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Antibiotic Residue Screening in Liver, Kidney, and Muscle Tissues of Non-Monitored Poultry Products, Cull-broiler and Cull, Sold in La Trinidad, Benguet

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Abstract

Cull-broiler and cull are popular poultry meat products in Cordillera Administrative Region (CAR), Philippines but these are not being monitored by authorities for antibiotic residue. This antibiotic residue could pose a direct threat to consumer's health; thus, this study deemed it necessary to detect the presence of antibiotic residue in the liver, kidney and muscle tissues of cull-broiler and cull being sold in La Trinidad, Benguet using disc diffusion technique or the Kirby-Bauer Disc Method. Results revealed presence of antibiotic residues in the three tissues of cull-broiler. The liver tissue samples had the highest number with positive results against the three test bacteria. This result implies the highest incidence of antibiotic residue in liver tissues followed by kidney and least in muscles. Additionally, cull-broiler has greater incidence of antibiotic residue than cull. Also, there is a significant difference in the level of antibiotic residue present between the tissues of cull-broiler and cull, as shown by the mean diameter (mm) of inhibition zone. Tissues of cull-broiler had the widest mean zone of inhibition as compared to the tissue of culled chicken. Moreover, the zone of inhibition is significantly affected by the type of chicken and the type of tissue indicating that the level of antibiotic residues depends on the type of chicken and the type of tissue. Though the presence of antibiotic residue in the different tissues is relatively small, it still indicates the potential for public health risk. Concerned agencies should not only check broiler for the presence of antibiotic residue but should also include cull-broiler and cull chicken sold in the market.

KEYWORDS

Antibiotic residue
cull-broiler chicken
culled chicken
Benguet
withdrawal period
disc diffusion technique

Introduction

Antibiotic residues occur in various types of foods of animal origin including milk, egg, and meat due to large-scale application of antibiotics in veterinary practice. An antibiotic residue is a portion of antibiotics that remains in the body after its use have been discontinued (Swatantra et al., 2014). Antibiotic residues present in edible poultry tissues even in trace amounts can affect

the public health (Donoghue, 2003; Mohamed, 2014) and it is one of the concerns of medical health professionals (Adams, 2001). Adverse effects could either be immediate or longer term side effects. Notable antibiotic families used in veterinary medicine include B-lactam, Tetracyclines, Chloramphenicols, Aminoglycosides, Nitrofuranes, Nitroimidazoles, Macrolides, Quinolones and Macrocyclics (Ward & Farris, 2001).

A withdrawal period is established to safeguard human from exposure of antibiotics added in food. Withdrawal time reflects the amount of time necessary for an animal to metabolize an administered product and the amount of time necessary for the product concentration level in the tissues to decrease to a safe, acceptable level before slaughter. Every approved drug or animal health product has a withdrawal period printed on the label or package insert that should be observed before slaughtering the animal. Withdrawal period includes bans on offering medicated feed or water to animals before slaughter. Withdrawal times are not the same for all drugs and could be extended when combinations of drugs are used or when used beyond the recommended rate (Porter et al., 2011).

Withdrawal time only applies when the drug is used according to label instructions and not following these instructions may cause antibiotic/ drug residue. Changing the dose, route, volume per injection site or duration of treatment can drastically change the required withdrawal time of a drug (Porter et al., 2011). Chemicals and drugs including antibiotics employed for chemotherapeutic and prophylactic purposes are also used as feed additives to promote growth, improve feed efficiency, and synchronize the reproductive cycle and breeding performance which may further lead to residue toxicity (Swatantra et al., 2014). Previous studies of Ezenduka et al. (2014), Hind et al. (2014) and Al-Ghamdi et al. (2000) have reported that broilers are positive of antibiotic residues.

In the Philippines, poultry producers and dealers sell chicken in the form of cull and cull-broiler. Culled chicken layers or spent hens refers to those non-laying, unproductive, or low producing hens from a laying flock. On the other hand, cull-broilers are 5-month-old broilers that are raised mainly for their flesh, where meat textures are less tough than culled hens but tougher than broiler meat.

