



***DIETARY EXPOSURE ASSESSMENT OF ANTIBIOTIC RESIDUES IN
CHICKEN MEAT FROM SELECTED CHICKEN SLAUGHTERHOUSES IN
PENINSULAR MALAYSIA***

MARZURA BINTI MD RODZI

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By

MARZURA BINTI MD RODZI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Master of Science**

November 2018

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chairman : Associate Professor Nor Ainy binti Mahyudin, PhD
Faculty : Food Science and Technology

Dietary intake of significant amounts of antibiotic residue can lead to adverse health effects and also lead to development of antimicrobial resistance in foodborne pathogens. The objectives of this study were to screen the presence of antibiotic residues in slaughtered chickens; to quantitate antibiotic residues concentrations and to estimate dietary intake (EDI) of antibiotic residues for children and adult. Antibiotic residues in a total of 637 slaughtered chicken sample from selected slaughterhouses in Peninsular Malaysia were screened using microbial inhibition test and quantitated with Liquid Chromatography Mass Spectrometry (LC-MS/MS). An exposure assessment was conducted to detect and quantitate tetracycline, sulfonamide and quinolones in slaughtered chickens in Peninsular Malaysia and the results were then applied to calculate the risk estimates using deterministic and probabilistic approaches. The result showed that 17 of 637 (2.7%) samples were positive by screening for antibiotic residues. Majority of the samples detected with tetracyclines residues (82.4%), followed by quinolones (29.4%) and sulfonamides (23.5%). The result from confirmatory method showed a total of 10 (1.6%) samples were positive for tetracyclines, while enrofloxacin was detected below maximum residue limits (MRLs) in one sample, whereas sulphonamide was not detected in any samples. Through deterministic approach, the dietary intake of tetracycline in adult was estimated to be 0.00921 µg/kg bw/day compared to 0.05919 µg/kg bw/day in children. The lower aged children groups (1<y<3 and 4<y<6) had the highest tetracycline intakes compared to the adult group. Exposure dietary intake (EDI) for the children and adults could reach up to 0.2% and 0.1% of the defined acceptable daily intake (ADI), respectively. Based on probabilistic approach, tetracycline residues intake was higher in children (ranged from 0.13779 µg/kg bw/day to 0.33935 µg/kg bw/day) compared to adult (ranged from 0.09002 µg/kg bw/day to 0.4333 µg/kg bw/day). EDI for the children and adults could reach up to 0.5%

to 1% and 0.1% to 0.3% of the defined ADI, respectively. The results produced by probabilistic approach were slightly higher compared to deterministic analysis as all possible data provided has been taken into account. Both approaches yielded the estimate risks lower than the ADI for tetracycline at 30 µg/kg bw/day. Based on the ADI limit, the results indicated that the toxicological risk associated to the consuming of chicken was insignificant and could be considered as safe. However, occurrence of high concentration of antibiotic residues in small number of samples still warrant a closer monitoring and management on the use of antibiotics in chicken farms in Peninsular Malaysia. It is crucial for the authorities to instigate policies for a better control of the potential risk of antibiotic residues in foods of animal origin.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PENILAIAN PENDEDAHAN SISA ANTIBIOTIK MELALUI PEMAKANAN
DAGING AYAM DARI PUSAT PEMROSESAN AYAM TERPILIH DI
SEMENANJUNG MALAYSIA**

Oleh

MARZURA BINTI MD RODZI

November 2018

Pengerusi : Profesor Madya Nor Ainy binti Mahyudin, PhD
Fakulti : Sains dan Teknologi Makanan

