Published with Open Access at Journal BiNET
Vol. 29, Issue 01: 2399-2406
Journal of Bioscience and Agriculture Research
Journal Home: www.journalbinet.com/jbar-journal.html

Screening of oxytetracycline & chlortetracycline residues in poultry feeds in Bangladesh

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Article received: 24.03.2022; Revised: 15.05.2022; First published online: 30 May, 2022

ABSTRACT

A total of 112 poultry feed samples were screened for oxytetracycline and chlortetracycline antibiotic residues by thin-layer chromatography (TLC). The samples were collected from Natore, Naogaon, Rangpur and Comilla districts of Bangladesh. Poultry feed samples were classified according to their product type (homemade, commercial) and types of poultry feed (broiler feed, layer feed and sonali feed). All the homemade poultry feed samples (100%) tested positive for oxytetracycline and chlortetracycline antibiotic residue when screened. About 95% of commercial poultry feed tested positive for antibiotic residue in all four districts. In this study, the positive screening for oxytetracycline and chlortetracycline antibiotic residue in broiler, layer and sonali poultry feed types are 98%, 95% and 90%, respectively. This study also observed that antibiotic residue remained in feed samples irrespective of temperature and time as they had been stored for about a year at room temperature. From this study, it was revealed that the use of antibiotics in poultry feeds was extensive. Moreover, the duration of sustaining in poultry feed also raised concerns regarding their use and effect on the environment. This study has also increased the concern about the strict monitoring of the use of antibiotics in the poultry industry.

***Key Words**: Oxytetracycline, Chlortetracycline, Poultry feed and TLC.*

Cite Article: Islam, P., Biswas, S. K., Hossain, M. I., Khatun, P., Sikder, M. H. and Islam, A. (2022). Screening of oxytetracycline & chlortetracycline residues in poultry feeds in Bangladesh. Journal of Bioscience and Agriculture Research, 29(01), 2399-2406.
Crossref: https://doi.org/10.18801/jbar.290122.291

I. Introduction

In Bangladesh, the poultry industry is one of the most essential food production sectors. It provides 22-27% of the total animal protein supply and 37% of total meat production in Bangladesh (WHO, 2001). It has a significant impact on our lives by providing direct and indirect employment opportunities, greater food security, and increased access to high-quality protein in people's diets (Hamid et al., 2017). The demand for poultry meat has increased due to population growth and the

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constant search for a healthy diet (Das et al., 2008). Moreover, poultry meat remained a cheap protein source constantly over a decade (Marangoni et al., 2015). Poultry meat is an excellent source of protein since it includes all of the essential amino acids in desirable concentrations (Magdelaine et al., 2014). The poultry industry has provided opportunities for people to improve their lifestyles and eating habits. It has also helped ensure food security by reducing the reliance on beef and mutton as animal protein sources (Ali and Hossain, 2012).

The increased demand for poultry meat has put farmers under constant pressure to produce as much poultry as possible in the shortest period (Apata, 2009). In this regard, Farmers and feed manufacturers use a variety of antibiotics as growth promoters in their feed (Chowdhury et al., 2009). Feed is a prerequisite for growing broiler and layers, and their body growth, maintenance and development are primarily dependent on feed (Tchounwou et al., 2012). Antibiotics in the feed are used as sub-therapeutic doses for Growth promotion (Marshall and Levy, 2011). Almost 90% of used antibiotics in poultry were administered at sub-therapeutic concentrations. About 70% of this was for disease prevention and 30% was for growth promotion (Jayalakshmi et al., 2017). When a drug administered is not completely absorbed from the gut and is therefore excreted in the feces. As a result, in agricultural land, such manure or farm effluents lead to the selection of resistant bacteria, as well as the development and transfer of antibiotic resistance genes in microbes (Boxall et al., 2003). The antibiotic residue below sub-therapeutic doses causes antimicrobial resistance and many health hazards in humans, such as carcinogenicity, mutagenicity, bone marrow toxicity and allergy (Doyle, 2006).