The people of Benguet and Mountain Province are the number one consumers of cull-broiler and culled layer hens in the Cordilleras with La Trinidad and Baguio City as two of the central livestock markets for these. At present, pre-roasted cull-broiler and culls are also sold in the wet market. These are patronized by the local folks because of their cheaper price compared to broiler and can feed a large family or a big number of people. The demand increases during festive occasions like Christmas. In fact, livestock stores sometimes run out of live chicken to sell during the holiday season.

Unfortunately, the National Meat Inspection Commission (NMIC) of the Philippines only monitors slaughtered broilers for the presence of antibiotic residue but is not including cull-broiler and cull. This lack of monitoring for cull-broiler and cull could pose a direct threat to the health of its consumers. Hence, the study was conceptualized to determine if cull-broilers and culls sold in La Trinidad, Benguet contain antibiotic residue using disc diffusion technique. It specifically sought to detect the presence of antibiotic residue in the liver, kidney, and breast muscles of cull-broiler and cull and to determine the effect of the type of chicken and the type of tissue on the inhibition zones. The study was limited to microbial test and did not include complex confirmatory text such as ELISA and HLPC (Tajik et al., 1998). Still the researchers found the study to be immediate due to the lack of monitoring for cull-broiler and cull which could pose a direct health hazard.

Materials and Methods

A total of 60 chickens were bought from various live poultry dealers, and meat stores selling dressed cull-broiler and cull. The tissue samples obtained from 30 cull-broiler (30 livers, 30 kidneys, 30 breast muscles) and 30 cull chicken (30 livers, 30 kidneys, 30 breast muscles) were considered in the study. These were taken from two different livestock stores; one in La Trinidad wet market and a poultry farm. About 10-15g of tissue samples were collected in sterile polyethylene bags and stored in a cooler with ice. The samples collected were then transported to the Microbiology Laboratory at Benguet State

University for isolation and analysis.

Samples were analyzed for the presence of antibiotic residue by using microbiological assay method specifically the Kirby-Bauer Disc Method or disc diffusion technique (Hudzicki, 2009). The test organisms were three species of bacteria namely; *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*. The positive test control was enrofloxacin suspension which was used to compare the zone of inhibitions on samples being tested.

The inoculums of the three species of bacteria were prepared by obtaining an 18-hour old broth culture. The inoculum was then adjusted to a turbidity equivalent to 0.5 McFarland standards (approximate cell density of $1.5x10^8$ CFU/ml). Finally, the turbidity of the suspensions was compared by placing the tubes in front of a white paper or file card with black lines (Coyle et al., 2005). The suspensions were inoculated on the sterile Mueller Hinton agar (MHA) plates and spread evenly with the use of a sterile glass L-rod.

Prior to the preparation of inoculums, sterile Whatman paper disc were soaked in the different tissue fluids. The sterile discs were inserted within the incised tissue samples of kidneys, liver and muscles. The soaked Whatman paper disc were strategically laid with sterile forceps in three replicates per plate. Three pieces of sterile Whatman paper discs were immersed in the suspension of the antibiotic, enrofloxacin, and placed on the inoculated MHA plates as positive control. The plates were then inverted and incubated at 37-38°C for 18 hours, taking care not to let the inoculated MHA stay at room temperature for not more than 15 minutes. After incubation, the plates were observed for the presence of inhibition (Hudzicki, 2009). The diameters of the zone of inhibition were measured with a vernier caliper. The data on the measurement of inhibition zones were analyzed with ANOVA and Tukey's test as post-hoc.

Results

Table 1 presents the results of antibiotic residue detection as indicated by the presence of the zone of inhibition from the liver, kidney, and breast muscle tissue samples of cull-broiler and cull sold in La Trinidad, Benguet. A total of one hundred eighty tissue samples were tested against the three test bacteria: *Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus*.

