OCCURRENCE OF TETRACYCLINES, SULPHONAMIDES AND QUINOLONES RESIDUES IN CHICKEN MEAT SAMPLE FROM SELECTED CHICKEN SLAUGHTERHOUSES IN PENINSULAR MALAYSIA

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ABSTRACT. The occurrence of veterinary drug residues in chicken meat originating from 320 small and medium scale chicken slaughterhouses in Peninsular Malaysia was determined. 637 chicken meat samples were examined for tetracycline (TCs), sulphonamide (SAs) and quinolone residues using a microbiological inhibition test and was further confirmed using liquid chromatography mass spectrometry (LC-MS/MS). The presence of TC residues were confirmed in 10 (1.6%) samples, and 1 (0.2%) sample was confirmed in compliance to the established maximum residue limit (MRL) for residues of quinolone. A total of 6 (0.9%) samples were above the MRL for TC. The samples were from Pulau Pinang, Terengganu and Kelantan. Among those tested in compliance, the main analytes found for TC and quinolone were chlortetracyclines (CTC), enrofloxacin and mixture of chlortetracycline (CTC) and oxytetracycline (OTC). No samples were found to contain sulfonamides residues.

Keywords: chicken meat, residues, small and medium scale chicken slaughterhouse

INTRODUCTION

Poultry products such as meat and eggs are a cheap source of protein for humans. In Malaysia, the average of self-sufficiency in poultry meat from 2011-2017 accounts for 104.3% (DVS, 2018). The poultry industry in Malaysia was the highest contributor in livestock industries as its gross output achieved RM9,058 million of the total value contributed by the livestock sub-sector in 2015 (DOS, 2017). In 2016, chicken production recorded the highest number among other livestock at 305.06 million, an increase of 6.4% from the previous year (DOS, 2017). The statistics significantly show that the poultry industry is one of the biggest players in the country and plays an important role to satisfy the increasing demand of meat for the domestic market. In order to suit the demand, the use of veterinary drugs is one of the solutions against poultry diseases and to improve productivity. The mode of drugs administered was either through feed or drinking water. These drugs are generally used for therapeutic and prophylactic

purposes, as growth promoter in order to enhance feed conversion efficiency and increase the lean meat to fat ratio (Kabir *et al.,* 2004; Milagro and Fidel, 2008). The subsequent use of the drug may result in a significant risk of drug residues remaining in the poultry products if chickens are slaughtered without observing the withdrawal period of drugs (Kabir *et al*., 2004; Mc Evoy, 2002; Milagro and Fidel, 2008; Darwish *et al*., 2013). Hence, the need to monitor the presence of veterinary drug residues in food of animal origins such as poultry meat is a must (Croubels *et al.,* 2004). In order to safeguard human health, the Malaysian government has set the tolerance level or MRL of 100 µg/kg for TTC, OTC, and/or CTC (parent drug, singly or in combination) and 100 µg/kg for doxycycline (DC) in all food-producing animal (Malaysian Food Regulation 1985, 2015). The Department of Veterinary Services Malaysia (DVS), as part of its mandate, monitors for the presence of antibiotic residues in poultry products under the Malaysian Drug Residues Monitoring Programme which covers certified chicken processing plant. The findings from 771 samples of 2010-2011 showed that irrational use of antibiotics was recorded in 3% of the total sample (Marzura *et al*., 2012). Other findings from Marni *et al*. (2017), showed that there was an increasing trend in rate of incompliance to veterinary drug residue of chicken samples in 2010-2016. Since the monitoring programme only covered chicken processing plants the more than 5,000 heads/day, the trend of antibiotics misuse in small and medium scale chicken slaughterhouses was unknown. Thus, this study was conducted primarily to determine the residue levels of TCs, SAs and quinolone in chicken meat originating from small and medium scale chicken slaughterhouses in Peninsular Malaysia.

