Research Article

Chloramphenicol Residues in Retailed Chicken in Davao City Public Markets

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Abstract | Chloramphenicol is a broad-spectrum bacteriostatic agent used against pathogenic microorganisms particularly members of the *Enterobacteriaceae* such as *Escherichia* spp. and *Salmonella* spp., which are responsible for broad range of infections in animals, making this drug prominently used in veterinary medicine. Concerns about chloramphenicol side effects such as genotoxicity, embryo toxicity and fetotoxicity, its carcinogenic potential to humans and the lack of a dose-response relationship for aplastic anaemia prompted authorities to ban chloramphenicol as a drug for food-producing animals in many countries. This study was conducted to screen the presence of chloramphenicol residues in retailed chicken sold in selected public markets in Davao City. Eighty-four (84) samples were tested, which consisted of 42 chicken breast and chicken liver. Screening was done using enzyme-linked immunoassay (ELISA) method. The results showed that six out of 42 (14%) chicken breast and five out of 42 (12%) chicken liver were positive for chloramphenicol residues. There is no significant difference between the number of positive samples in chicken breast and liver. Overall, 11 out of 84 samples (13%) of the retailed chicken breast and liver sold in Davao City public markets were positive for chloramphenicol residues with concentrations ranging from 0.03 to 0.29 ng/g. Based on the results, it can be inferred that there is still continued use of this drug for food-producing animals despite the prohibition from food regulatory authorities. The researcher recommended the prudent use of antimicrobials to food-producing animals and appropriate screening of antibiotics by food regulatory authorities to ensure food safety and eliminate food-associated hazards to consumers.

Keywords | Chloramphenicol, Chickens, Food safety, ELISA, Philippines.

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INTRODUCTION

Chloramphenicol is a broad-spectrum bacteriostatic antibacterial substance. It is actively used against Gram-positive organisms and some other Gram-negative bacteria particularly organisms under *Enterobacteriaceae* which includes *Escherichia* spp. and *Salmonella* spp. which are responsible for a broad range of infections in both humans and animals making this drug prominently used as a treatment in veterinary medicine (Bishop, 2005).

Genotoxicity, embryo toxicity and fetotoxicity, its carcinogenic potential to humans and the lack of a dose-response relationship for aplastic anaemia are concerns expressed about chloramphenicol intake (Wongtavatchai et al., 2004). With its non-dose dependent toxicity in humans and possible residues that will remain in animal food products, chloramphenicol in food-producing animals have been legally banned with a maximum residue level of zero being expected in any animal meat products (Aduweyi et al., 2011). Both regulatory authorities, the Department of Agriculture (DA) and the Department of Health (DOH), banned the usage of chloramphenicol as a drug for food-producing animals in the Philippines. This has been stipulated in DA Administrative Order No. 60, Series 1990 and DOH Administrative Order No.91, Series 1990.

In Asia, where small farmers with low technical knowledge dominate the poultry industry, antibiotics are often available in the open market in a wide range of commercial products with dubious quality guaranty. Health support to the farmers is insufficient and diagnostic services for disease outbreaks, susceptibility testing, antimicrobial prescription, and technical back up are not readily available. The under-regulation or the insufficient enforcement of antimicrobial use regulations leads to excessive and inappropriate use of antibiotics (Alday et al., 2006).

Conversely, the Philippines attained self-sufficiency in its poultry industry. It is a prolific producer of poultry products, with about 800 million broilers produced annually (Pe, 2014). In Davao City, chicken is the second in demand poultry product with an annual per capita consumption of 7.85 kilograms (kg) (Deligero, 2012). Making it one of the biggest chicken consumers in the country. However, there is a paucity of data on the extent of use of prohibited antibiotics which is one of the major concerns in the food safety industry nowadays. In light of this, the proposed study will determine the presence of chloramphenicol residue in retailed chicken sold in selected Davao City public markets giving awareness to the public of the possible toxic effects brought by these drug residues and for regulatory authorities to maintain its strict policy of drug prohibition.