For *B. subtilis*, seven (7) liver tissue samples of cull-broiler tested positive for antibiotic

Table 1

Number of tissue samples obtained from cull-broiler and culled chicken that exhibited the zone of inhibition against the 3 test bacteria

Test bacteria	Tissue samples N=30	No. of tissue samples w/ positive results		Percentage (%)	
		Cull-broiler	Cull	Cull-broiler	Cull
Bacillus subtilis	Kidney	5	4	16.67	13.33
	Liver	7	4	23.33	13.33
	Muscle	0	4	0.00	13.33
Escherichia coli	Kidney	0	0	0.00	0.00
	Liver	5	0	16.67	0.00
	Muscle	0	0	0.00	0.00
Staphylococcus aureus	Kidney	5	0	16.67	0.00
	Liver	10	0	33.33	0.00
	Muscle	3	0	10.00	0.00

residue while five samples for kidney tissue. Unexpectedly, there was no zone of inhibition with the muscle tissue samples for *B. subtilis*. In cull, all tissues have four samples each that tested positive with zone of inhibition.

For the tissue samples tested against *E. coli*, only cull-broiler tested positive for antibiotic residue and it is only the liver tissue samples that showed a zone of inhibition. Lastly for *S. aureus*, only tissues from cull-broiler tested positive for antibiotic residue. Highest positive occurrence was recorded in liver tissues (10) followed by kidney and muscle tissue samples at 5 and 3, respectively. Notably, among the three tissues tested, the liver tissue samples had the highest number with positive results against the three test bacteria. This result implies the highest incidence of antibiotic residue in liver tissues followed by kidney and least in muscles. Additionally, cull-broiler has greater incidence of antibiotic residue than cull.

Figure 1 illustrates the total proportion of all the samples collected and tested between cull-broiler and culled chicken for the presence of inhibition zones. The liver tissue samples obtained from the cull-broiler had the highest percentage of inhibition zones at 24.44% followed by kidney samples at 11.11% and least in the muscle tissues at 3.33%. On the other hand, the tissue samples from the culled chicken had 4.44% positive samples for each kind of tissue.

The overall mean diameter of inhibition zone from the different tissue samples taken from cull-broiler, cull and the positive control, enrofloxacin, is shown in Figure 2. The liver tissue samples of cull-broiler had the highest mean of inhibition zone of 8.98 mm among all the samples tested, followed by kidney tissue of the same with 6.00mm. The muscle tissue samples had the lowest mean of inhibition zone at 0.25mm. On the other hand, the kidney, liver, and muscle tissue samples of culled chicken, had inhibition zone diameter of 2.72mm, 2.57mm and 2.78mm, respectively. Interestingly, cull-broiler muscle tissue samples with positive results had the lowest mean diameter of inhibition zone. The tissue samples from both kinds of chicken showed lower mean diameter compared to the 14.66mm result of the positive control, enrofloxacin.

Highly significant differences on the zone of inhibition between the types of chicken was observed (Table 2). Cull-broiler marked a higher zone of inhibition at 8.92mm than culled chicken at 8.07mm mean inhibition zone. Additionally, highly significant differences were recorded on the zones of inhibitions between the types of tissues. Chicken liver had the highest zone of inhibition recorded at 8.91mm followed by kidney and muscle at 8.68mm and 8.0mm respectively. With reference to the test bacteria used, *B. subtilis* had the highest zone of inhibition at 8.98mm followed by *S. aureus* and *E. coli* with mean