Antibiotic resistance is a public-health crisis that is rapidly approaching (Boyce, 2008). The WHO has now recognized it as one of the top health challenges in this modern era (Apata, 2009). The European Union and other regulatory bodies have defined maximum residue limits (MRL) and withdrawal periods for antibiotics in poultry and poultry products to protect consumer health. Antibiotic stewardship campaigns have been developed at the international, national, and local levels with the purpose of preserving antibiotic effectiveness for severe and life-threatening infections (Belongia et al., 2005). Bangladesh Animal Feed Act 2010 banned antibiotics as growth promoters in feeds. Many scientific studies found antibiotic residue in poultry meat and eggs (Muaz et al., 2018) and it was also indicated that antibiotics are directly related to poultry production (Mund et al., 2017). So it is necessary to know the presence of antibiotic residue in poultry feed. Thus this study was designed to detect antibiotic residues in poultry feed from different areas of Bangladesh by Thin-Layer Chromatography (TLC).

II. Materials and Methods

Collection and storage of samples

Samples were collected from Comilla, Natore, Naogaon, and Rangpur Sadar Upazila between December 2019 and February 2020. A total of 112 different branded and non-branded feeds of broiler, layer and sonali birds were collected. The samples were sealed in the individual zipper bag labelled with collection area, farmer's name, feed type, feed company name, collection date etc. Then they were kept in a cartoon to store in the Post-graduate laboratory-2, Department of Pharmacology, Bangladesh Agricultural University, Mymensingh, at a temperature of 30°C for further analysis.

Processing and categorizing of samples

After a certain period, samples were removed from the zipper bag and exposed to sunlight to remove moisture content. A total of 112 feed samples were selected for the experiment, and a blindfold serial number was set for them. Among 112 samples, 28 samples were collected from Natore, 38 samples from Naogaon, 38 samples from Rangpur and eight samples from Comilla. Again the feed samples were categorized according to production types (commercial and homemade) and poultry types (broiler, layer, and sonali). According to poultry types, 48 broilers, 43 layers, and 21 sonali feed samples were collected from Natore, Naogaon, Rangpur and Comilla districts. Further, according to feed types, a total of 28, 36, 34, 8 samples of commercial and 0, 2, 4, 0 samples of homemade were collected from Natore, Naogaon, Rangpur and Comilla districts, respectively (Figure 01).

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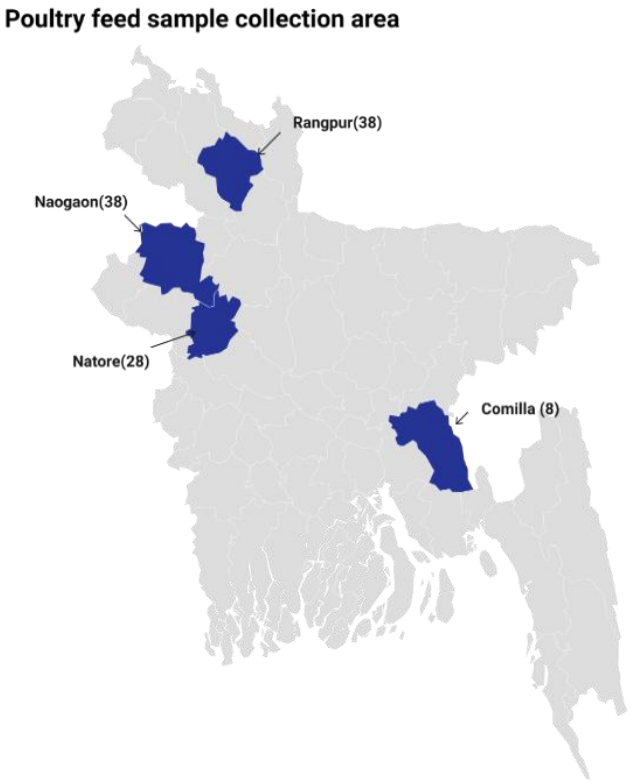

Figure 01. Map showing sample collection area

Chemical and Reagents

In this study, analytical and HPLC grade chemicals and reagents were used. All standard chemicals and reagents have a purity of at least 99 percent. The phosphate buffer (Merck, Germany), methanol (Merck, Germany), trichloroacetic acid (TCA) (Merck, Germany), acetonitrile (Duksan Pure Chemicals, Korea), and diethyl ether (RCI Labscan-Thailand) were used and all the chemicals were HPLC grade. Oxytetracycline and chlortetracycline were obtained from Sigma-Aldrich. Syringe filters (0.45µm) were obtained from Merck, Germany.

