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Article

Detection and mitigation of antibiotic residues in poultry products and byproducts

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Abstract: Majority of the people in Bangladesh are still not aware about the health hazards of antibiotic residues. In this research, farmer's awareness has been investigated by a questionnaire in different poultry farms, retail sellers and poultry markets and spread out the knowledge of public health hazards of antibiotics residues. Poultry farmers were found well educated about health hazards of antibiotic residues and aware about the judicial use of antibiotics before selling the poultry for human consumptions. During this survey, sufficient samples (thigh muscle, breast muscle, liver etc., n=100) were collected from different poultry farms, retail sellers and poultry markets. Randomly 50 livers, 50 thigh muscle and 50 breast muscle samples respectively were evaluated by TLC analysis. Out of 50 samples for each 2 liver, 2 breast muscle and 2 thigh muscle samples were found positive for Amoxicillin antibiotic; 5 liver, 3 breast muscle and 3 thigh muscle samples were found positive for Ciprofloxacin; 3 liver, 3 breast muscle and 3 thigh muscle samples were found positive for Cefalexin; 2 liver, 2 breast muscle and 2 thigh muscle samples were found positive for Enrofloxacin; 4 liver, 2 breast muscle and 2 thigh muscle samples were found positive for Oxytetracycline. Gentamicin and Neomycin were found negative for any samples. Further investigation was done in indoor discriminate and indiscriminate use of antibiotics (Cefalexin) in broilers. Indoor experiment was investigated in poultry chicks. Day old chicks were collected and reared up to 30 days. On day 14th, the chicks were randomly divided into three groups namely control group (n=10), discriminate antibiotic group (n=10) and indiscriminate antibiotic group (n=10). Discriminate antibiotic group was treated with antibiotics (Cefalexin) for one week followed by withdrawal period for one week, whereas; indiscriminate antibiotic group was treated with antibiotic (Cefalexin) for two weeks until the day of sacrifice. Liver, thigh muscle and breast muscle samples were collected and evaluated by TLC method. Control and discriminate antibiotics birds were found negative for any antibiotics residues. On the other hand, ten liver samples, eight thigh muscles and eight breast muscles were found positive for Cefalexin in indiscriminate group. Indiscriminate uses of antibiotic indicated that antibiotic residue mitigation for human safe meat production depends on withdrawal period and farmers awareness. Therefore, poultry treated with antibiotics are required for specific withdrawal period until all residues are depleted to safe levels before human consumption.

Keywords: TLC; withdrawal period; human health; indiscriminate use, discriminate use

1. Introduction

Antibiotics are the most widely used veterinary drugs in poultry industry (Simon and Baxter, 2006). They are used by the poultry industry and veterinarians to enhance growth rates, health, feed efficiency, egg production,

or for therapeutic reasons and prophylaxis measures to reduce the incidence of poultry diseases (Donoghue, 2003; Nisha, 2008). In Bangladesh, a large population of poultry farming is done by the illiterate poultry farmers who do not care veterinary prescription and supervision and resulting indiscriminate use of antibiotics in poultry farming. Human consumption of toxic levels of antibiotic residues in poultry origins had caused several pathological defects in human that are of public health importance (Dipeolu, 2004; Muhammad *et al.*, 2009; Shareef *et al.*, 2009). Therefore, poultry treated with antibiotics are required for specific withdrawal period until all residues are depleted to safe levels before their tissues can be used for human consumption (Kukanich *et al.*, 2005; Olatoye *et al.*, 2010). Now a day, appearance of antibiotics residue in edible animal tissues remain a global problem (Geidam *et al.*, 2009).

Now a day, different analytical and screening methods are available. Chromatographic analysis like thin layer chromatography, liquid chromatography, gas chromatography, and more advanced mass spectrometry have been replacing the conventional microbiological and immunological detection and quantification of antibiotic residue from food and environmental samples as these methods provided more recovery rate even several-fold higher (Moats, 1985). Hence, chromatographic techniques are preferred to other analytical techniques even though they are quite expensive and sophisticated. Therefore, the proposed work was undertaken to investigate the antibiotics residues in edible poultry and to mitigate the antibiotics residues in poultry product and byproducts.

2. Materials and Methods

2.1. Ethical approval

The experimental broilers were used ethically and at the end of the experiment sacrificed humanely following the ethical and welfare guidelines set by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University.

2.2. Study design

A preliminary survey on poultry farming was made in the north area of Bangladesh necessary samples (liver, thigh muscles and breast muscles) were collected for laboratory investigation. On the other hand, indoor experiments also done on discriminate and indiscriminate use of antibiotics in broiler birds. At the end of experiments, liver, thigh muscles and breast muscles were collected &preserved under -20°C until TLC evaluations.

