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Research Paper *Open Access*

'ANTIBIOTIC RESIDUE FREE BROILER MEAT'; PREVALENCE OF ANTIBIOTIC RESIDUES IN BROILER MEAT AND RESISTANT BACTERIA IN POULTRY LITTER IN SRI LANKA AND AWARENESS ON ANTIBIOTIC USAGE

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Antibiotics are broadly being used in poultry industry where the residual effect and antibiotic resistance have become major environmental impacts. In this study, a questionnaire survey was carried out with thirty three buyback farmers and employees of closed house farms from six broiler chicken producers in Sri Lanka. Eighty four whole birds were randomly selected at processing plants and tested for antibiotic residues in liver, kidney and breast, using the Six Plate Assay method (SPA). Litter samples from twenty five farms were cultured in Tryptic Soy Agar (TSA) with 3 treatments; T1: TSA+0 ppm Antibiotics, T2: TSA+10 ppm Enrofloxacine and T3: TSA+10 ppm Tetracycline. The survey revealed that the managers and supervisors of the closed house farms and the buyback farmers had a substantial knowledge on antibiotics and their adverse effect whereas the laborers of closed houses were not well aware. All the litter samples contained resistant bacterial populations where the Tetracycline resistant population was always higher ($P<0.05$) than that of Enrofloxacine. The broiler meat was free from detectable amounts of antibiotic residues. In conclusion, broiler chickens (meat, livers and kidneys) of the surveyed producers were free from antibiotic residues. However, broiler litter samples consisted of antibiotic resistant bacterial populations.

Keywords: Antibiotic residues, Antibiotic resistance, Tetracycline, Broiler meat, Litter

INTRODUCTION

Due to the presence of essential of substantial amounts of amino acids and the higher palatability, animal-based food products have a significant value in daily human diets (Mumtaz *et al.*, 2000). In Sri Lanka poultry industry is a major subsector in livestock farming where broiler chicken meat is considered as one of the main outputs (De Silva, 2012). Animal feed is the major input in poultry sector where antibiotics are known to be one of the major feed additives. Generally antibiotics are known as a subset of antimicrobial agents derived or molded from other microorganisms, which have the capability of slowing down or killing another targeted bacteria (National Veterinary Accreditation Program, 2012). These antibiotics are used in the poultry industry with the aim of therapy, prophylaxis and growth promotion.

Although there are many beneficial sides in antibiotic usage, World Health Organization has taken the leadership in reducing the use of human antibiotics in livestock animals including broiler chicken, due to the adverse negative effects (Graham *et al.*, 2007). According to the studies, residual effects of antibiotics and development of antibiotic resistance have been identified as the major negative

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outcomes of antibiotic use, to humans as well as to the environment (Redding *et al.*, 2014).

According to recent surveys, less attention on the withdrawal period has become the major reason for antibiotic residues accumulation in meat, in lower and middle-income countries (Redding *et al*. 2014). Due to these residues, the antibiotic resistance has been developed over years, following the inability of successive results of using antibiotics (Ricke *et al.*, 2012). Antibiotic resistance has a great effect on human health and food safety where the nonpathogenic bacteria can transfer the resistant genes to pathogenic microorganisms.

Therefore, this study was carried out with the aim of assessing the awareness of commercial level closed house and buyback broiler chicken farmers on usage and impacts of antibiotics, while evaluating the presence of antibiotic residues in broiler chicken meat produced in commercial broiler chicken farms in Sri Lanka and the incidence of resistant bacterial populations in litter material.

MATERIALS AND METHODS

The current study was conducted with six leading broiler chicken producers in Sri Lanka. One company operated only with closed housed broiler chicken farms and the other five companies are operated with closed house farms and buyback farms. The farms were mainly located in the Western and North Western provinces of Sri Lanka.

Questionnaire Survey

Three, pre-tested structured questionnaires were used for three different groups involved (i.e., closed house farm managers, closed housed supervisors/laborers, and buyback farmers). In brief, the questionnaires included general management aspects of the farm, details on medicinal drugs usage in the farm, antibiotic usage in disease control and growth promotion, management practices in antibiotic application, awareness on impacts of antibiotic usage.

