Journal of Advanced Veterinary and Animal Research
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2018.e257
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

June 2018
Vol 5 No 2, Pages 140-145.

Original Article

Screening of antibiotic residues in chicken meat in Bangladesh by thin layer chromatography

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• **Received:** March 3, 2018 • **Revised:** April 20, 2018 • **Accepted:** April 23, 2018 • **Published Online:** May 22, 2018

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ABSTRACT

Objective: Screening of antibiotic residues in broiler chicken meat and liver collected from farms and local markets in Bangladesh.

Materials and methods: A total of 160 samples (breast, thigh muscle and liver) were collected from markets and farms from different region of Bangladesh. PBS buffer system with trichloracetic acid and diethyl ether based sample extraction was performed. For comparison the standard antibiotics; Ciprofloxacin (CIP), Enrofloxacin (ENR), Oxytetracycline (OTC), Amoxicillin (AMOX) and Doxycycline (DOX) were prepared by dissolving in methanol. Samples were pointed on TLC plates transferred to TLC tank containing acetone-methanol (1:1) as mobile phase. Retention factor (Rf) was calculated after observing the chromatograms on UV light at 256 nm. Same Rf value of standard and sample considered similar compound.

Results: Liver sample were mostly positive for antibiotics residue followed by thigh muscles and breast muscle. The frequency of antibiotic residues was highest in liver followed by thigh muscles and breast muscle. Among the antibiotics CIP ranked top in all types of sample. In breast muscle highest antibiotic was CIP (39%) followed by DOX (26%), AMOX (24%), OTC (23%) and lowest was ENR (21%). In thigh muscle, 42, 29, 28, 27 and 24% sample was positive for CIP, OTC, DOX, AMOX and ENR, respectively. Highest number of liver samples were shown positive result for all screened antibiotics (CIP-52%, OTC-46%, DOX-43%, AMOX-42% and ENR-36%).

Conclusion: This study ascertained those antibiotics residues are present in chicken muscle and liver which causes serious health hazards to consumers. So proper steps should be taken to control emergence of antibiotic resistance in human being as well as in the environment.

KEYWORDS

Antibiotic residues; Broiler; Liver; Meat; TLC

How to cite: Sarker YA, Hasan MM, Paul TK, Rashid SZ, Alam MN, Sikder MH. Screening of antibiotic residues in chicken meat in Bangladesh by thin layer chromatography. Journal of Advanced Veterinary and Animal Research. 2018; 5(2):140-145.

INTRODUCTION

Veterinary Antibiotics (VAs) are commonly being used by the farmers and veterinary practices, that may lead to drug residues from food animals to human, and subsequently adverse health hazards may develop to the consumer (Chanda et al., 2014). Significant level of exposure of antibiotic residues from animal food products to individual may modulate immunological responses and can detrimentally affect intestinal microbiota in susceptible individual (Ramatla et al., 2017).

However, farmers are intended to use of VAs in different pattern as prophylactic, therapeutic, growth promoter and sometimes both prophylactic and therapeutic purposes all over the world (Wadoum et al., 2016). In Canadian poultry industry for prophylaxis and growth promotion different types antibiotic are used (Diarra and Malouin, 2014). Massive magnitudes of antibiotics are used in Bangladesh annually. A huge ration of this used tactlessly under circumstances of inadequate or no medical supervision and in most cases without prior tests on documentation of the disease causing organism and fortitude of its sensitivity to the antibiotic prescribed (Sarker et al., 2016).

Therefore, antibiotics are extensively used as growth promoters as like other developing countries in Bangladesh in poultry to control diseases and facilitated feed conversion. The concept rely on maintaining poultry fewer days on feed before reaching slaughter weight to make the production system profitable (Sarker, 2016; Gelband et al., 2015). Generally, animal burns 70-85 percent calories to maintain body function, therefore shorter times on feed improve feed efficiency per kg of weight gain. All consequences cause serious health hazards with emergence of antibiotic resistance (Hassan et al., 2014).

Fluoroquinolones including ciprofloxacin, enrofloxacin, nalidixic acid, etc. have been widely used for treatment and prophylaxis (Er et al., 2013). Ciprofloxacin is found effective where microorganisms are resistant to aminoglycosides, tetracycline's, macrolides and β-lactams (Sultan, 2014). Tetracycline, a broad-spectrum antibiotic is used to treat infections and is as growth promoter in animals. About 60% of an ingested dose of oxytetracycline is absorbed from the gastrointestinal tract and widely spread in the body (Doyle, 2006; Mund et al., 2017).

In recent years, many studies have shown that antibiotics administered to poultry and livestock were accumulated in liver, kidney, muscle and bones exceeding the Maximum Residual Limits (MRL) (Sarker et al., 2016).