2.3. Indoor antibiotics experiments on broiler birds

A total of 35 apparently healthy day-old chicks (Cobb-500) were collected from the hatchery of CP Bangladesh Company Ltd, Bhaluka, Mymensingh. The birds were already vaccinated from the hatchery. The experiment was done as described by Trisa *et al.* (2021). On the day 14th, chicks were randomly divided into three groups namely control group (group A, n=10), discriminate Cefalexin antibiotic group (group B, n=10) and indiscriminate Cefalexin antibiotic group (group C, n=10).

Trade name	Generic name	Formulation	Pack size	Dose
CEFA^{TM}-1vet (Popular Pharmaceuticals Ltd.)	Cefalexin monohydrate	Powder	100gm	2gm/Liter drinking water

2.4. Medication of the birds with Cefalexin

Group A was kept as untreated control and received no medicated water. Group B and C were administered Cefalexin antibiotics. After 7 days, at the age of day 21; antibiotic supply was stopped in the group-B followed by withdrawal period. In group C antibiotic was continued till the day of sacrifice.

2.5. Edible tissue samples and screening of antibiotic residues

At the end of experiment, birds were sacrificed and liver, breast muscles and thigh muscles were collected and preserved at -20°C until examination.

The experiment was conducted in the Department of Pharmacology, Bangladesh Agricultural University (BAU), Mymensingh. Collected samples were brought to the Postgraduate Laboratory for investigation of antibiotic residues by Thin Layer Chromatography (TLC) technique. All chemical used were analytical grade.

2.6. Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a sensitive and exact-reliable method for monitoring low amounts of different biological and chemicals. TLC was done as described by Islam *et al.* (2021). In case of field samples;

antibiotics residues of Amoxicillin, Ciprofloxacin, Cefalexin, Enrofloxacin, Oxytetracycline, Zentamycin and Neomycin have been screened out on the other hand in case of indoor experiment; Cefalexin antibiotic has been screened out by TLC analysis.

2.7. Data analysis

Statistical analysis was done. Appropriate statistical analysis was applied as needed. Results were expressed as the mean ± standard error of mean (S.E.M.).

3. Results and Discussion

A total of fifty livers, fifty thigh muscles and fifty breast muscles respectively were analyzed in the Pharmacology Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh. The antibiotics screening test were done on the very popular & mostly used antibiotics namely amoxicillin, ciprofloxacin, cefalexin, enrofloxacin, oxytetracycline, gentamycin and neomycin. TLC analysis revealed that out of fifty liver samples only two samples were positive for amoxicillin, five samples were positive for ciprofloxacin, three samples were positive for cefalexin, two samples were positive for enrofloxacin, four samples were positive for oxytetracycline and gentamycin & neomycin were found negative for any samples (Figure 1).