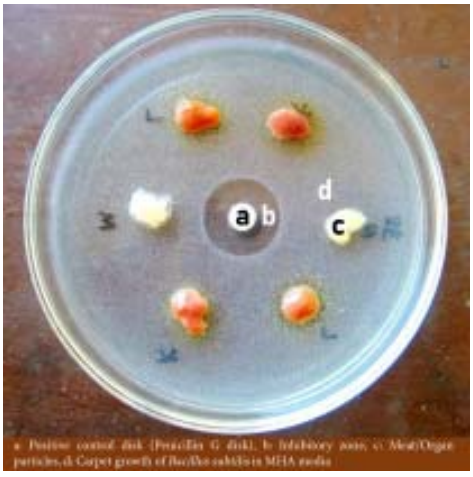
Analysis of Broiler Chicken Meat for Antibiotic Residues

Eighty-four birds representing the six companies were used in the current study. From each bird, three sub samples were taken; approximately 150 g of breast muscle without bones, whole liver and whole kidney. Meat samples were separately packed and kept in an iced cooler box while transporting.

Bio-assay procedure was conducted at the Food Safety and Quality Assurance Laboratory, Department of Veterinary Public Health And Pharmacology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka. Six plate assay was used to detect/ screen antimicrobial residues in meat samples (Hathurusinghe, 2004). Pre-tested *Bacillus subtilis* strain was used to conduct the assay.

Mueller Hinton agar was used as the culture medium for *Bacillus subtilis*. From each tissue sample to be analyzed, a cylindrical core of 8 mm diameter was removed and from those cylinders, 2 mm thick slices were cut, avoiding any fat particles and one assay plate of a bird was prepared as the plate I, placing two identical slices from the same muscle at opposite sides (Plate 1). Prepared plates were incubated at 30 °C for 18 hours and the screening of residues was done by observing the occurrence of inhibitory zones in each plate. By comparing/measuring the size or the diameter of the inhibitory zones, the severity of the presence of antibiotic residues was taken.

Plate 1: Photograph of a Cultured and Incubated Plate of the Bio Assay



Analysis of Litter for Presence of Resistant Bacteria

From the selected cages, a representative sample of litter (approximately 150 g) was taken, and stored in an iced cooler box while transporting. All together 25 samples from 22 buyback farms and 3 closed housed farms were collected for analysis. Three treatments were used for the analytical process and from each treatment two replicates were done. The three treatments were, enumeration of bacteria on 0 ppm

antibiotics, 10 ppm enrofloxacine (*Enrocare; enrofloxacine 10% oral solution, Hayles Agriculture, Sri Lanka*) and 10 ppm tetracycline (*Wolicyclin, 20% oxy-tetracycline, Vetoquinol)*. The effectiveness of two antibiotics were verified before use by testing against HPLC grade Enrofloxacine and Oxytetracycline purchased from Sigma Aldrich, USA. Tryptic Soy Broth was used as the growth medium and Bacteriological Agar was used to solidify the medium. Prepared Tryptic Soy Agar (TSA) medium was also used to isolate selected bacteria. Before the actual culturing was done, a preliminary test was done and the dilution levels were selected as, 10^{-7} for the antibiotic treated plates and 10^{-9} for the control plates. After incubation of the culture plates for 48 hours at 28-30 °C, Colony Forming Units (CFU) were counted and recorded with their significant characteristics. From the cultured plates, few bacterial colonies were selected and then isolated in separate TSA plates.

After preparing 1:5 litter: distilled water slurry, pH was measured using a standard pH meter and another 1:10 litter: distilled water slurry was used to measure the Electrical Conductivity (EC), using an EC meter (*Eutech cond6+ EC meter*). Moisture content was determined using the oven dried method.

Statistical Analysis

SPSS 16.0 (Statistical Package for Social Sciences) and Microsoft Excel 2007 edition were used to analyze the survey data.

RESULTS AND DISCUSSION

Questionnaire Survey

During the current study, 33 buyback farmers and employees of 6 closed house farms were interviewed. The Table 1 describes the major differences observed between the two systems. All the farms surveyed in the current study were using three commercial feed types available in the market, which contain antibiotic growth promoters, according to the information label provided by the manufacturer. However, only 56.5% buyback farmers knew about those in-feed antibiotics. In addition to that, more than 40% of the farmers were not aware of any antibiotic feed additives. Although 21% of the whole group knew that antibiotics are associated with growth promotion in broiler chicken feed, the farming community must be well aware on what they really feed to the birds.

