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Article

Discriminate and indiscriminate use of amoxicillin antibiotic and detection of its residue in poultry edible tissue by thin layer chromatography (TLC) method

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Abstract: Antibiotic residue is a burning question in the present world. Antibiotic remain in edible tissues of poultry as a residue due to indiscriminate use in the veterinary field. Human health is at risk of antibiotics due to poultry edible tissues. In this study, we used broiler chicks as a laboratory animal to investigate the judicial use of amoxicillin antibiotic for human health concern. Chicks were reared accordingly and on day 14 the chicks were randomly divided into three groups (n=6) namely control (group A), discriminate (group B) and indiscriminate (group C). At the age of day 16, amoxicillin treatment was started and continued for seven days for discriminate group (Group B) and 15 days for indiscriminate group. In case of discriminate group, seven days withdrawal period was properly maintained, whereas, no withdrawal period was maintained in case of indiscriminate group. In control group, no positive samples were detected by thin layer chromatography (TLC) analysis, whereas, the amoxicillin intensity in liver, kidney, thigh muscle and breast muscle were positive by TLC as 57.82%, 52.30%, 45.18% and 49.96% respectively for indiscriminate group. Similarly 46.81% liver, 44.65% kidney, 29.27% thigh muscle and 32.73% breast muscle were the amoxicillin intensity in discriminate group. The level of amoxicillin were found significantly different between control & discriminate, control & indiscriminate and discriminate & indiscriminate groups by TLC analysis. Therefore, amoxicillin residue present in both discriminate and indiscriminate group but the intensity percentage (%) was highest in case of indiscriminate group indicates high residual concentration.

Keywords: amoxicillin; antibiotic residue; broiler

1. Introduction

Poultry production is the most rapidly growing industries around the globe, and poultry is one of the major sources of meat (Muaz *et al*., 2018). Antibiotics are used largely for three purposes in poultry industries, therapeutic use to treat sick poultry, prophylactic use to prevent infection in poultry and as growth promoters to improve feed utilization and production for their growth promoting properties they are routinely used at sub-therapeutic levels as poultry feed additives (Guetiya Wadoum *et al*., 2016). But, indiscriminate and irrational use of antibiotics resulting in antibiotic residues in foodstuff which can pose hazards to human health and among them are sensitivity to antibiotics, allergic reactions and imbalance of intestinal microbiota are very common (Javadi *et al*., 2011). Antibiotic residues in foods of animal origin are one of the sources of concern among the public and medical health professionals (Singh *et al*., 2014). Amoxicillin is a broad-spectrum, pharmacologically active beta-lactam antibiotic (Koutoulis *et al*., 2015) used largely for treatment of bacterial infection in poultry industries (Alswayeh *et al*., 2015; Khan *et al*., 2018). Despite the important therapeutic use of amoxicillin in veterinary practices, relatively little has been known on the pharmacokinetic behavior and residual status of this drug in birds (Dorrestein *et al*., 1986). Therefore, the present study was undertaken to find

out the antibiotic residues in broiler meat and aware the people about these residues to adopt preventive measures for their own health and welfare of the animals.

2. Materials and Methods

2.1. Experimental design

18 DOCs were collected as laboratory animal from commercial hatchery. Chicks were reared for 15 days without using any antibiotic; only feed and water. At the age of day 15 chicks were grouped in 3 experimental groups (A, B and C); each group having 10 chicks. Group A was kept as untreated control and received no antibiotic medicated water, group B is discriminate group and group C is indiscriminate group. Antibiotic treatment was started from 16^{th} day. Group B was administered with amoxicillin at recommended therapeutic dose @ 10 mg/kg through drinking water as described in (Anadón *et al*., 1996). In group C the dose of amoxicillin was indiscriminate and more than the therapeutic dose (10mg/kg). After 7 days, at the age of day 23; antibiotic supply was stopped in the group-B and withdrawal period was maintained as drug nomenclature (7 days). In group C withdrawal period was not maintained and antibiotic continued till 30^{th} day. Liver, kidney, thigh muscle and breast muscle were collected from every bird at 30^{th} day.