MATERIALS AND METHOD

Sample collection

Whole chicken samples (without internal organs) were collected from January to April in 2016. A total of 640 whole chicken samples were collected by Veterinary Authority Officers from 320 small and medium scale chicken slaughterhouses (<5,000 head/day) throughout Peninsular Malaysia. The sampling area was divided into four zones according to Figure 1. Table 1 showed the number of samples collected by the state in each representative zone. The samples were immediately kept in polystyrene boxes containing ice pack upon collection and during transportation to a Veterinary Public Health Laboratory (VPHL, 2014a; VPHL, 2014b; VPHL, 2014c). The samples were stored at -20 °C prior to analysis.

Sample processing

The whole chicken samples were further sub-sampled in the laboratory. The skin and fat were removed. Only chicken meat was taken for further laboratory analysis.

Chemicals and reagents

Reference and internal standards (Table 2) were obtained from Sigma-Aldrich (St Louis, MO, USA), Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Riedel-de Haen (Seelze,

Figure 1. Map of Peninsular Malaysia indicating the four representative zones where the samples were collected.

Table 1. Number of samples collected according to the state for each representative zone

Zones	State	No. of samples collected
North	Perlis	18
	Kedah	44
	Pulau Pinang	46
	Perak	51
	Total	160
Central	Selangor	86
	W. P. Kuala Lumpur	2
	Melaka	24
	Negeri Sembilan	48
	Total	160
East	Pahang	56
	Terengganu	52
	Kelantan	52
	Total	160
South	Johor	160
	Total	160
Grand Total		640

Germany). All chemical and chromatographic reagents used were of liquid chromatographic (LC) grade or analytical grade. Acetonitrile (ACN), methanol (MeOH), tetrahydrofuran and di-sodium hydrogen phosphate dihydrate were purchased from Merck (Darmstadt, Germany), di-sodium ethylenediaminetetraacetate dihydrate (EDTA), oxalic acid, trichloroacetic acid, formic acid and citric acid were purchased from Fisher Scientific, UK. Ultrapure water was filtered through a Milli-Q® Integral, Millipore water system (Millipore, Bedford, MA, USA). Purified nitrogen and purified argon used for mass spectrometry system were from Malaysia Oxygen (MOX). The antibiotic discs standard of penicillin G, streptomycin, erythromycin, sulphadimidine, oxytetracycline and ciprofloxacin were purchased from MAST Diagnostics, UK. Bacteria used in microbiological screening test are *B. cereus*, *B. subtilis*, *E. coli* and *K. rhizophila* obtained from Microbiologics, USA.

Instrumentation

The LC system consisted of a 2695 Alliance Separations Module equipped with a 2695 microvacuum degasser, a 2695 thermostated autosampler and column compartment (Waters, Manchester, UK) was connected to a Waters Quattro Ultima Pt. triple-quadrupole mass spectrometer (Micromass Co. Inc. Manchester, UK). The mass spectrometer was operated in the positive electrospray ionisation (ESI) mode. MS/MS conditions and the specific monitoring parameters for the TCs, SAs and quinolone are detailed out in Table 2 and Table 3.

Analytical methods

Screening test

All the samples were first analysed using the microbiological inhibition test, Six Plate Test (MKAV/C031). This screening test was used to rapidly detect samples suspected to be non-compliant. This test is carried out as an agar diffusion test in which bacteria that are relatively sensitive to a particular class of antibiotic present in the sample will be inhibited from growth which is demonstrated by the formation of zones of inhibition. Positively detected chicken samples were stored at -20 °C for confirmatory analysis.

Confirmatory test

Suspected positive chicken samples were thawed to room temperature and lean meat portion (fat removed) were individually minced in a domestic food processor. The procedure for TCs, SAs and quinolones are presented in Table 4.

Validity parameters

To ensure the validity of confirmatory test results, the following measures were taken. Prior to sample analysis, standards were analysed to verify adequate system performance. Each batch of sample analysis was prepared to include reagent blank and sample blank in duplicate to control for background contamination, and spiked samples in triplicate to confirm satisfactory drug recovery in the acceptable range, 70% to 110% (EC, 2002). In addition, the

Table 2. TCs, SAs and quinolones LC-MS/MS method parameters.