Furthermore, Amoxicillin, Enrofloxacine and Thylosin were used in farms as prophylactic sub-therapeutic treatments, within the first 5-6 days as a prophylactic measure. From the interviewed buyback farmers, 54.5% were aware of the withdrawal period of antibiotics, but only 45.5% knew its relevance and importance. Among the buyback farmers, more than 50% had misconceptions about antibiotics; impacts on hormonal regulation on humans and various organ related illnesses such as liver and kidney. Therefore, it is advisable to the field officers to educate the farmers with recent findings of antibiotic usage in broiler industry. Furthermore, 73.9% of the buyback farmers mentioned that antibiotics are used only for disease control out of which only 13% knew the growth promoting effect. This indicates less exposure of buyback farmers to the scientific importance of antibiotics. However, more than 60% of the buyback farmers were observed to be aware of the animal feed act; banned and registered antibiotics. It was noticed that in the present circumstances, broiler birds are being slaughtered after 35-36 days of rearing if the birds have gained their marketable weight. However, in both closed houses and buyback farms, antibiotic treatments are given

Table 1: A Brief Comparison Between Closed House Systems and Buyback Systems Observed in the Farmer Survey

Observed Parameter	Close House System	Buyback System
Production capacity	Higher	Lower
Number of birds per cage	>20, 000	1000-2000
Number of cages	3 to 14	1 to 3
Daily monitored by;	Farm manager, supervisors and line leaders	Farmer and field officer
Cage details	Controlled environment	Inner environment not controlled
Feeding, watering etc.	Fully automated, monitored by line leader or supervisor	Manual, monitored by farmer
Litter material	Paddy husk/hull or wood shavings	Paddy husk
Location	Remote, away from human habitat	Inside villages, in own land

within first 18-24 days of age, to ensure the meat is safe from residual antibiotics.

Through all the management practices in the farm along the growing time; 30 to 35 days, the chicken is assumed to be free from any harmful contaminations. The meat producers must be convinced to conduct an assay to ensure their meat quality to assure the consumer food safety (Schweihofer, 2013).

Analysis of Broiler Chicken Meat for Antibiotic Residues

All the analyzed samples (84 livers, 84 kidneys and 84 breast muscles) were negative for antibiotic residues; no inhibitory zones were detected. Results from a previous study revealed that none of the broiler chicken meat samples available in Sri Lanka were contaminated with detectable amounts of antibiotic residues of Penicillin, Amoxicillin, Doxycycline, Erythromycin, Ciprofloxacin, Streptomycin Sulphate and Sulphadiazine compounds (Hathurusinghe, 2004). This ensures that the meat supplied from the farms which were undertaken to this study, produce broiler meat without contaminations of detectable antibiotic concentrations. However, since the above method is a qualitative test, it could be argued that the study doesn't prove that the meat is 100% antibiotic free (Table 2).

In the current study, one whole bird was analyzed for three different muscle parts in the bio assay procedure.

Plate 2: TSA+10 ppm Tetracycline (Dilution Level at 10^{-7})

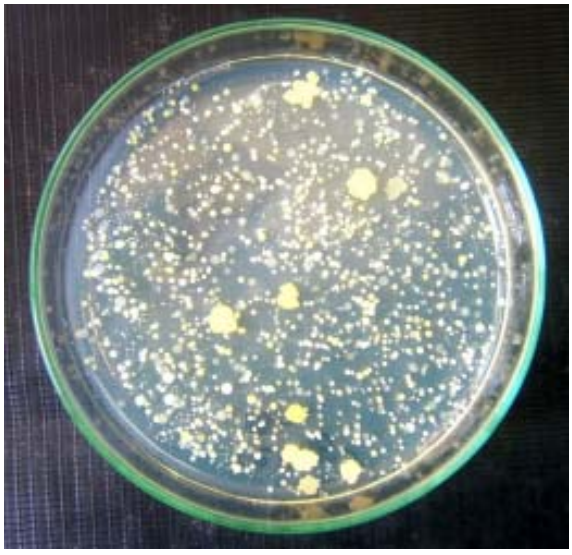


Table 2: Detectable Antibiotics by Six Plate Assay, their Minimum Detectable Concentrations and Maximum Residue Level in Foods (Hathurusinghe, 2004)

Antibiotic	MDC (µg/disk)	MRL (µg/100 mg)
Penicillin G	0.00025	0.005
Amoxicillin	0.00025	0.005
Tetracycline	0.00025	0.01
Doxycycline	0.00025	0.01
Erythromycin	0.0025	0.04
Ciprofloxacin	0.00025	0.01
Streptomycin Sulphate	0.0025	0.05
Sulphadiazine	0.00025	0.01

Note: MDC; Minimum detectable concentration, MRL; Maximum residue level.