2.2. Chemical and standard drugs

Purity of all standard chemicals and reagents was at least 99%. HPLC grade methanol (Merck-Germany), trichloracetic acid (TCA), diethyl ether, butanol, distilled water and acetic acid. The standard for amoxicillin was prepared by dissolving 0.1 gm of amoxicillin powder in 4 mL solution of methanol. Standard solution was stored in -4°C and every month fresh solution was prepared (Sarker *et al*., 2018).

2.3. Sample preparation

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. Total procedure was performed as the reference cited by (Popelka *et al*., 2005).

2.4. Preparation of the mobile phase

In order to perform Thin Layer Chromatography (TLC) along with the stationary phase, mobile was prepared as directed in the references (Hancu *et al*., 2013). The composition of mobile phase was Butanol: distilled water: acetic acid (60:20:20).

2.5. Thin layer chromatography (TLC)

2.5.1. 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; Butanol: distilled water: acetic acid = 60:20:20) 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 (R_f).

2.5.2. Calculation of R_f values

These measurements are the distance travelled by the solvent, and the distance travelled by individual sample spots. Same R_f value of standard and sample considered similar compound.

2.6. Data analysis

Experimental data were introduced and stored in Microsoft Excel-2010 and results were analyzed statistically using Graph Pad Prism Version 6.

3. Results

3.1. Liver sample

Amoxicillin residue in liver sample of three different groups is presented in Table 1 and Figure 1. There was no positive sample in control group, however, in both discriminate and indiscriminate groups positive samples were found by TLC analysis. The multiple pairwise comparisons during one way ANOVA (Bonferroni) showed that there was significant difference between discriminate and indiscriminate groups ($P<0.05$, $P=0.049$).

3.2. Kidney sample

Table 2 and Figure 2 represent amoxicillin residue in kidney sample of three different groups. In control group, we could not find any positive kidney samples. On the other hand positive samples were found by TLC analysis in both discriminate and indiscriminate groups. The multiple pairwise comparisons during one way ANOVA (Bonferroni) showed that there was significant difference between discriminate and indiscriminate groups ($P<0.05$, $P=0.026$).

3.3. Thigh muscle sample

Amoxicillin residue in thigh muscle sample of three different groups is presented in Table 3. The highest amoxicillin intensity (%) was recorded in the indiscriminate group (45.18± 3.054). The amoxicillin intensity (%) of control and discriminate groups was 0.00± 0.00 and 29.27± 3.548 respectively. There was significant difference among control, discriminate and indiscriminate group ($P<0.001$).

Figure 3 represents amoxicillin antibiotic residue in thigh muscle. In control group all thigh muscle samples were negative, however, in both discriminate and indiscriminate groups positive samples were found by TLC analysis. The multiple pairwise comparisons during one way ANOVA (Bonferroni) showed that there was significant difference between discriminate and indiscriminate groups ($P<0.01$, $P=0.003$). The other two groups (Control & discriminate and control & indiscriminate) also showed significant difference ($P<0.001$).

3.4. Breast muscle

Amoxicillin residue in breast muscle sample of three different groups is presented in Table 4. The amoxicillin intensity (%) in indiscriminate group was 49.96±4.024 whereas, in discriminate group it was 32.73±2.826 and in control group it was 0.00±0.00. The highest amoxicillin intensity (%) was obtained from indiscriminate group. During one way ANOVA there was significant difference among control, discriminate and indiscriminate group ($P<0.001$).

Figure 4 represents amoxicillin antibiotic residue in breast muscle. In control group all thigh muscle samples were negative, however, in both discriminate and indiscriminate groups positive samples were found by TLC analysis. The multiple pairwise comparisons during one way ANOVA (Bonferroni) showed that there was significant difference between discriminate and indiscriminate groups ($P<0.01$, $P=0.002$). The other two groups (Control & discriminate and control & indiscriminate) also showed significant difference ($P<0.001$).

Table 1. Amoxicillin residue in liver sample of three different groups.

Name of group	Amoxicillin intensity in liver by TLC (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	0.00± 0.00		
Group-B (Discriminate group)	46.81± 2.76	<0.001	***
Group-C (Indiscriminate group)	57.82± 4.15		

Table 2. Amoxicillin residue in kidney sample of three different groups.