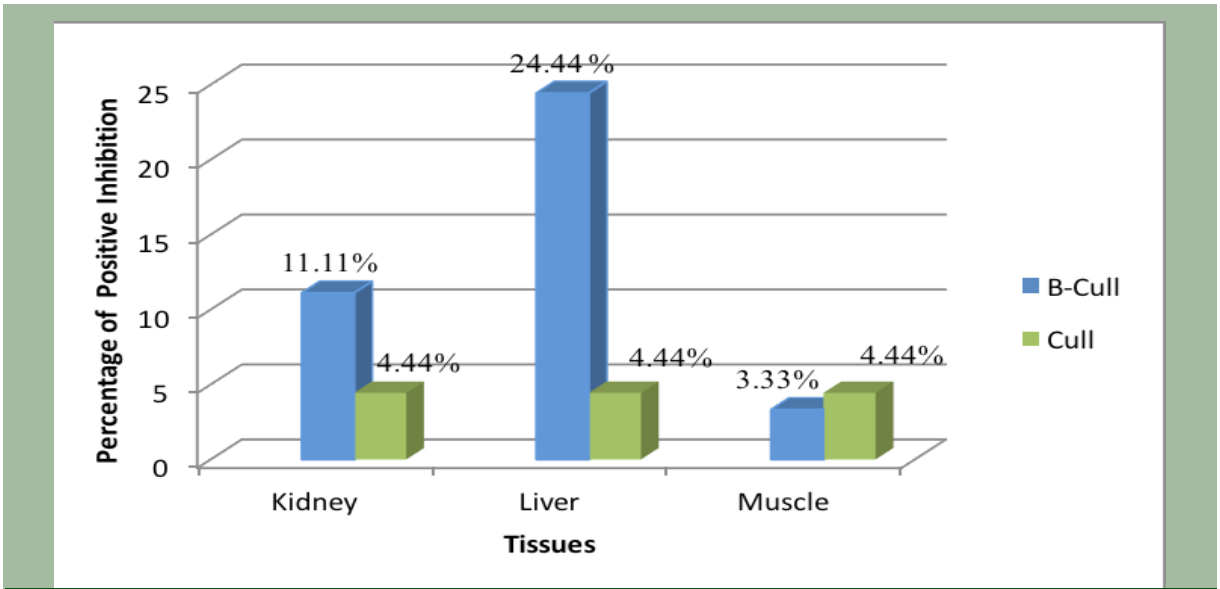

Figure 1. Proportion of tissue samples effecting inhibition zones

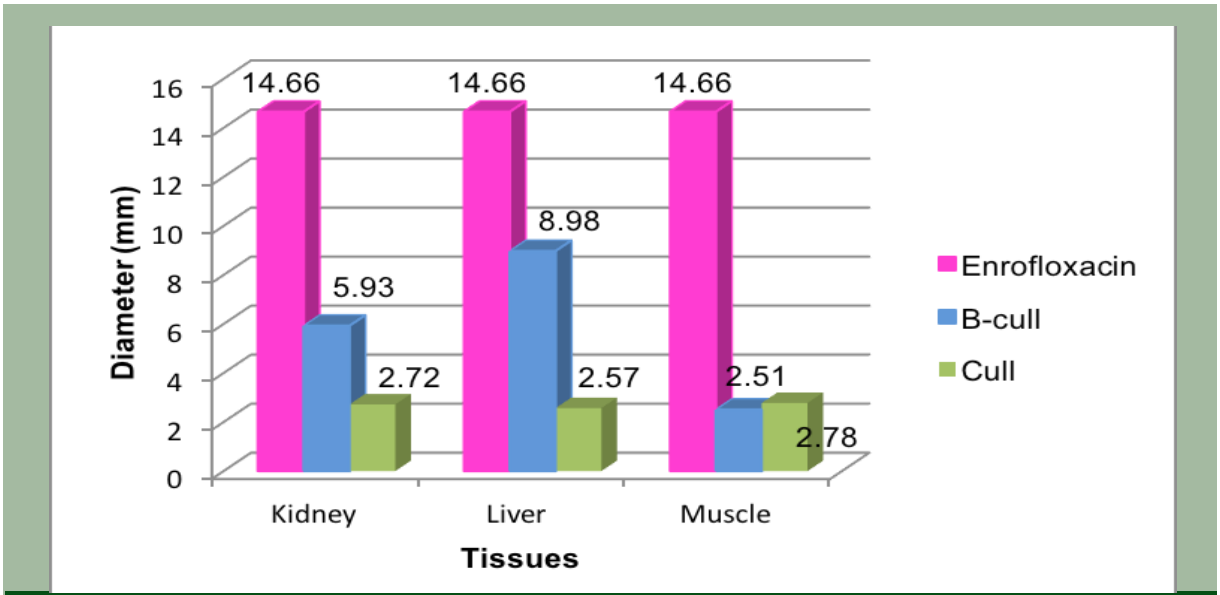

Figure 2. Over all Mean Diameter of inhibition zones effected by tissue samples and the positive control (enrofloxacin).

inhibition zone of 8.70mm and 8.49mm respectively. These results show that the zone of inhibition was affected significantly by interaction between types of chicken, type of tissues and species of microorganisms used.