Standard preparation

The individual stock solution of all standard antibiotics was prepared by dissolving the chemicals in methanol. The stock solutions of oxytetracycline and chlortetracycline were made in methanol at a 10 mg/ml concentration, respectively. By serial dilution, a working standard solution of 2 mg/ml was made from the stock solution. For future usage, the stock was kept at a temperature of -4°C. The mobile phase of all the antibiotics was the same and consisted of acetonitrile and methanol at the ratio of 1:1, respectively.

Sample extraction

The extraction of feed samples for antibiotics analysis was carried out following Popelka et al. (2005). The samples were crushed with mortar and pestle and made a fine powder by sieving. The ground 2 gm sample was placed in a falcon tube, and 5 ml phosphate buffer saline was added (pH-7.2). The sample was then vortex (Vortex- XHC, Wincom, China) for one minute. After mixing with 1 mL 30% TCA, the samples were centrifuged for 10 minutes at 5000 rpm (Hettich D-78532, Germany). To remove the solid portion of an extract, Whattmann filter paper no. 12 was used. The filtered fluid was collected in a new falcon tube with the same amount of diethyl ether and defatted for 10 minutes at room temperature. The upper oily layer was discarded, but only the bottom layer was collected, and these combinations were then separated from one another. Diethyl ether was used two more times to extract the supernatant. Then the extracted solution was ready for TLC analysis.

Thin-Layer Chromatography (TLC)

TLC apparatus and Chromatographic condition

This study used a TLC plate (MN-Germany), a TLC tank, and a UV detection box (UV light: F18W-Germany). With few modifications, the TLC plate was prepared in accordance with Tajick and Shohreh

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(2006). From a 20x20 cm TLC plate, the necessary size (10x6.5 cm) was cut. A pencil was used to draw a straight line across the plate, 1.5 cm from the bottom. Below 1 cm from the plate's upper edge, a second straight line was drawn across the plate. On the bottom line, five (5) places of equal distance were marked. The left-most spot was marked for oxytetracycline standard, and the rightmost spot was for chlortetracycline standard, whereas the middle three spots were the triplicate of the same sample. Standards and samples were applied to the plate using micropipettes. For spotting, a volume of 2 µl was used for spotting. After two minutes of air drying, the plate was ready to run in the mobile phase.

Running and visualization

The TLC plate was placed in the TLC jar containing the mobile phase and the glass lid was closed over it. The mobile phase was below the bottom line on the TLC plate. The plate remained in the jar until the mobile phase reached the top line. The run time for oxytetracycline and chlortetracycline was approximately 13 and 15 minutes. After that, the TLC plate was removed from the chamber and kept horizontally for air drying. Then the plate was placed in the UV chamber (UV light: F18W-Germany) and observed under the UV light (λ=254 nm). The sample was considered positive if the sample spot ran along with the standard spot in the plate or if the sample spot had the same Rf value as the standard. Then the photo of the plate was captured for further analysis.

Measurement of R_f values

R_f values are the measurements of distance traveled by the solvent and the distance traveled by individual sample spots. A compound with the same Rf value as the standard is considered similar compound.

$$Rf = \frac{\text{Distance traveled by sample (a)}}{\text{Distance traveled by solvent (b)}}$$

Data analysis

The experiment results were analyzed statistically using SPSS IMB 20 for descriptive statistics and stored in Microsoft Excel-2010 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20, Armonk, New York USA: IBM Corp).

III. Results

112 poultry feed samples were screened for oxytetracycline and chlortetracycline antibiotic residue. After the blindfold experiment, the samples were categorized according to the place of collection, feed production, and poultry types. Among 112 samples, 28 samples were collected from Natore, 38 samples from Naogaon, 38 samples from Rangpur and 8 samples from Comilla (Table 01). The samples from Naogaon and Comilla showed 100% positive results for oxytetracycline and chlortetracycline antibiotic residue and Rangpur and Natore showed 94.73% & 89.28% positive results, respectively.