For the overall perspective, it could be stated these antibiotic residues might be caused potential health hazards to human and animal and a great constraint to export meat in developed countries. From this assessment, this research work was done to detect antibiotic residues in broiler meat and liver from different farms and local markets for the presence of residues of ciprofloxacin, enrofloxacin, oxytetracycline, doxycycline and amoxicilin antibiotics by thin layer chromatography (TLC).

MATERIALS AND METHODS

Sample collection: A total of 160 samples (breast, thigh muscle and liver) were collected from markets and farms from different region of Bangladesh. Each sample was placed into a separate plastic zipper bag transferred to laboratory by ice bag plastic container and stored at -20°C until extraction.

Chemicals and Standard Drugs: Purity of all standard chemicals and reagents was at least 99%. HPLC grade methanol (Merck-Germany), trichloracetic acid (TCA), diethyl ether and acetone (RCI Labscan-Thailand) was used. Ciprofloxacin (CIP), enrofloxacin (ENR), oxytetracycline (OTC), amoxicillin (AMOX) and doxycycline (DOX) were obtained from Sigma-Aldrich via Renata Limited, Bangladesh. The standard for the selected antibiotics; CIP, ENR, OTC, AMOX and DOX were prepared by dissolving 0.1 gm of powder in 4 mL solution of methanol. Standard solution was stored in -4°C and every month fresh solution was prepared.

Sample preparation: Sample extraction was performed according to Poppelka et al. (2005). Four gram of each sample was cut into small pieces, grinded and blended. 10 mL Phosphate Buffer Saline (pH-6.5) was added and mixed by vortexing (Vortex- XHC, Wincom, China). Centrifuged (Hettich D-78532, Germany) @ 60000 rpm for 20 min was done after mixing with 2 mL 30% TCA. Supernatant was collected and filtered by Whatman filter paper and funnel. Filtrated fluid was collected in another falcon tube and same amount of diethyl ether was added and left for 10 min in room temperature. The bottom layer was collected and supernatant extraction was repeated twice using diethyl ether. Final volume of the

extracts were pooled carefully into screw cap vial and kept into refrigerator for future analysis.

Thin Layer Chromatography (TLC): TLC apparatus: TLC plate (MN-Germany), TLC tank and UV detection box (UV light: F18W-Germany) were used. TLC was performed according to Tajick and Shohreh (2006) with some required adjustments. TLC plate was cut into appropriate size (4x5 cm) from 20x20 cm. A straight line was drawn across the plate approximately 2 cm from the bottom by a pencil. Another straight line was drawn across the plate below 1 cm from the upper edge of the plate. Desired spots marking were marked on the bottom line where analytes were dropped. Spots were applied to the plate using thin capillary glass pipettes. A volume of 50 µl was used for spotting. Plate was placed in TLC tank (contained mobile phase; Acetone and Methanol: 1:1) and covered by lid and it was left until the mobile phase reached the upper line. Spots were visualized in UV detection box at 256 nm. Spots marking were done by pencil for calculation of retention factor (Rf). Calculation of Rf values: These measurements are the distance travelled by the solvent, and the distance travelled by individual sample spots. Same Rf value of standard and sample considered similar compound.

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

RESUTS AND DISCUSSION

Five different types of antibiotic were screened by Thin Layer Chromatography (TLC). The presence of antibiotic residues in the studied sample was highest in liver followed by thigh muscles and breast muscle respectively. Among the antibiotics CIP ranked top in all three types of sample (breast, thigh meat and liver). In breast muscle highest antibiotic was CIP (39%) followed by DOX (26%), AMOX (24%), OTC (23%) and lowest was ENR (21%). In case of thigh muscle, 42, 29, 28, 27 and 24% sample was positive for CIP, OTC, DOX, AMOX and ENR respectively. Highest number of liver samples were shown positive result for all screened antibiotics (CIP-52%, OTC-46%, DOX-43%, AMOX-42% and ENR-36%). Results are shown in **Figure 1**.

Presence of various antibiotic residues significantly higher in meat and offals of chickens getting prophylactic and or therapeutic without respect of recommended withdrawal periods have been revealed by different scientists including TLC methods (Hind et al., 2014). To separate and identify the CIP, ENR, OTC, AMOX and DOX in chicken meat TLC was used in our research project. In this study, liver sample were mostly positive of antibiotic residues than other samples due it is the organ where detoxifies various metabolites including antibiotics occurs which has supported the report of Naeem et al. (2006), Islam, (2009), Attari et al. (2014) and Ramatla et al. (2017) that chicken liver contained the highest level of CIP and ENR residues than muscles. Amjad et al. (2005) and Aslam et al. (2016) also performed similar study to separate and identify CIP and ENR residues from chicken liver, kidney and muscles.