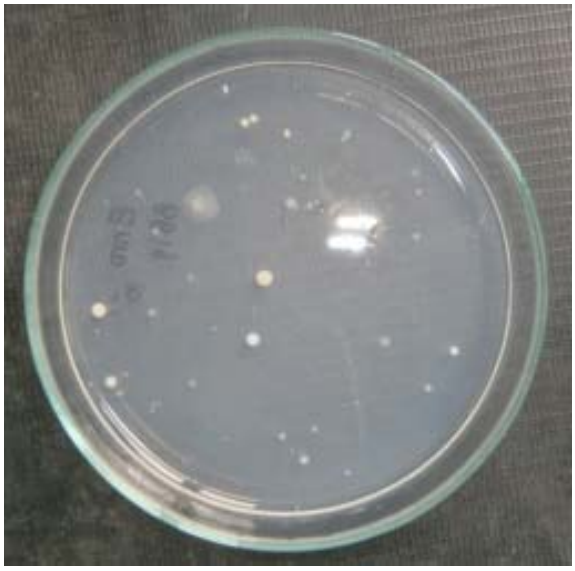
Breast muscle is a major edible portion and the compositions of other muscles are more or less similar to that of the breast muscle. Furthermore, the kidney was used with the aim of screening any water soluble antibiotic residues. In the same way, the liver was analyzed with the aim of screening fat soluble antibiotics. The accumulation of antibiotic residues in broiler meat is followed mainly due to misuse of antibiotics and paying less attention on the withdrawal period.

Analysis of Litter Samples for Prevalence of Resistant Bacteria

After culturing litter samples, it was observed that resistant bacterial populations were existed in litter material. Furthermore, the total culturable bacterial growth was observed to be always higher than resistant bacterial growth (Figure 1) where the Enrofloxacin resistant population was always lower than that of Tetracycline (Plate 2 and Plate 3). A moderate positive correlation was observed with the total culturable Enrofloxacine resistant bacteria and that of Tetracycline ($p<0.01$, Figure 3). This proves the existence of multi-antibiotic resistant bacteria in litter. Antibiotic resistance has become a great issue when it comes to the environmental risk analysis. Moreover, such antibiotic compounds are stable and can survive in the outer environment which are also considered as environmental pollutant agents (Anadon and Larranaga, 1998).

There are two key methods to assess the risk of antibiotic residual impact. That is either to assess the

Plate 3: TSA+10 ppm Enrofloxacine (Dilution Level at 10^{-7})