Table 01. The district-based total collected sample

District	Collected sample
Natore	28
Naogaon	38
Rangpur	38
Comilla	8
Total	112

Further, the feed samples were classified on the poultry type (broiler, layer, sonali). From Natore, Naogaon, Rangpur and Comilla areas total of 48 broilers, 43 layers, and 21 Sonali feeds were screened, and after the TLC procedure, 47 broilers (97.91%), 41 layers (95.34%), and 19 Sonali (90.47%) feed samples were found as the positive sample. In Natore, 11 broiler, 7 layers and 10 sonali feed were collected, and after TLC screening, 100% of samples tested positive for broiler, 85.71% positive for layer and 90% positive results for sonali birds. In Naogaon, 21 broilers, 13 layers and 4 sonali feeds were collected where 95.23% of samples tested positive for broiler and 100% positive results for layer and sonali feeds. In Rangpur 16 broilers, 15 layers, and 7 sonali feed were collected and after the TLC procedure 100% of samples tested positive for broiler, 93.33% positive for layer and 85.71% positive

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for sonali feed. In the Comilla area, only 8 layers feed samples were collected, and TLC results showed 100% positive for layer feed samples. The results are presented in (Table 02) and illustrated in (Figure 02).

Table 02. Feed sample results based on poultry type

District	Total broiler	Broiler positive	Total layer	Layer positive	Total sonali	Sonali positive
Natore	11	11	7	6	10	9
Naogaon	21	20	13	13	4	4
Rangpur	16	16	15	14	7	6
Comilla	0	0	8	8	0	0
Total	48	47	43	41	21	19

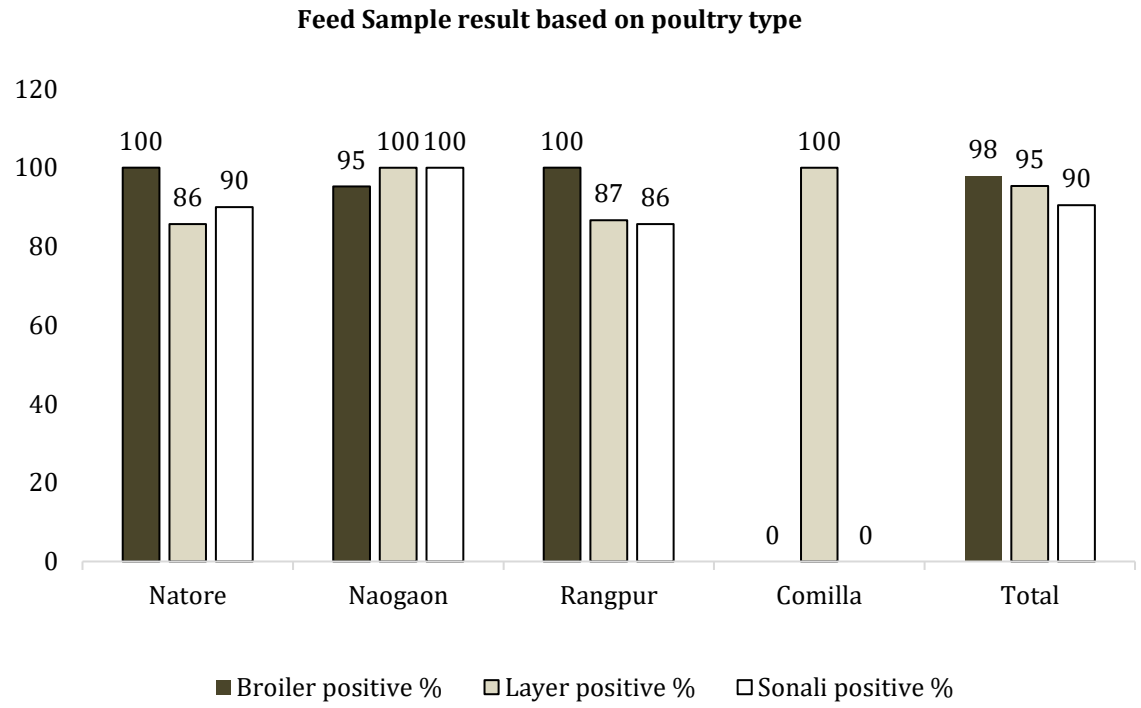

Figure 02. Feed Sample result based on poultry type

Again, the poultry feeds sample was categorised based on feed production (commercial and homemade production). A total of 106 commercial feed and 6 homemade feed were collected, where after the TLC procedure, 101 commercial feed (95.28%) gave positive results, and homemade feed showed 100% positive results. In Natore, the collected homemade feed number was zero, and among 28 commercial feeds, TLC showed positive results in 26 feed samples (92.85%). In Naogaon and Rangpur, collected homemade feed showed 100% positive results, whereas commercial feed samples showed 35 (97.22%) positive results for Nagogaon and 32 (94.11%) positive results for Rangpur district. In Comilla district, only 8 commercial feeds were collected and after TLC examination, it showed 100% positive results for antibiotic residue for commercial feeds. The whole results are presented in (Table 03) and illustrated in (Figure 03).