RESEARCH METHODOLOGY

Research Design

This research uses a descriptive research design, in which laboratory procedures were performed to detect or measure the chloramphenicol residues in retailed chicken in Davao City public markets.

Research Locale

The retailed chicken samples were purchased from three public markets in Davao City, which are located in three geographical districts in the city. These are the public markets in District I, District II, and District III. The public markets were selected using a sample frame obtained from the Davao City Economic Enterprise Office.

Sampling Design and Sample Size

Stratified random sampling with proportionate allocation was employed in the study. Each of the three public markets from the three districts was considered as stratum. Chicken stalls served as the sampling unit and chicken breast or liver served as the elementary unit. An interval systematic sampling was used in choosing the stalls where chicken breast or liver were purchased. Proportionate allocation of samples from each market was employed.

The sample size was obtained using OpenEpi ver. 2.3.1. The assumed prevalence of 17.5% was taken from the study of Tajik et al. (2010). Based on a confidence level of 95%, 42 samples each of chicken breast and chicken liver was collected. A total of 84 chicken samples were analyzed. It consisted of 12 samples each of chicken breast and liver from Market A, 11 samples each of chicken breast and liver from Market B and 19 samples each of the chicken breast and liver from Market C.

Procedure of the Study

Enzyme-linked immunosorbent assay (EISA) was used to determine the presence of chloramphenicol residue in retailed chickens (Tajik et al., 2010). A competitive enzyme immunoassay test kit (Max Signal TM; Bioo Scientific Corp., Austin, Texas, 2011) was utilized in this study.

Sample Preparation

A homogenized amount of sample was used for the extraction of chloramphenicol residues in chicken breast and liver using ethyl acetate as the extracting solution as indicated in the manufacturer's manual (MaxSignalTM; Bioo Scientific Corp., Austin, Texas, 2011).

Sample Assay

A 100uL of extract was tested using ELISA at 450nm wavelength. Five calibration standard solutions were used in the study. A negative control was also run. The dilution factor of the assay is 0.5. The ELISA method used has a reported limit of detection (LOD) of 0.025 ppb (0.025ng/g) and only values above this concentration were detectable and considered chloramphenicol residue-positive.

RESULTS AND DISCUSSION

Chloramphenicol residues were detected in 11 out of 84 samples (13%) of retailed chicken breast and liver from three public markets in Davao City (Table 1). The concentration of chloramphenicol residues from the 11positive samples tested ranges from 0.03 to 0.29ng/g. However, a zero tolerance level in animal-derived food products had been established worldwide by food regulatories. Administration of the drug is prohibited in food-producing animals due to its non-dose toxic effects in humans. Thus, any concentration detected in this study is considered a violation.These findings indicate the wide-spread and uncontrolled use of antibiotics that may lead to the development and spread of antimicrobial-resistant organism. These antimicrobial-resistant organisms pose a potential threat to human health as they are poised to enter the food supply and could be widely disseminated in food products particularly poultry products (Donoghue, 2003).

Table 1: Chloramphenicol Residues in Retailed Chicken from three Public Markets in Davao City, ng/g (ppb)

Public Market	Chicken Part					
	Breast		Liver		Breast and Liver	
	No. of Positive Samples (%)	Concentration, ng/g	No. of Positive Samples (%)	Concentration, ng/g	No. of Positive Samples (%)	Concentration, ng/g
Market A	0 (0)	ND	1 (8)	0.05	1(4)	0.05
Market B	1 (9)	0.04	2 (18)	0.04-0.20	3 (14)	0.04-0.20
Market C	5 (26)	0.03-0.29	2 (11)	0.05-0.06	7 (18)	0.03-0.29
Total	6 (14)	0.03-0.29	5 (12)	0.04-0.20	11(13)	0.03-0.29