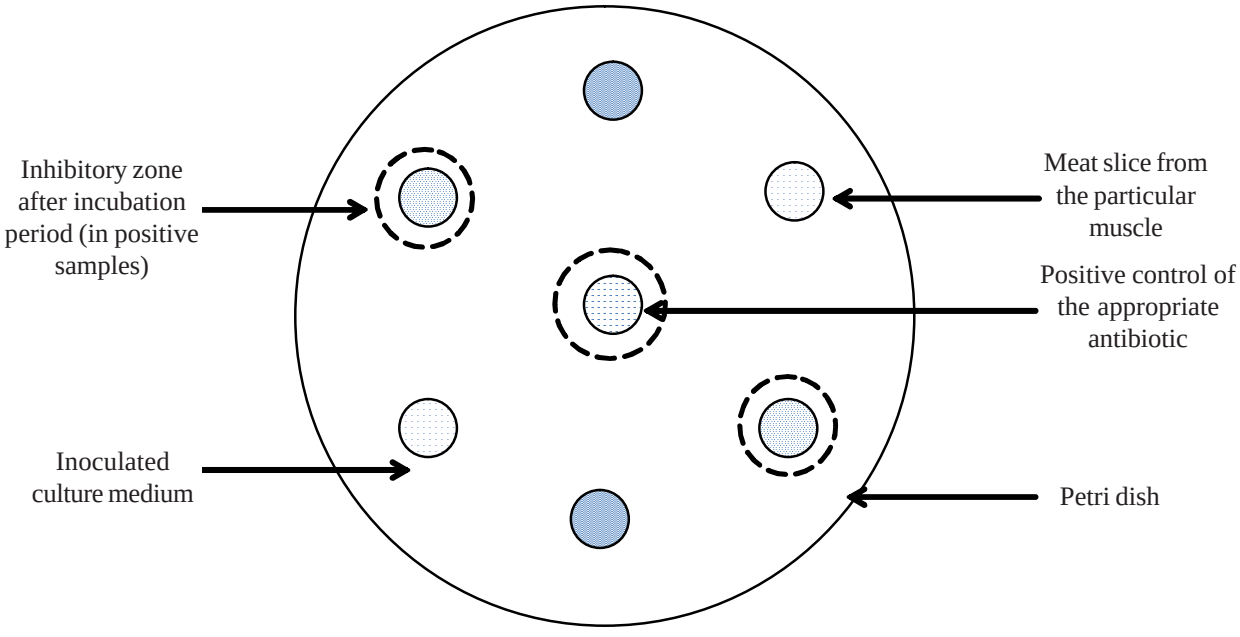


analysis for antibiotic residues and litter analysis for resistant bacteria. Farm survey was conducted to support and prove the results obtained, regarding the usage of antibiotics.

Since the litter is composed of mainly bird droppings and litter material, the origin of the bacterial populations might be the bird's gut or the litter material. The farms undertaken to this particular study were using new bedding for each new batch. Hence the exterior environment might be the origin of most of the bacteria in the litter (Cressman *et al.*, 2010). However, antibiotics could also be added to the litter by the means of bird droppings and these compounds remain stable when they are added to the litter and the environment. It is found that some of these antibiotic agents are naturally degraded where as some stable compounds lies on soil with a half-life time of 2-3 weeks even at 20 °C.

In this study, commercially available Enrofloxacine and Tetracycline were used and according to the farmer survey, it was investigated that the modern broiler industry is using

Figure 1: Layout of a Prepared and Incubated Culture Plate of the Assay Procedure



presence of antibiotic residues in broiler meat (direct and immediate impact) or, to evaluate the litter for antibiotic residues or resistant bacteria (indirect and long term impact). This study was done based on them; meat

Enrofloxacine while minimizing the use of Tetracycline. It emphasize that the development of resistance to Tetracycline has alerted the broiler industry to look for another replacement antibiotic for Tetracycline.