Pengambilan makanan yang mengandungi residu antibiotik boleh menyebabkan kesan kesihatan yang buruk dan juga membawa kepada pembangunan rintangan antimikrob dalam patogen bawaan makanan. Objektif kajian ini adalah untuk melihat kehadiran antibiotik residu dalam ayam yang disembelih; untuk mengukur kepekatan antibiotik residu dan untuk menganggarkan pendedahan pengambilan makanan (EDI) mengandungi residu antibiotik oleh kanak-kanak dan orang dewasa. Sejumlah 637 sampel ayam yang di sampel dari rumah penyembelihan terpilih di Semenanjung Malaysia telah diuji melalui ujian perencatan mikrob dan kemudiannya di sahkan dengan Kromatografi Cair-Spektrometri Massa (LC-MS / MS). Penilaian pendedahan dilakukan untuk mengesan dan menghitung kehadiran tetrasiklin, sulfonamid dan quinolon dalam ayam yang disembelih di Semenanjung Malaysia dan keputusan tersebut kemudiannya digunakan untuk mengira anggaran risiko melalui pendekatan deterministik dan probabilistik. Keputusan menunjukkan bahawa 17 daripada 637 (2.7%) sampel positif ujian saringan residu antibiotik. Majoriti sampel dikesan dengan residu tetrasiklin (82.4%), diikuti oleh quinolon (29.4%) dan sulfonamid (23.5%). Melalui keputusan pengesanan, sebanyak 10 (1.6%) sampel positif untuk tetracyclines, manakala enrofloxacin dikesan di bawah had residu maksimum (MRLs) dalam satu sampel, manakala sulfonamida tidak dikesan dalam sebarang sampel. Melalui pendekatan deterministik, anggaran pengambilan makanan terhadap tetrasiklin adalah dari 0.00921 µg/kg bw/hari (dewasa) hingga maksimum 0.05919 µg/kg bw/hari (kanak-kanak). Pengambilan tetrasiklin adalah tinggi bagi kumpulan kanak-kanak yang berumur lebih rendah ($1 < y < 3$ dan $4 < y < 6$) berbanding kumpulan orang dewasa. Pendedahan pengambilan makanan (EDI) untuk kanak-kanak dan orang dewasa boleh mencapai sehingga 0.2% dan 0.1% daripada pengambilan harian yang boleh diterima (ADI). Berdasarkan pendekatan probabilistik, pengambilan

residu tetrasiklin adalah lebih tinggi pada kanak-kanak ($0.13779 \mu\text{g}/\text{kg bw}/\text{hari}$ - $0.33935 \mu\text{g}/\text{kg bw}/\text{hari}$) berbanding dewasa ($0.09002 \mu\text{g}/\text{kg bw}/\text{hari}$ - $0.4333 \mu\text{g}/\text{kg bw}/\text{hari}$). EDI untuk kanak-kanak dan orang dewasa masing-masing boleh mencapai 0.5% hingga 1% dan 0.1% hingga 0.3% daripada ADI. Berdasarkan analisa deterministik, keputusan ini adalah lebih tinggi kerana kesemua data telah diambil kira. Kedua-dua pendekatan menghasilkan risiko anggaran yang lebih rendah daripada ADI tetrasiklin pada $30 \mu\text{g} / \text{kg bw} / \text{hari}$. Berdasarkan ADI, hasil menunjukkan bahawa risiko toksikologi yang dikaitkan dengan pemakan ayam adalah tidak penting dan dianggap selamat. Walaupun begitu, kepekatan residu antibiotik yang tinggi dalam sebilangan sampel masih memerlukan pemantauan lebih intensif dalam pengurusan dan penggunaan antibiotik di ladang ayam di Semenanjung Malaysia. Adalah penting bagi pihak berkuasa untuk membuat dasar bagi mengawal risiko residu antibiotik yang berpotensi dalam makanan berasaskan ternakan.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Nor Ainy binti Mahyudin, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Ungku Fatimah Ungku Zainal Abidin, PhD

Senior Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Chai Lay Ching, PhD

Senior Lecturer
Faculty of Science
Universiti Malaya
(Member)

Marni binti Sapar, MSc

Research Officer
Veterinary Public Health Laboratory
Department of Veterinary Service
(Member)

ROBIAH BINTI YUNUS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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Signature: _____ Date: _____

Name and Matric No: Marzura Binti Md Rodzi, GS45107

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Signature: _____

Name of Chairman
of Supervisory
Committee:

Associate Professor Dr. Nor Ainy binti Mahyudin

Signature: _____

Name of Member
of Supervisory
Committee:

Dr. Ungku Fatimah Ungku Zainal Abidin

Signature: _____

Name of Member
of Supervisory
Committee:

Dr. Chai Lay Ching

Signature: _____

Name of Member
of Supervisory
Committee:

Dr. Marni binti Sapar

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LIST ABBREVIATIONS

ADI	Acceptable Daily Intake
ANOVA	Analysis of Variance
DVS	Department of Veterinary Services
EDI	Exposure Dietary Intake
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ESI	Electrospray Ionisation
FAO	Food and Agricultural Organization
IPCS	International Program on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS/MS	Liquid Chromatography Mass Spectrometry
LOD	Limit of Detection
MANS	Malaysian Adult Nutrition Survey
MRLs	Maximum Residue Limits
SOP	Standard Operating Procedure
VPHL	Veterinary Public Health Laboratory
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In general, antibiotics are used for therapeutic and prophylactic purposes in human medicine, fish agriculture or in veterinary medicine that can be administered in the feed or in the drinking water (McEvoy, 2002). Antibiotics are used to prevent propagation and destroy bacteria but not against viral and fungal pathogens. Classifications of antibiotics are based on the bacteria species which they act, family chemical characteristic and their mode of action (Mehdi et al., 2018).