Name of group	Amoxicillin intensity in kidney by TLC (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	0.00± 0.00		
Group-B (Discriminate group)	44.65± 2.54	<0.001	***
Group-C (Indiscriminate group)	52.30± 1.94		

Table 3. Amoxicillin residue in thigh muscle sample of three different groups.

Name of group	Amoxicillin intensity in thigh muscle by TLC (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	0.00± 0.00		
Group-B (Discriminate group)	29.27± 3.548	<0.001	***
Group-C (Indiscriminate group)	45.18± 3.054		

Table 4. Amoxicillin residue in breast muscle sample of three different groups.

Name of group	Amoxicillin intensity in breast muscle by TLC (Mean ± SEM)	P Value	Level of Significance
Group-A(Control group)	0.00± 0.00		
Group-B(Discriminate group)	32.73± 2.826	<0.001	***
Group-C(Indiscriminate group)	49.96± 4.024		

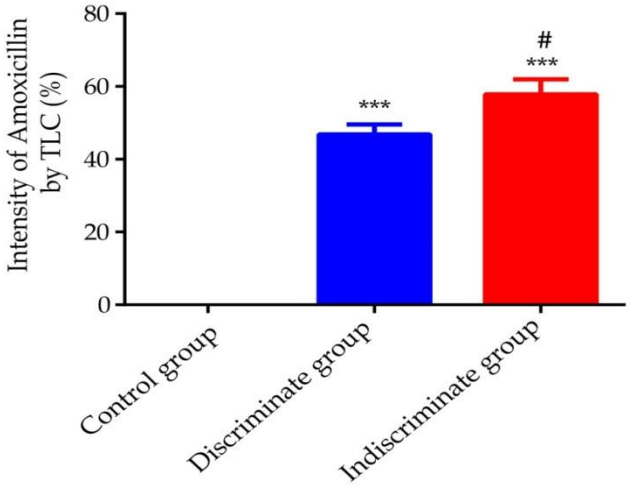

Figure 1. Amoxicillin antibiotic in liver.

***, Significantly difference between control & discriminate groups and control & indiscriminate groups (P<0.001).

#, Significantly different between discriminate and indiscriminate groups (P<0.05).

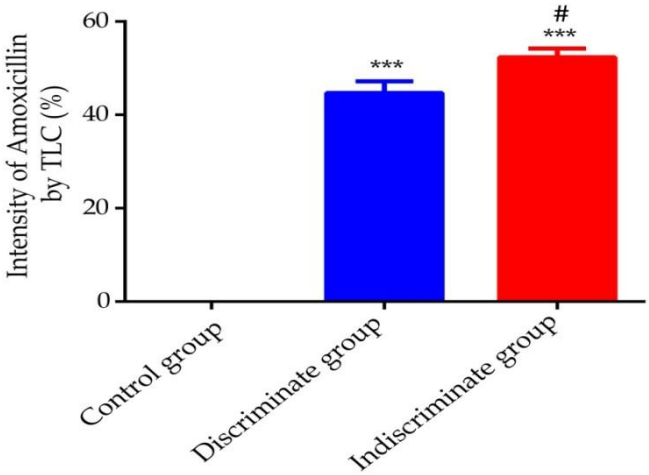

Figure 2. Amoxicillin antibiotic in kidney.

***, Significantly difference between control & discriminate group and control & indiscriminate groups (P<0.001).

#, Significantly different between discriminate and indiscriminate groups (P<0.05).

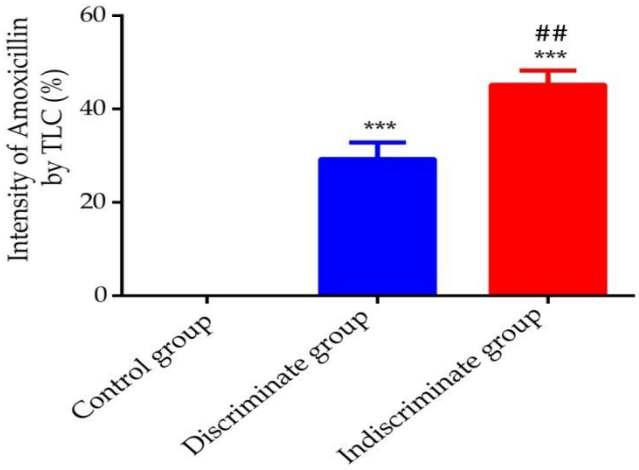

Figure 3. Amoxicillin antibiotic in thigh muscle.