Table 03. Feed sample results based on feed production

District	Total commercial	Commercial positive	Total homemade	Homemade positive
Natore	28	26	0	0
Naogaon	36	35	2	2
Rangpur	34	32	4	4
Comilla	8	8	0	0
Total	106	101	6	6

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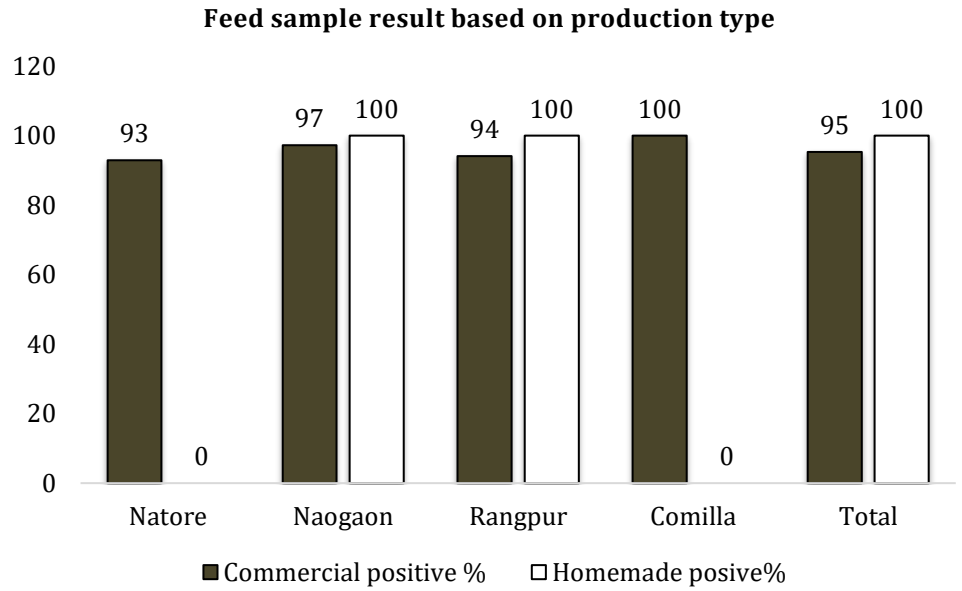

Figure 03. Feed sample result based on production type

IV. Discussion

112 collected poultry feed samples were screened for oxytetracycline and chlortetracycline antibiotic residue. In poultry feed samples, the oxytetracycline and chlortetracycline residue were determined using the thin-layer chromatography (TLC) method. Thin-layer chromatography is a simple non-expensive and qualitative technique that can execute easily in most laboratories. Among chromatographic techniques, HPLC is a high-accuracy chromatographic technology with significant drawbacks. The TLC analysis of oxytetracycline and chlortetracycline residue on poultry feed could not be differentiated due to the same detection method.

The use of indiscriminate antibiotics in poultry feed nowadays imposes potential risks for antibiotic residue. In order to reduce morbidity and promote growth, feed producers have played a key role in the indiscriminate use of antibiotics in poultry feed. The majority of feed producers do not reveal the data about the addition of antibiotics in feed. Unfortunately, a considerable number of the farmers who produce homemade feed mixes antibiotics to protect their poultry from diseases and growth promotion. Possible reasons to observe tetracyclines residue in this study might be due to the feed producers' broad spectrum of activity, availability, and relative affordability.

It was published that in Nigeria, the poultry farms (100%) are screened positive for using oxytetracycline (OTC) (Kabir et al., 2003; Nonga et al., 2009; Ezenduka et al., 2011). Another piece of evidence reported that 20 out of 23 layer farms (about 87%) used medicated feed containing chlortetracycline (CTC) in Trinidad (Adesiyun et al., 2005). A study revealed in Saudia Arabia that Chlortetracycline(CTC) was the most commonly used antimicrobial in poultry feed for prophylactic purposes (Al-Mustafa and Al-Ghamdi, 2002). Our study results with screened poultry feed positive for oxytetracycline and chlortetracycline also supported these studies.