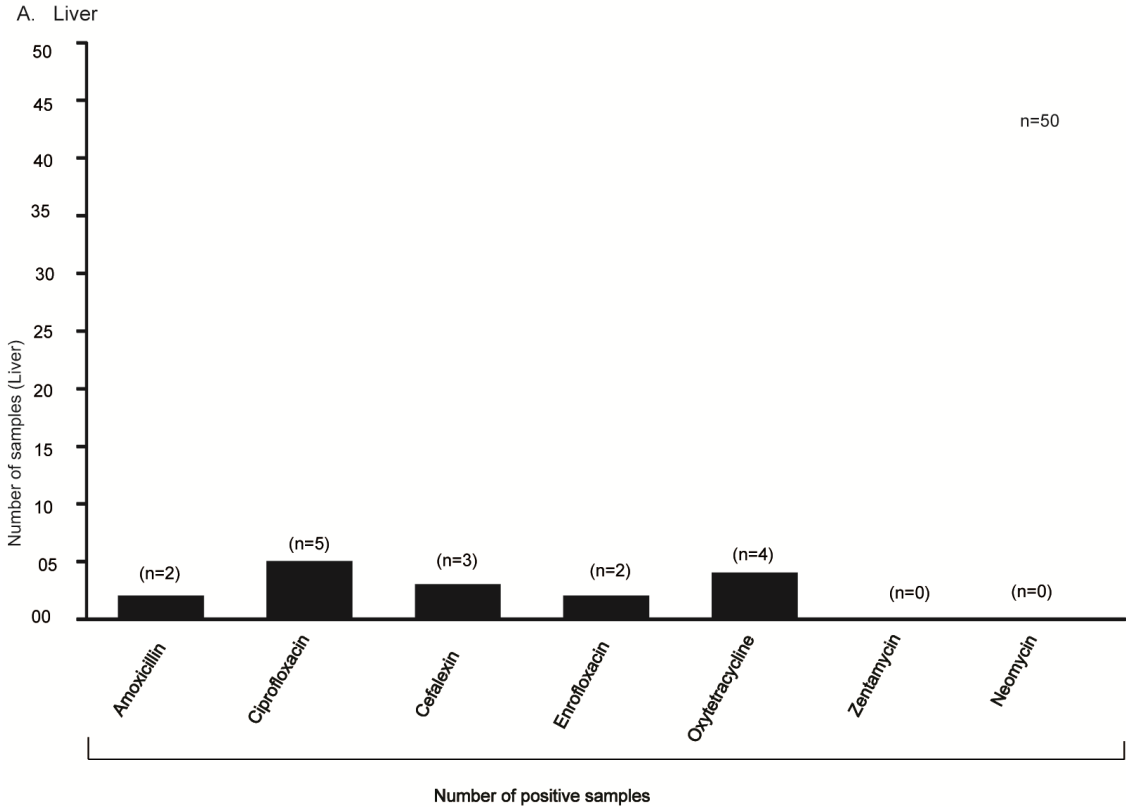

Figure 1. Antibiotics residues in liver. Number of positive samples for amoxicillin, ciprofloxacin, cefalexin, enrofloxacin, oxytetracycline, zentamycin and neomycin are shown. n=50.

In case of thigh muscles, out of fifty samples, two samples were positive for amoxicillin, three samples were positive for ciprofloxacin, three samples were positive for cefalexin, two samples were positive for enrofloxacin, two samples were positive for tetracycline and gentamycin & neomycin were found negative for any samples (Figure 2).

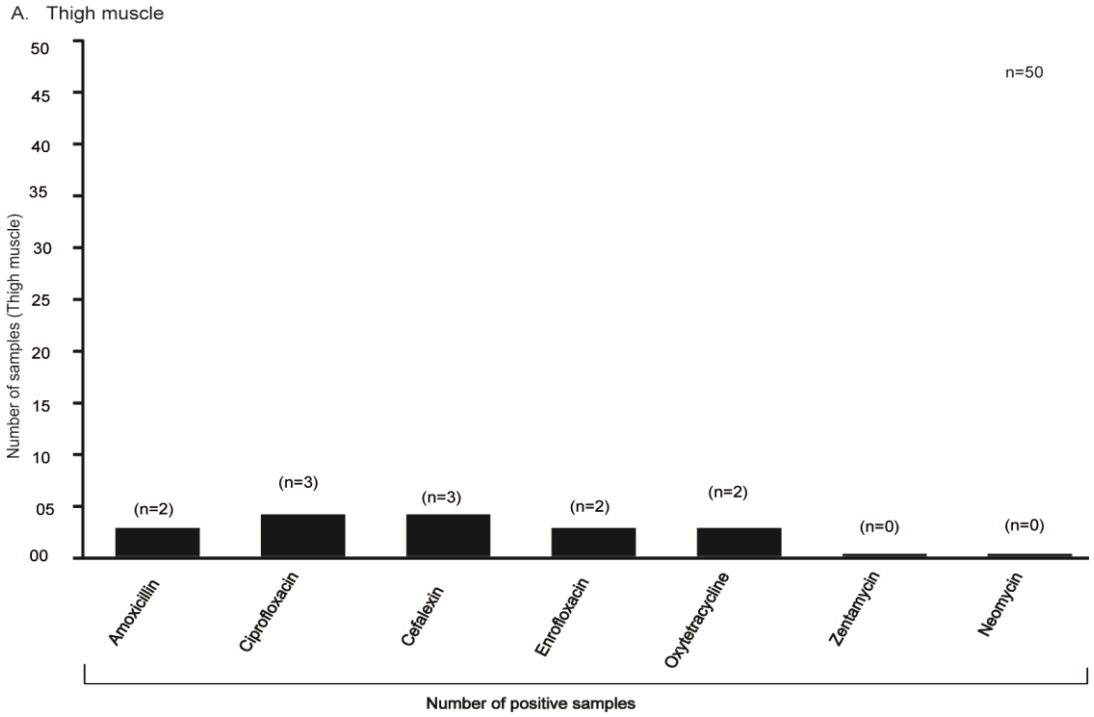

Figure 2. Antibiotics residues in thigh muscle. Number of positive samples for amoxicillin, ciprofloxacin, cefalexin, enrofloxacin, oxytetracycline, zentamycin and neomycin are shown. n=50.