***, Significantly difference between control & discriminate group and control & indiscriminate groups (P<0.001).

##, Significantly different between discriminate and indiscriminate groups (P<0.01).

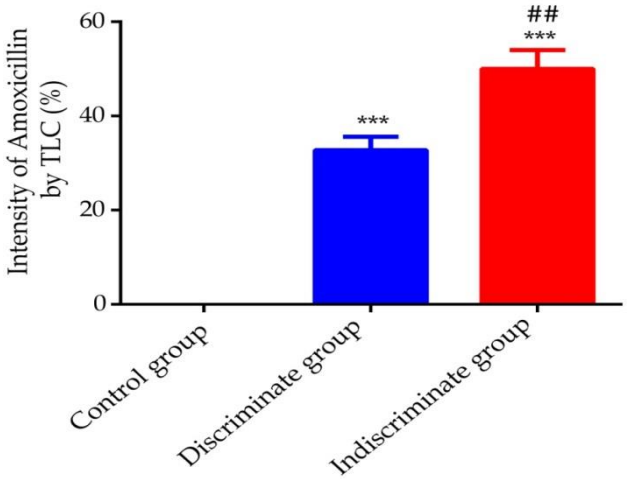

Figure 4. Amoxicillin antibiotic in breast muscle.

***, Significantly difference between control & discriminate group and control & indiscriminate group (P<0.001).

##, Significantly different between discriminate and indiscriminate group (P<0.01).

4. Discussion

Chicken meat is a good source of high quality protein along with important vitamins and mineral and contains low fat (Givens, 2005). Also chicken production is quicker and cheaper than other meat sources (Ivanovic, 2003). Demand for chicken meat increasing day by day resulting increase use of antibiotics, hormone and growth promoter to cope with the increasing demand (Paryad and Mahmoudi, 2008). In TLC analysis the indiscriminate group showed high amoxicillin residual intensity. Chicken liver and kidney contained the highest of proportion of antibiotic residues than the rest of samples. This finding has similarities with the report of Sattar *et al*. (2014); Sarker *et al.* (2018); chicken liver contained the highest level of amoxicillin residues than muscles and other viscera. As the liver and kidney is the main metabolic and excretory organs, all drug are metabolized and excreted via liver and kidney chiefly (Sattar *et al.*, 2014). So, even in discriminate groups, the presence of antibiotics residue was found in liver and kidney with high intensity. There are other evidences of similar results where kidney and liver was considered the predilection site of antibiotics residues and analyzed accordingly (Metli *et al*., 2015). The amoxicillin 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. Data on antibiotic residues in chicken is relatively low in Bangladesh. However, periodic sampling is being carried out in many countries to detect antibiotic residue in the food cycle (Weiss *et al*., 2007;

Zhao *et al*., 2009). Due to the high risk of veterinary drug residues in foods of animal origin, the maximum residues limit (MRL) regulation for use of each pharmacologically active substance has been developed by European Union (EU). The MRL regulation determines the maximum concentrations of residues which are permitted in foods of animal origin and should be followed for public health food safety (Myllyniemi, 2004; Reyes-Herrera *et al*., 2005). Our study confirms the existing presence of antibiotic residue in our food chain. However, it is necessary to investigate how antibiotic residues enter the human food chain via different ways.

5. Conclusions

The deposition and subsequent detection of amoxicillin residue in poultry edible tissue is really a matter of concern. 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. This research finding is reporting that once antibiotics are administered to animal body, antibiotic residues are present in high or low concentrations in their products. However, it mainly depends on the duration of the administration of antibiotics and maintenance of withdrawal period. After the administration of antibiotic, concentration of their residues gradually reduces mostly after maintenance of withdrawal period secretion of residues is negligible. Hence, the withdrawal time of drugs should be strictly followed. Thus, by observing proper scientific guidelines and precautions we can minimize the harmful effects of antibiotic residues.

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Conflict of interest

None to declare.

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