Compound	Standard source	Precursor (*m/z*)	Cone (V)	Dwell time (s)	Base fragment (*m/z*)	Collision energy (eV)	Secondary fragment (*m/z*)	Collision energy (eV)	Internal standard
Tetracycline (TCs)									
Oxytetracycline	Sigma-Aldrich	461.27	35	0.25	426.29	22	443.32	15	demeclocycline
Tetracycline	Sigma-Aldrich	445.36	35	0.25	410.44	23	427.08	13	demeclocycline
Chlortetracycline	Sigma-Aldrich	479.00	35	0.25	444.54	26	462.18	21	demeclocycline
Doxycycline	Dr. Ehrenstorfer GmbH	445.29	35	0.25	321.39	37	428.31	22	demeclocycline
Demeclocycline	Dr. Ehrenstorfer GmbH	464.88	35	0.25	447.99	18	-	-	-
Sulphonamide (SAs)									
Sulfisomidine	Sigma-Aldrich	279.04	35	0.25	124.10	22	186.00	16	sulfapyridine
Sulfadiazine	Sigma-Aldrich	251.07	35	0.25	108.18	20	156.07	14	sulfapyridine
Sulfathiazole	Sigma-Aldrich	255.88	35	0.25	108.06	24	155.92	16	sulfapyridine
Sulfamerazine	Sigma-Aldrich	265.09	35	0.25	156.03	15	172.02	15	sulfapyridine
Sulfamethazine	Sigma-Aldrich	279.05	35	0.25	156.00	20	186.00	17	sulfapyridine
Sulfamethizole	Sigma-Aldrich	271.13	35	0.25	108.20	19	156.08	11	sulfapyridine
Sulfamethoxypyridazine	Sigma-Aldrich	281.09	35	0.25	126.08	20	156.01	18	sulfapyridine
Sulfapyridine	Sigma-Aldrich	250.08	35	0.25	156.05	12	-	-	-
Quinolone									
Ofloxacin	Sigma-Aldrich	362.23	35	0.1	261.23	22	318.31	15	D_5-enrofloxacin
Enrofloxacin	Riedel-de Haen	360.31	35	0.1	245.30	25	316.40	15	D_5-enrofloxacin
Danofloxacin	Riedel-de Haen	358.29	35	0.1	96.26	20	314.36	14	D_5-enrofloxacin
Sarafloxacin	Riedel-de Haen	386.21	35	0.1	299.23	20	342.28	15	D_5-enrofloxacin
Difloxacin	Riedel-de Haen	400.29	35	0.1	299.32	25	356.39	15	D_5-enrofloxacin
Ciprofloxacin	Riedel-de Haen	332.22	35	0.1	245.24	20	288.29	15	D_5-enrofloxacin
D_5-enrofloxacin	Sigma-Aldrich	325.28	35	0.1	281.37	15	-	-	-

Table 3. LC-MS/MS conditions for monitoring the TCs, SAs and quinolone residues.