According to Diarra and Malouin, 2014, antibiotic such as tetracycline, bacitracin, tylosin, salinomycin, virginiamycin and bambarmycin are often used in intensive poultry farming in North America. In European Union (EU), among antimicrobial administered to animals, 37% are from tetracyclines group (Carvalho and Santos, 2016). According to Du and Liu, 2012, tetracyclines were the most commonly used in animal farming followed by sulfonamides and macrolides. In Malaysia, currently veterinary biologics and vaccines are registered under the Department of Veterinary Services Malaysia, while veterinary drugs are under the jurisdiction of the National Pharmaceutical Regulatory Agency (NPRA), Ministry of Health. According to Health Action International Asia Pacific (HAIAP), 2013, there are 97 different antibiotics registered by NPRA for use mostly in poultry and pig farms. Those antibiotics are b-lactam, cephalosporins, tetracycline, sulphonamide, macrolide, aminoglycoside and fluoroquinolone.

Improper use of antibiotics has resulted in residues of drugs in animal product and could have impact on consumer health (Gonzalez Ronquillo and Angeles Hernandez, 2017). Antibiotic residues may also be due to contamination from animal feedstuffs (McEvoy, 2002) and many of these substances may exert some imperative toxic effects such as endocrine effects, genotoxic, immunotoxic and/or carcinogenic effect when administered to animal for other purpose like growth promotion (Milagro et al., 2008) causing an important consumer's health risk (Croubels et al., 2004). Sulphonamides recorded as the highest violation rate in the USA (Dey, et al., 2003). Some quinolones, are increasingly involved in antibiotic resistance phenomena, characterizing both animal and human isolates (Engberg, et al., 2001). Tetracyclines was found to cause teeth development problem in young children (Kummerer, 2009). Thus, safe and appropriate use of antibiotics remains the upmost challenging public health concerns.

Qualitative and/or quantitative determination of the drug residues levels in marketed poultry products such as liver, eggs and meat is the initial step in assessing the safety and proper use of antibiotics in poultry production. Whereas the level of antibiotics residues in poultry products is regularly observed in the developed country throughout the world, Malaysia such as the developing nations, still lack of comprehensive and intensive study that could urge the enforcement of the preeminent use of antibiotics in poultry husbandry practices. Besides, there is also a lack of structured risk assessment in Malaysia and therefore this study is conducted. As an initial step to assess the possible health risk posed by veterinary drug residues in Malaysian poultry meat, this study determined the residue levels of 3 groups of antibiotics namely tetracycline, sulfonamide and quinolones in the tissue of marketed-ready chicken muscle originating from small and medium scale chicken slaughterhouses in Peninsular Malaysia.

1.2 Objectives of Study

A study was carried out to assess the risk to antibiotic residues from chicken meat intake particularly in adults and children consumer. The study was carried out in Peninsular Malaysia involving small and medium scale (≤ 5000 chicken/day) chicken slaughterhouses monitored by the Malaysian Department of Veterinary Services (DVS). Routinely, DVS monitored the antibiotic residues level in chicken meat from certified poultry processing plan and it was estimated to cover 30% of overall Malaysia's poultry production, whereas another 70% are from small and medium scale chicken slaughterhouses, would be the first reported in the study. Furthermore, this study was also conducted to support the DVS function in assuring the safe food production before the food is marketed to consumers. The main objective of this study is to determine the current status of chicken meat safety in Malaysia and the potential exposure (intake) of consumers to antibiotic residues in chicken meat.

Thus, the specific objectives of this study were as follows:

- (i) To screen the presence of antibiotic residues in slaughtered chickens collected from selected slaughtered houses in Peninsular Malaysia.
- (ii) To quantitate the antibiotic residues concentrations from suspected non-compliant slaughtered chicken samples collected from slaughtered houses in Peninsular Malaysia.
- (iii) To assess the public health risk by estimating the exposure (EDI) of children and adult of various age groups to antibiotic residues via consumption of chicken in Peninsular Malaysia using deterministic and probabilistic approaches.

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