. Current Opinion in Microbiology. 19(1): 30-36.

Kiilholma, J. (2007). Food-safety concerns in the poultry sector of developing countries In. Lesna, I., Wolfs, P., Faraji, F., Roy, L.,Komdeur, J. and Sabelis, M.W. (2009). Candidate predators for biological control of the poultry red mite Dermanyssus gallinae. Experimental and Applied Acarology. 48(1-2): 63-80.

Kim, D.P., G. Degand, C. Douny, G. Pierret, P. Delahaut, V.D. To, B. Granier, M.L. Scippo (2013a). Preliminary Evaluation of Antimicrobial Residue Levels in Marketed Pork and Chicken Meat in the Red River Delta Region of Vietnam. Food and Public Health. 3(6): 267-276.

Kim, D.P., C. Saegerman, C. Douny, T.V. Dinh, B.H. Xuan, B.D. Vu, N.P. Hong and M. Scippo (2013b). First Survey on the Use of Antibiotics in Pig and Poultry Production in the Red River Delta Region of Vietnam. Food and Public Health. 3(5): 247-256.

Kodimalar, K., R.A. Rajini, S. Ezhilvalavan and G. Sarathchandra (2014). As survey of chlortetracycline concentration in feed and its residue in chicken egg in commercial layer farms. Journal of Biosciences. 39: 425-431.

Kotaiah, T. (2016). Poultry Production in India - The Current Scenario. FnBnews.com.http://www.fnbnews.com/Poultry/poultry-production-in-india-the-current-scenario-38620. Last accessed on 20.01.2019.

Laxminarayan, R. and R.R. Chaudhury (2016). Antibiotic Resistance in India: Drivers and opportunities for Action. PLoS Medicine. 13(3): e1001974.

Marcinèák, S., K. Hussein, N. Zdolec, J. Janošová (2006). Premi®test - Fast screening test for detection of sulphadimidine residues in poultry tissues. Meso (VII) rujan - listopad br. 5.

Maria, C., V. Trifan, G. Paraschiv and A. Rotaru. (2007). The depletion of doxycycline residues in poultry meat. Bulletin USAMV-CN. 64: 1-2.

McGettigan, P., P. Roderick, A. Kadam and A. Pollock (2018). Threats to global antimicrobial resistance control: Centrally approved and unapproved antibiotic formulations sold in India. British Journal of Clinical Pharmacology. Published Online: February. 5. 2018.

Nikaido, H. (2009). Multidrug Resistance in Bacteria Annual Review of Biochemistry. 78: 119-146.

Okerman, L., S. Croubels, S. De Baere, J. Van Hoof, P. De Backer and H. De Brabander (2001). Inhibition tests for detection and presumptive identification of tetracyclines, beta-lactam antibiotics and quinolones in poultry meat. Food Additives and Contaminants. 18(5): 385-393.

Oyekunle, M.A., S.A. Shodiya and I.K. Jimoh (2003). Reservoir of antimicrobial Resistant *Salmonella*e among poultry in a Local Government area in Ogun State, Nigeria. Asset Journal International Series A. 3(4): 71-80.

Refsdal, A.O. (2000). To treat or not to treat: a proper use of hormones and antibiotics Anim. Reprod. Sci. 60(61): 109-119.

Sakdinun, P., T. Paethaisong and S. Sirilux (2006). Chlotetracycline and Oxytetracycline in Animal Feed from 4 Western Provinces Thai-NIAH eJournal: http://www.dld.go.th/niah. 1(2): 143 -146.

Salama, N.A., S.H. Abou-Raya, A.R. Shalaby, W.H. Emam and F.M. Mehaya (2011). Incidence of tetracycline residues in chicken meat and liver retailed to consumers. Food Additives and Contaminants: Part B. 4(2): 88-93.

Sattar, S., M.M. Hassan, S.K.M.A. Islam, M. Alam, M.S.A. Faruk, S. Chowdhury, A.K.M. Saifuddin (2014). Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. Veterinary World. 7(9): 738-743.

Senyuva, H., T. Ozden and D.Y. Sarica (2000). High-Performance Liquid Chromatographic Determination of Oxytetracycline Residue in Cured Meat Products. Turkish Journal of Chemistry. 24: 395-400.

Singh, R.P., Y.P. Sahni, S. Bharti and N. Chandra (2016). Determination of Residual Concentration of Doxycycline In Chicken Meat. Journal of Cell and Tissue Research. 16(3): 5825-5828.

Siwczyńska, M.P., E. Patyra, M.C. Purchała, B.Kozak and K. Kwiatek. (2015). Occurrence of tetracyclines in feedingstuffs-results of a two-year study within the official control of feed. Bulletin

of the Veterinary Institute in Pulawy. 59: 527-532.

USDA (2015). Global Agricultural Information Network- Poultry and Poultry Products Annual Report. GAIN Report No IN5124 (2015).

Waghamare, R.N., Paturkar, A.M., Vaidya, V.M., Zende R.J., Dubal, Z.N., Dwivedi, A., Gaikwad, R.V. (2018). Phenotypic and genotypic drug resistance profile of *Salmonella* serovars isolated from poultry farm and processing units located in and around Mumbai city, India, Veterinary World. 11(12): 1682-1688.

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