In case of breast muscles, out of fifty samples, two samples were positive for amoxicillin, three samples were positive for ciprofloxacin, three samples were positive for cefalexin, two samples were positive for enrofloxacin, two samples were positive for tetracycline and gentamycin & neomycin were found negative for any samples (Figure 3).

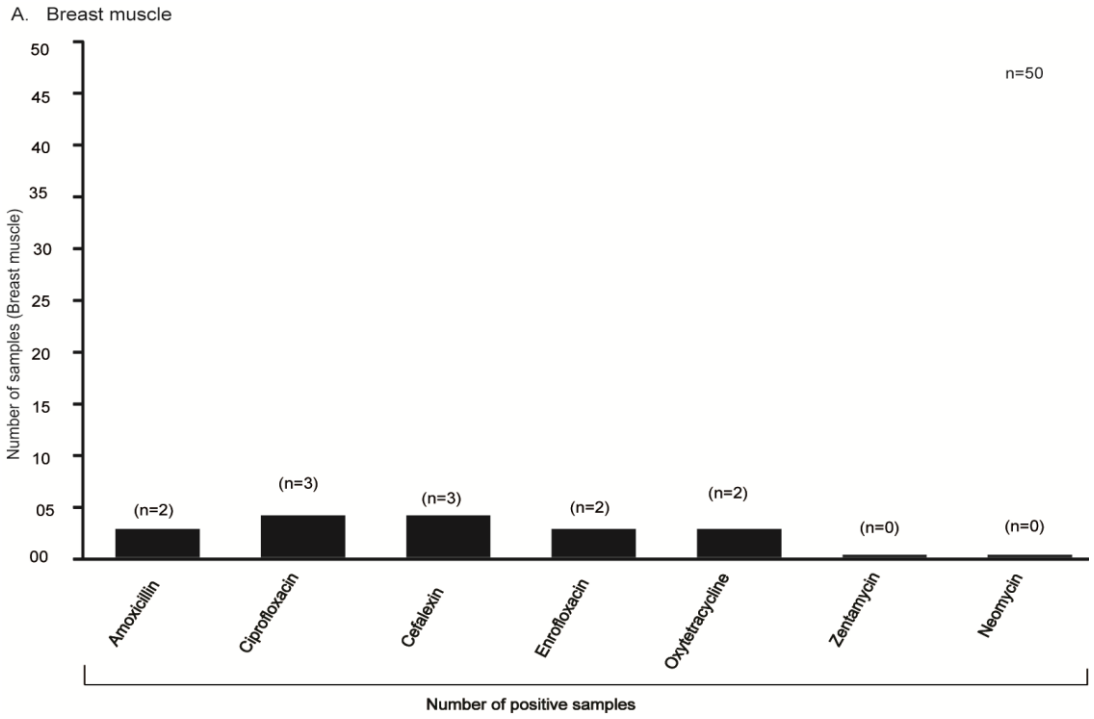

Figure 3. Antibiotics residues in breast muscle. Number of positive samples for antibiotic amoxicillin, ciprofloxacin, cefalexin, enrofloxacin, oxytetracycline, zentamycin and neomycin are shown. n=50.

On the other hands, discriminate and indiscriminate use of Cefalexin antibiotic experiments were also done and analyzed. Samples such as liver, breast muscles and thigh muscles were collected and analyzed by TLC techniques. Control and discriminate groups of birds were found negative for any antibiotics as shown in Figure 4. However, indiscriminate antibiotics group birds demonstrated antibiotics residues positive in liver, thigh muscle and breast muscle. Out of ten samples for each; ten liver, eight thigh muscles and eight breast muscles were found positive for Cefalexin (Figure 4). Antimicrobial resistance is becoming a burning question due to indiscriminate use of antibiotic imposing greater risk to human health. In Bangladesh, Large number of protein is supplied from poultry meat. But in poultry industry, farmers use antibiotic as therapeutic as well as growth promoter without maintaining proper withdrawal period resulting antimicrobial resistance (Ferdous *et al.*, 2020).