In Iraq that 52% of samples were positive for antibiotics (oxytetracycline and sulfadiazine) in stored poultry products, whereas 28% were positive for oxytetracycline (Shareef et al., 2009) which could be evidence that antibiotics could remain irrespective of temperature or time. In this study, feed samples were kept in storage for a longer period to observe whether the antibiotic residue would fade off. After storing them at room temperature for almost a year, the feed samples tested positive for oxytetracycline and chlortetracycline. This result indicated that these antibiotics could sustain in the feed samples irrespective of temperature and time. This is an alarming indication that antibiotics can remain in nature or feed for longer than our expectations. This can also be an issue to consider during the use of antibiotics from an environmental perspective.

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So from our study, it is clear that antibiotic residue exists in poultry feed in our country irrespective of time, storage and type of poultry feed. The high prevalence of residues in poultry feed indicates excessive usage and misuse of antibiotics in poultry production. However, a complete investigation should be conducted in Bangladesh to detect and quantify all antibiotics used in broiler, sonali, and layer chickens to take appropriate precautions to safeguard humans and the environment from antibiotic residual dangers. Moreover, to control these levels, the respective authority should implement strict regulatory measures as soon as possible.

V. Conclusion

In this study, the prevalence of antibiotic residues in poultry feed was incredibly high. Antibiotics used indiscriminately and irrationally in poultry feed without a withdrawal time may result in unwanted residues in animal feed. In Bangladesh significant amount of antibiotics are used in poultry farming, but concerns have been raised that tissues of food animals contaminated with antimicrobial residues may cause adverse side effects in consumers. Our study confirmed the presence of antibiotic residues in poultry feed samples collected from Natore, Naogaon, Rangpur, and Comilla. This may pose a potential hazard to public health. Thus, it is recommended that rules should be taken to ensure maintaining proper withdrawal periods before marketing and drug control in veterinary use. The respective authorities should take the necessary steps to increase awareness among the community. It needs to train poultry producers to restrict the abuse of antibiotics in poultry feeds and its consequences on consumers. Many samples from wider sources need to be analysed to know the actual concentration of antibiotics residues to identify drug resistance bacterial load in poultry to provide safe food for poultry.

VI. References

[1]. Adesiyun, A., Offiah, N., Lashley, V., Seepersadsingh, N., Rodrigo, S. and Georges, K. (2005). Prevalence of antimicrobial residues in table eggs in Trinidad. Journal of Food Protection, 68(7), 1501–1505. https://doi.org/10.4315/0362-028X-68.7.1501

[2]. Ali, M. M. and Hossain, M. M. (2012). Problems and prospects of poultry industry in Bangladesh: an analysis. Dhaka: American International University Bangladesh (AIUB).

[3]. Al-Mustafa, Z. H. and Al-Ghamdi, M. S. (2002). Use of antibiotics in the poultry industry in Saudi Arabia: Implications for public health. Annals of Saudi Medicine, 22(1–2), 4–7. https://doi.org/10.5144/0256-4947.2002.4

[4]. Apata, D. F. (2009). Antibiotic Resistance in Poultry. International Journal of Poultry Science, 8(4), 404–408. https://doi.org/10.3923/ijps.2009.404.408

[5]. Belongia, E. A., Knobloch, M. J., Kieke, B. A., Davis, J. P., Janette, C. and Besser, R. E. (2005). Impact of Statewide Program To Promote Appropriate Antimicrobial Drug Use. Emarging Infectious Diseases, 11(6), 912–920. https://doi.org/10.3201/eid1106.050144

[6]. Boxall, A. B., Fogg, L. A., Blackwell, P. A., Blackwell, P., Kay, P., Pemberton, E. J. and Croxford, A. (2004). Veterinary medicines in the environment. Reviews of environmental contamination and toxicology, 1-91. https://doi.org/10.1007/0-387-21729-0_1

[7]. Boyce, J. M. (2008). Community-Associated Methicillin-Resistant Staphylococcus aureus as a Cause of Health Care-Associated Infection. Clinical Infectious Diseases, 46, 795–798. https://doi.org/10.1086/528717

[8]. Chowdhury, R., Haque, M. N., Islam, K. M. S. and Khaleduzzaman, A. B. M. (2009). A review on antibiotics in an animal feed. Bangladesh Journal of Animal Science, 38(1–2), 22–32. https://doi.org/10.3329/bjas.v38i1-2.9909

[9]. Das, S. C., Chowdhury, S. D., Khatun, M. A., Nishibori, M. and Isobe, N. (2008). Small-Scale Family Poultry Production Poultry production profile and expected future projection in Bangladesh. World's Poultry Science, 64(1), 99–118. https://doi.org/10.1017/S0043933907001754

[10]. Doyle, M. E. (2006). Veterinary drug residues in processed meats-potential health risk. Food Research Institute, 1-16.