Legend: ND Not detected at LOD = 0.025ng/g

The prevalence obtained from this study (13%) is lower than the estimated population proportion compared to the study of Tajik et al. (2010). The assumed prevalence of 17.5% was taken from the study conducted in Iran. This variance in the results may have been caused by different factors such as diverse environment of poultry farms, level of monitoring of the prohibited drugs by food authorities, and regulation of antibiotics used in poultry farms. Nevertheless, in the Philippines, chloramphenicol must not be detected in the chicken samples as specified in DA Administrative Order No. 60, Series of 1990 and DOH Administrative Order No. 91, Series of 1990.

Three public markets in Davao City were used as demographic areas of study. These were the areas where the chicken samples tested were purchased. For confidentiality, the markets were coded as Market A, B, and C. Market C obtained the highest frequency of positive samples from the total samples tested. The market with the highest percentage of positive samples is Market C (18%) followed by Market B (14%) and Market A (4%). Market C is the biggest public market selling chicken. It has the highest number of chicken retail stalls, leading to the highest sample size in this study. As a center of trading, the bulk of the chicken supply falls in this market. Six out of 42 samples (14%) of chicken breast and 5 out of 42 (12%) chicken liver samples were positive with chloramphenicol residues (Table 2). The number of the chicken breast and chicken liver with detected chloramphenicol residues were tested for significant difference using chi-square test. Result of the chi-square is presented in Table 3. In testing for significant difference between the number of chicken breast and liver samples that turned positive for chloramphenicol residues, a chi-square ($\chi 2$) value of 0.105 was obtained, with a p-value of 0.746. With a 1 df, $\chi 2$= 3.84 is significant at α=0.05. Based on the chi-square test, there is no significant difference between the number of chicken liver and chicken breast samples with detected chloramphenicol residues. This means that the number of positive samples is not related to the chicken part tested. Hence, the number of positive breast samples is not significantly more than the number for liver samples.

Table 2: Chicken Sample Part: Laboratory Result Cross tabulation

Count		Negative	Positive	Total
Chicken Part	Breast	36	6	42
	Liver	37	5	42
Total		73	11	84

Table 3: Chi-square Test between the Number of Chloramphenicol Residue-Positive Chicken Breast and Liver

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.105[a]	1	.746		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.105	1	.746		
Fisher's Exact Test				1.000	.500
N of Valid Cases[b]	84				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.50.

b. Computed only for 2x2 table

This finding is in disagreement with the study of Tajik et al. (2010) where 22 chicken liver, 21 kidney and 14 chicken muscle samples (breast, thigh) were detected for chloramphenicol residues. The liver, kidney and muscle samples came from one and the same chicken. Their findings demonstrate that metabolism of chloramphenicol by cytochrome P450 system takes place in three organs and the produced metabolites are distributed in the entire body of the chicken. Since chloramphenicol is mainly conjugated in the liver for excretion in the urine or bile, it can be expected that chloramphenicol should be detected in the liver in higher concentration. Thus, it would need a longer time for the drug residue or its metabolites to be eliminated from the liver than from the breast or thigh.

It can be concluded that chloramphenicol is still being used as an antibiotic agent for food-producing animals, particularly in poultry. This is despite the prohibition of its usage by different food regulatories in the Philippines. Non- compliance to the banned use of the drug poses a public health risk to consumers of retailed chicken regardless of the chicken part (breast or liver) consumed. Thus, upholding prudent use of antimicrobials to food-producing animals and appropriate screening of antibiotics by food regulatory authorities is recommended to ensure food safety and eliminate food-associated hazards to consumers.

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CONFLICT OF INTEREST

No conflict of interests are declared by authors for the contents in this manuscript.

AUTHORS CONTRIBUTION

Kimberly Marie S. Develos designed and carried out the experiment and prepared the draft manuscript. Ludivina M.Porticos provided guidance, technical support and edited the manuscript.

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