Discussion

The results obtained in the detection of antibiotic residue in the liver, kidney and muscle tissues of cull-broiler and culled chicken (Figure 1) corroborates with the study of Shahid et al. (2007), where liver had the highest percentage of positive results followed by kidney then muscle tissues. However, our results contradict those of Ezenduka et al. (2014) and Pavlov et al. (2008) who reported that kidney had the most number of samples with antibiotic residue followed by liver in broilers sold at poultry markets and broilers slaughtered at abattoirs. Our results shown lower incidence of antibiotic residue compared to the study of Hind et al. (2014) who detected 21.4–28.3% incidence across liver, kidney and muscle chicken tissues in Khartoum State, Sudan. Our results would imply presence of misuse of antibiotic in cull-broiler and cull in the area but not as prevalent as feared. Still, these results should still to be alerted to authorities for proper action.

Presence of the inhibition zone indicates the presence of antibiotic residue in the tissue samples of kidney, liver, and muscles from cull-broiler and culled chicken. The specific antibiotic residues though are unknown since the method employed does not allow differentiation of the type of drugs. Though there is a significant difference in the mean diameter of inhibition zone in the tissue samples studied with the positive control, it is still of concern that the samples studied contain antibiotic residue as shown by the presence of zone of inhibitions. The result on the inhibition zone may be affected by the sensitivity of the various bacteria towards the different antibiotics that may be present in the samples. It has been reported that B-lactam antibiotics gave inhibition zones only with *Micrococcus luteus* while tetracyclines can be detected up to the MRL level with *Bacillus cereus* and flouroquinolones with *Escherichia coli* (Hind et al., 2014).

There is a strong significant difference in the zone of inhibition of the antibiotic residues between the type of chicken and the type of organ/tissue (Table 2). This affirms previous study conducted by Al-ghamdi et al. (2000) who reported that younger chicken such as broiler has a higher percentage of samples with antibiotic residue compared to layer hens or cull. The presence of antibiotic residue may also be traced back to the feeds and drugs given to the poultry. The uncontrolled and/or prolonged use of antibiotics

Table 2

Effect of the type of chicken and type of tissue/organ on inhibition zones

	Factors	Inhibition (mm)
Types of Chicken	Cull-broiler	8.92*
	Cull	8.07*
Types of Tissue	Kidney	8.68[b]
	Liver	8.91[a]
	Muscle	8.00[b]
Types of Bacteria	*Bacillus subtilis*	8.98[b]
	Escherichia coli	8.49[a]
	Staphylococcus aureus	8.70[b]

Means with the same letter in a group (type) are not significantly different at 5% Tukey's test

**- significantly different*

in poultry farms to prevent diseases, both in the therapeutic setting recommended by the veterinarian or in cases of poultry farmers giving medications to their chicken without proper prescription, and the lack of awareness by the farmers regarding the recommended withdrawal period of drugs may all contribute to the presence of inhibition zone that indicates the presence of antibiotic residues. The lower percentage of samples with antibiotic residue in cull might be due to the practice of some poultry farmers wherein they feed the culled chicken with plain corn grits and the stop adding antibiotic to their drinking water.

The high demands of cull-broiler for "pinikpikan" during holidays like Christmas compel producers to sell their livestock without completing the prescribed withdrawal period of the antibiotics given to their livestock. The study was conducted during the Christmas season; hence, this factor could have also contributed to the results of this study.

In this study, *B. subtilis* detected more positive samples followed by *S. aureus* and least in *E. coli*. This agrees with the earlier report that *B. subtilis* can detect more positive samples than *E. coli*. Hind et al. (2014) reported that when using *Bacillus* as test bacteria, muscle tissue cannot be used as test material for screening oxytetracycline, enrofloxacin, and ciprofloxacin residue, while penicillin G can be screened from muscle tissue.