CIP was found highest both in muscle and liver, this occurs due to abusive use and prescription pattern of CIP in Bangladesh. Another reason behind the excessive presence of CIP in chicken meat in Bangladesh because of changes in the chemical structure of quinolones significantly in their antimicrobial activity. ENR is known to be transformed to CIP by deethylation after treatment (Andreu et al., 2007; Trouchon and Lefebvre, 2016). This is another reason of higher CIP and lower ENR frequencies. Furthermore, CIP could be further metabolized to other degradation products (Rao et al., 2000).

AMOX and DOX residues were detected in in liver, breast muscles and thigh muscles with various percentages. AMOX and DOX residues in poultry meat were also detected by Poppelka et al. (2005); Hussain et al. (2013) identified amoxicillin residues in poultry meat by using TLC. Jank et al. (2017) screened the most frequent antibiotic in poultry muscle was doxycycline.

OTC residues were found in livers, thigh and breast muscles samples. Salehzadeh et al. (2006) and Jayalakshmi et al. (2017) also reported the highest percentage of antibiotic residues in liver (95.55%) followed by muscle (27.77%).

Awkwardly, it is possible that abuse of antibiotics could result in poultry products containing residues exceeding the MRL. Examples of abuse could include the intentional, illegal shipment of animals to market without maintaining a required withdrawal period and higher dosing of antibiotic or not understanding the requirements for a proper treatment.

Researches on antibiotic residues in chicken is relatively scare in Bangladesh. However, periodic sampling is being carried out in many countries to detect antibiotic residue

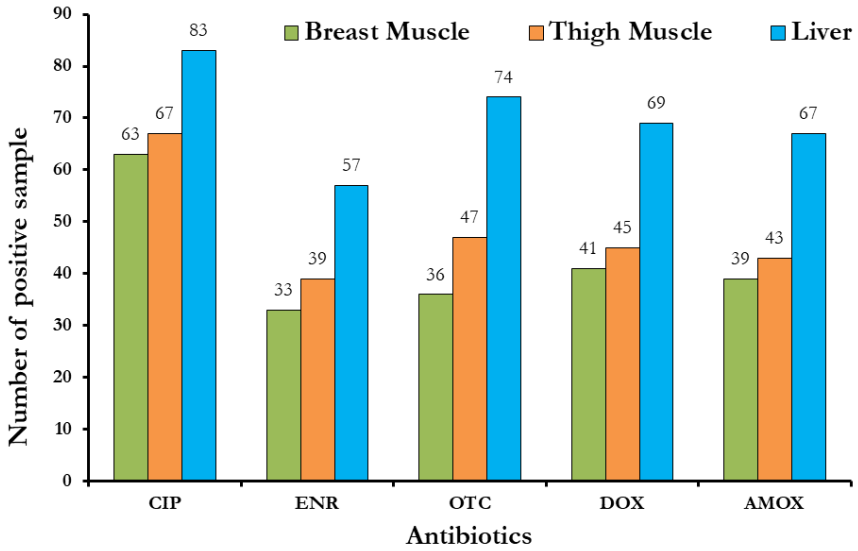

Figure 1. Presence of antibiotic residues in chicken muscle and liver (N=160). CIP: Ciprofloxacin, ENR: Enrofloxacin, OTC: Oxytetracycline, DOX: Doxycycline, AMOX: Amoxicillin.

in the food cycle (Weiss et al., 2007; Salehzadeh et al., 2007; Zhao et al., 2009; Pena et al., 2010; Sattar et al., 2014). Our study confirms the existing presence of antibiotic residue in our food chain. However, it is necessary to investigate how the antibiotic residues enter into the food chain directly via foodstuff and indirectly via vegetables and crops due to application of manure containing antibiotic residues in agricultural farmlands and causes human food chain contamination and in the long run emergence of antibiotic resistance phenomena.

CONCLUSION

The presence of antibiotic residues percentages in muscle and liver in local market is higher in comparison of our experiment. Indiscriminate and irrational use of antibiotics in poultry without following withdrawal period may result in unexpected residues in animal food and could cause serious health hazards to consumers. Adequate withdrawal period should be observed in all poultry farms following therapeutic use of antibiotics to check antimicrobial resistance phenomena and maintain their potency for use in human medicine.

ACKNOWLEDGEMENT

Nothing to disclose.

CONFLICT OF INTEREST

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

AUTHORS' CONTRIBUTION

YAS designed the study, performed sample collection, analysis, interpreted the data, and critically prepared the manuscript. MMH performed sample collection, preparation and analysis. TKP and SZR performed sample collection and analysis, MNA was involved in sample collection and storage. MHS supervised the research work. All authors read and approved the final version of manuscript.

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