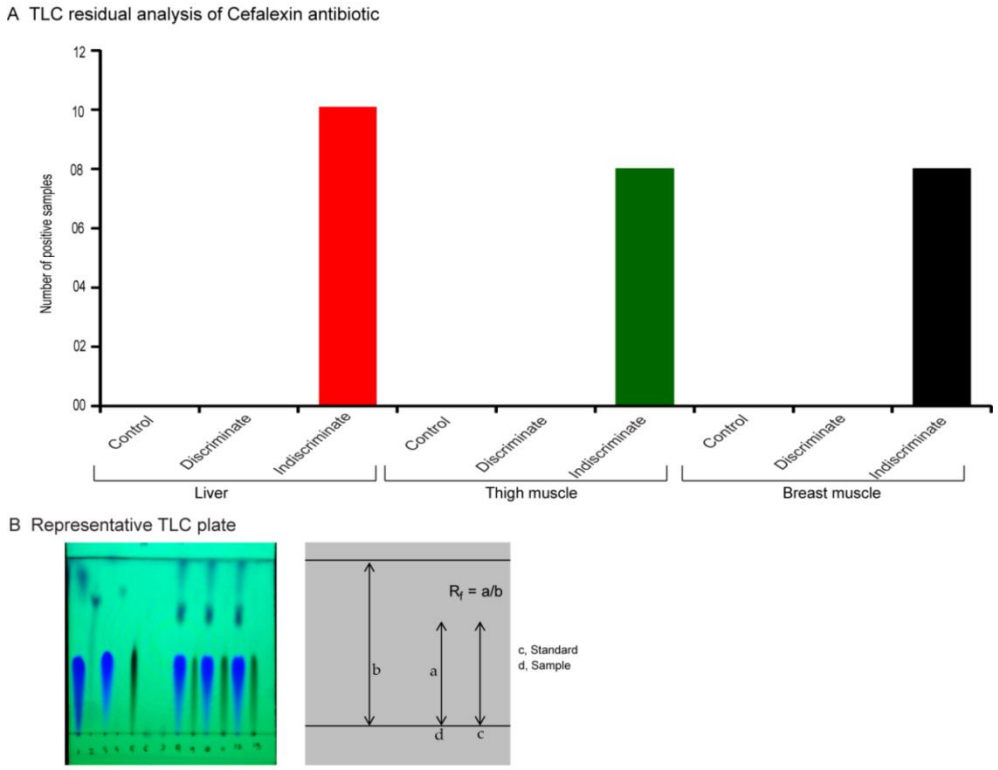

Figure 4. TLC analysis. A, Cefalexin antibiotic residue in liver, thigh muscle and breast muscle. B, representative TLC plate. n=10.

The finding is also similar to Ali *et al.* (2020) who reported that indiscriminate antibiotic group intensity of residues for liver, kidney, spleen, thigh muscle and breast muscle samples were found 50.67%, 50.33%, 39.50% and 48.17% respectively, which is also similar to our research finding. Our previous report also on indiscriminate use of amoxicillin antibiotic demonstrated similar intensity of amoxicillin in liver, kidney, thigh muscle and breast muscle as 57.82%, 52.30%, 45.18% and 49.96% respectively for indiscriminate group (Islam *et al.*, 2019). This finding have similarities with the report of Sarker *et al.* (2018); Sattar *et al*. (2014) that chicken liver contained the highest level of antibiotic residues than muscles and other viscera. As liver and kidney are the main metabolic and excretory organs, all drugs are metabolized and excreted via liver and kidney chiefly (Sattar *et al*., 2014). So, in both antibiotic treated groups, residue was found in liver and kidney with high intensity. There are other evidences of similarities where liver and kidney were considered as the predilection site of antibiotic residues and analyzed accordingly (Metli *et al.*, 2015). The mean residual intensity was lowest in thigh muscle and breast muscle compared to liver and kidney. Relevancy of our result has been found with Al-Mashhadany *et al*. (2018) with some variations.

Drugs rules & regulations should be taken properly to ensure proper maintenance of withdrawal periods before slaughtering and marketing of poultry. Besides, a monitoring policy should be implemented to ensure the conformity of poultry meat with international standards. National authorities should adopt a proactive approach that promotes programs aimed at reducing the need for antimicrobials in food animals and ensuring their prudent use.

4. Conclusions

Poultry samples both outdoor and indoor were investigated by TLC analysis. Interestingly, only negligible number of outdoor samples was found positive for antibiotics residues. On the other hand, indiscriminate use of antibiotic group birds demonstrated antibiotic residue almost in all edible tissues. But, in case of discriminate use of antibiotic in birds, residue was not detected. Therefore, discriminate use and maintenance of withdrawal period is important tool for mitigation of antibiotics residues in poultry product and byproducts.

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Data availability

All relevant data are within the manuscript.

Conflict of interest

None to declare.

Authors' contribution

Md. Shafiqul Islam: study conception, design, research conduction, data analysis and article writing; Sabbya Sachi: data interpretation and analysis; Sharmy Dash: cooperation in research; Md. Shakil Islam: Manuscript preparation. All authors have read and approved the final manuscript.

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