The overall result of this study may be explained by the massive, uncontrolled and prolonged antibiotic use in poultry farms as preventive measure against bacterial diseases. This can also be due to the disregard for the prescribed withdrawal periods between the time of administering antibiotics and time of slaughtering/selling the chicken. This could also be attributed to the high demand of these poultry product during the time of the study (being a holiday season) such that the prescribed withdrawal periods was not observed. Other factors that could also contribute are the presence of antimicrobials in feedstuff and drinking water given even in low concentration (Swantantra et al., 2014; Donoghue, 2003; Mohamed, 2014). A survey conducted on the use of antibiotics in the semi-industrial poultry farms accounted that 73% of owners do not seek veterinary assistance (Hakem et al., 2013).

The presence of drug or antibiotic residues in food above the maximum allowable level are recognized worldwide by various public health authorities. It is illegal and their consumption could result in public health hazards including development of resistant strains of microorganisms, hypersensitive reaction in sensitized individuals and distortion of intestinal microflora. Moreover, antibiotic residues could cause economic losses in the food industry especially in interfering with starter culture in yoghurt and cheese production (Ezenduka et al., 2014).

Though there is a small number of tissue samples positive with antibiotic residue in the study, it still indicates the potential for public health risk. Based on the report of Swatantra et al. (2014), microbial resistance to antibiotics results from prolonged ingestion of meat with small amount of antibiotic residues. Many of the antibiotics used to treat bacterial infections in humans have veterinary applications and antibiotic resistance is a global public health concern today. The U.S. Centers for Disease Control and Prevention has described resistance as one of the world's most pressing health problems, because the number of bacteria resistance to antibiotics has increased in the last decade and many bacterial infections are becoming resistant to the most commonly prescribed treatment. The World Health Organization (WHO) has identified antibiotic resistance as one of the three greatest threats to human health. Microorganism's resistance to

antibiotic drugs emerged and spread soon after the introduction of those drugs and in parallel with their use. Many well-known antibiotics are no longer effective to treat common infections such as otitis, pneumonia, gonorrhea, and tuberculosis. Thus, microbial resistance is the main cause of newly emerging and reemerging infectious diseases. Not only is there a risk to human health from direct toxicity and from allergic reactions in persons sensitized to antibiotics but antibiotic residues may also interfere with any microbial examinations which may be necessary in assessing the fitness of the carcass. The WHO has recommended that antibiotics that are licensed in human medicine should not be used any more as growth promoters in livestock.

Enrofloxacin (brand name Baytril), a fluoroquinolone drug related to ciprofloxacin, is used worldwide in the poultry industry. Baytril is authorized in the UK for treatment only for respiratory and digestive system infections in pigs, cattle, and poultry, including calves and piglets, and is administered to poultry in drinking water (NOAH, 2016). The fluoroquinolones are classified by the WHO as critically important antibiotics for human medicine, and their effectiveness needs to be protected. One of the main fluoroquinolones in human medicine is ciprofloxacin (brand name Cipro), which is a first line drug for treatment of severe Salmonella and Campylobacter infections in adults. It is also effective against plague, anthrax, and potential biological weapons. Poultry are 'a major source of human exposure to fluoroquinolone resistance via food', according to EFSA's (European Food Safety Authority) Panel on Biological Hazards.

When animals are administered with antibiotic that is closely related to an antibiotic used in human medicine, cross-resistance occurs and disease-causing bacteria become resistant to the drug used in human medicine. The world's veterinary and medical experts generally agree that it is dangerous and unjustifiable to use antibiotics that are related to drugs of critical importance in human medicine (NOAH, 2016).

Conclusions

Result of the study showed presence of antibiotic residue in different tissues of cull-broiler and cull where higher incidence of antibiotic residue was recorded in cull-broiler than in cull. Because the study was done during Christmas season where there is the high demand of poultry, there is the possibility that proper withdrawal time of drugs was not observed. Though the presence of antibiotic residue in the different tissues is relatively small, it still indicates the potential for public health risk. Still, these results should still to be alerted to authorities for proper action.

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