Figure 2: Total Culturable Bacterial Growth of Individual Farms

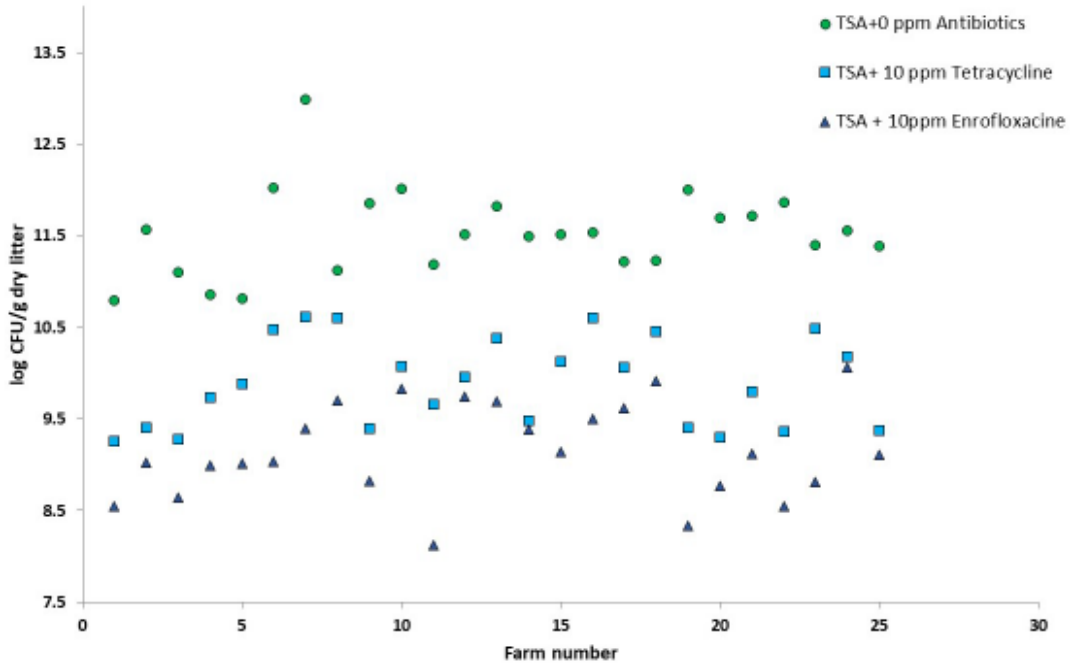
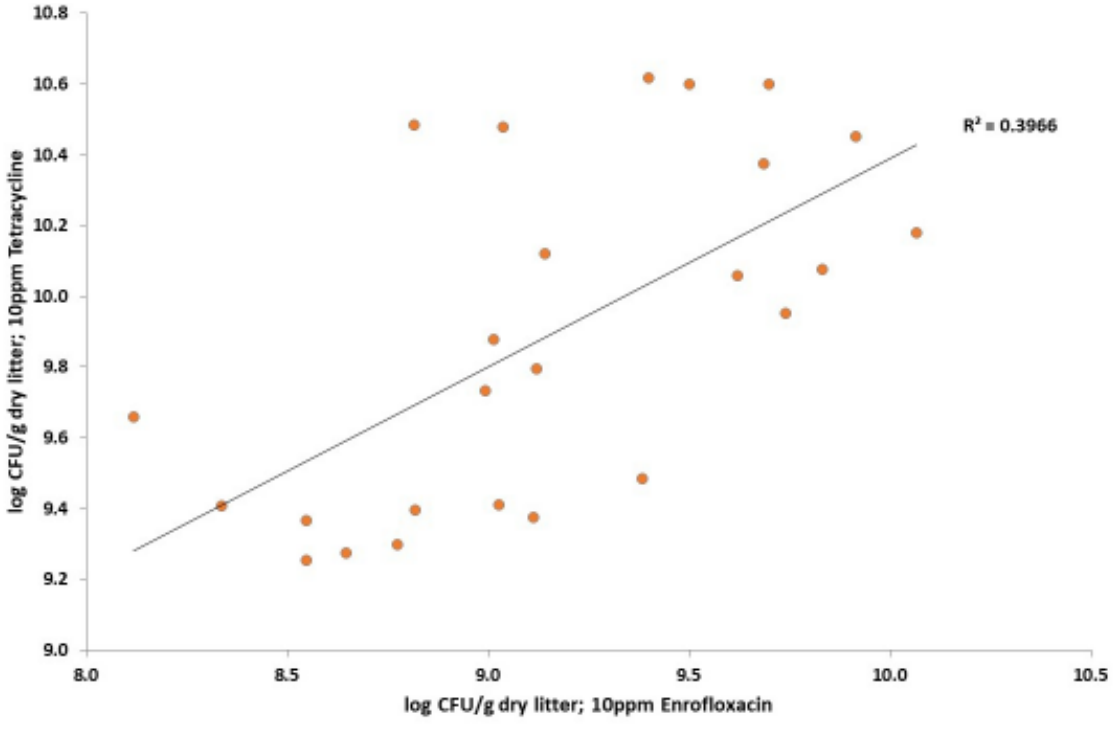


Figure 3: Relationship Between Total Culturable Enrofloxacin Resistant Bacterial Growth and Tetracycline Resistant Bacterial Growth of Litter Samples from Different Farms



CONCLUSION

The closed house farm managers and supervisors were having a substantial knowledge on antibiotic usage and its impacts and farm laborers had a poor knowledge. Buyback farmers were well monitored and supervised by the field officers and had a considerable knowledge on antibiotic usage. Broiler chicken meat of the broiler producers in this study were producing meat free from detectable amounts of antibiotic residues. The litter samples were harboring resistant bacterial populations for Enrofloxacine and Tetracycline where Tetracycline resistant population was always higher than that of Enrofloxacine.

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