[11]. Ezenduka, E. V., Oboegbulem, S. I., Nwanta, J. A. and Onunkwo, J. I. (2011). Prevalence of antimicrobial residues in raw table eggs from farms and retail outlets in Enugu State,

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Nigeria. Tropical animal health and production, 43(3), 557-559. https://doi.org/10.1007/s11250-010-9730-z

[12]. Hamid, M. A., Rahman, M. ., Ahmed, S. and Hossain, K. (2017). Status of Poultry Industry in Bangladesh and the Role of Private Sector for its Development. Asian Journal of Poultry Science, 11(1), 1–13. https://doi.org/10.3923/ajpsaj.2017.1.13

[13]. Jayalakshmi, K., Paramasivam, M., Sasikala, M. and Sumithra, A. (2017). Review on antibiotic residues in animal products and its impact on environments and human health. Journal of Entomology and Zoology Studies, 5(3), 1446–1451.

[14]. Kabir, J., Umoh, V. J., Audu-Okoh, E., Umoh, J. U. and Kwaga, J. K. P. (2004). Veterinary drug use in poultry farms and determination of antimicrobial drug residues in commercial eggs and slaughtered chicken in Kaduna State, Nigeria. Food Control, 15(2), 99-105. https://doi.org/10.1016/S0956-7135(03)00020-3

[15]. Magdelaine, P., Spiess, M. and Valceschini, E. (2014). Poultry meat consumption trends in Europe. World's Poultry Science, 64(1), 53–64. https://doi.org/10.1017/S0043933907001717

[16]. Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., Lucchin, L. and Poli, A. (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: An Italian consensus document. Food and Nutrition Research, 59(July), 1–11. https://doi.org/10.3402/fnr.v59.27606

[17]. Marshall, B. M. and Levy, S. B. (2011). Food animals and antimicrobials: Impacts on human health. Clinical Microbiology Reviews, 24(4), 718–733. https://doi.org/10.1128/CMR.00002-11

[18]. Muaz, K., Riaz, M., Akhtar, S., Park, S. and Ismail, A. (2018). Antibiotic residues in chicken meat: global prevalence, threats, and decontamination strategies: a review. Journal of food protection, 81(4), 619-627. https://doi.org/10.4315/0362-028X.JFP-17-086

[19]. Mund, M. D., Khan, U. H., Tahir, U., Mustafa, B. and Fayyaz, A. (2017). Antimicrobial drug residues in poultry products and implications on public health : A review. International Journal of Food Properties, 20(7), 1433–1446. https://doi.org/10.1080/10942912.2016.1212874

[20]. Nonga, H. E., Simon, C., Karimuribo, E. D. and Mdegela, R. H. (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from smallholder poultry keepers in Morogoro municipality, Tanzania. Zoonoses and Public health, 57(5), 339-344. https://doi.org/10.1111/j.1863-2378.2008.01226.x

[21]. Popelka, P., Nagy, J., Germuška, R., Marcinčák, S., Jevinova, P. and De Rijk, A. (2005). Comparison of various assays used for detection of beta-lactam antibiotics in poultry meat. Food additives and contaminants, 22(6), 557-562. https://doi.org/10.1080/02652030500133768

[22]. Shareef, A. M., Jamel, Z. T. and Yonis, K. M. (2009). Detection of antibiotic residues in stored poultry products. Iraqi Journal of Veterinary Sciences, 23(3), 45-49.

[23]. Tajick, M. A. and Shohreh, B. (2006). Detection of antibiotics residue in chicken meat using TLC. International Journal of Poultry Science, 5(7), 611–612. https://doi.org/10.3923/ijps.2006.611.612

[24]. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K. and Sutton, D. J. (2012). Heavy metal toxicity and the environment. Molecular, clinical and environmental toxicology, 133-164. https://doi.org/10.1007/978-3-7643-8340-4_6

[25]. World Health Organization (2001). Antibiotic resistance: synthesis of recommendations by expert policy groups/Alliance for the Prudent Use of Antibiotics; J. L. Avorn ... [et al.]. World Health Organization. https://apps.who.int/iris/handle/10665/66895

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