Compound	Column	Mobile phase	Gradient program	MS/MS parameter	
Tetracycline (TCs)[a]	XTerra® C18 (150 mm × 2.1 mm, 5 µm particle diameter), Waters UK.	A: 0.2% formic acid with 0.1mM oxalic acid (v/v). B Acetonitrile with 0.2% formic acid	0–5.5 min linear gradient from 10 to 70% B; 5.5-9.1 min maintain at 70% B and 9.1-20 min held at 10% B in order for the column to re-equilibrate before the next injection. Column temperature: 35 °C. Flow: 0.25 ml/min	Ionisation mode	ESI+
				Capillary voltage (kV)	3.23
				Cone voltage (V)	35
				Source temperature (°C)	120
				Desolvation temperature (°C)	350
				Cone gas flow (L/Hr)	60
				Desolvation gas flow (L/Hr)	550
				Multiplier voltage (V)	650
Sulphonamide (SAs)[b]	XTerra® C18 (150 mm × 2.1 mm; 5 µm particle diameter), Waters, UK.	A: 0.2% formic acid B: 0.2% formic acid in ACN	0-0.5 min linear gradient from 10 to 30% B; 0.5-3.0 min increased to 60% B and maintained until 8.0 min; 8.0-10.0 min reduced to 10% B and held at 10% B until 16.0 min in order for the column to re-equilibrate before the next injection. Column temperature: 25 °C. Flow: 0.20 ml/min	Ionisation mode	ESI+
				Capillary voltage (kV)	3.23
				Cone voltage (V)	35
				Source temperature (°C)	120
				Desolvation temperature (°C)	300
				Cone gas flow (L/Hr)	60
				Desolvation gas flow (L/Hr)	550
				Multiplier voltage (V)	650
Quinolone[c]	Atlantis d C18 (150 mm × 2.1 mm; 3 µm particle diameter), Waters, UK.	A: 0.2% formic acid B: 0.2% formic acid in ACN	0-6.6 min linear gradient from 1 to 10% B; 6.6-12.0 min increased to 45% B; 12.0-14.0 min further increased to 55% B; 14.0-14.1 min reduced to 1% B and 14.0-21.0 min held at 1% B in order for the column to re-equilibrate before the next injection. Column temperature: 25 °C. Flow: 0.25 ml/min	Ionisation mode	ESI+
				Capillary voltage (kV)	3.23
				Cone voltage (V)	35
				Source temperature (°C)	120
				Desolvation temperature (°C)	350
				Cone gas flow (L/Hr)	50
				Desolvation gas flow (L/Hr)	500
				Multiplier voltage (V)	650

Note: Refer to the method MKAV/ C038[a], MKAV/ C039[b] and MKAV/ C032[c]

Table 4. Extraction procedure for the determination of TCs, SAs and quinolone using LC-MS/MS

Analyte	Extraction method
Tetracycline (MKAV/C038)	Samples were weighed in at 2.00 ± 0.04 g in 50 ml centrifuge tubes. 200 µl of 4.5 µg/ml demeclocyline (DMC) was added as internal standard together with 800 µl of water. The tubes were shaken vigorously for 5 minutes at room temperature. A 7 ml of McIlvaine/EDTA buffer solution was added to the tube. The tissue was mixed with end-over-end rotator for 10 minutes for maximum extraction. Following centrifugation (4000 rpm, 15 minutes at 4 °C), sample supernatant was collected into a new tube. 1 ml of 20% trichloroacetic acid was added slowly and mixed with vortex stirrer. The extract was placed in the refrigerator at -20 °C for 15 minutes. Next, the extract was centrifuged at 4000 rpm for 15 minutes at 4 °C and filtered through a filter paper to facilitate the SPE extraction. The SPE cartridges were conditioned by 1 ml methanol, 1 ml water and 1 ml McIlvaine/EDTA buffer. After application of the extract solutions, the cartridges were cleaned with 1 ml of water twice and air dried by aspiration. The analytes were eluted with 600 µl of 2% oxalic acid in methanol twice and collected in a 3 ml glass tube. The eluates were evaporated to dryness under a nitrogen stream at 40 °C. The dried residues were reconstituted in 200 µl of mobile phase A. The solution was transferred into 1.5 ml microcentrifuge tube and centrifuged at 13,000 rpm for 5 min. The lower layer was collected and transferred to an injection vial and 15 µl was injected into the LC system.
Sulphonamide (MKAV/C039)	Samples were weighed in at 2.00 ± 0.04 g in 50 ml centrifuge tubes. A volume of 200 µl of 1 µg/ml sulfapyridine (SP) was added as internal standard. The tubes were shaken vigorously for 5 minutes at room temperature, after which they were allowed to sit for 15 minutes on the bench to allow the drug to penetrate into the homogenized tissue. A 5 ml acetonitrile was added to the tube. The tissue was mixed with end-over-end rotator for 5 minutes for maximum extraction. The homogenized extract was centrifuged at 4000 rpm for 10 minutes at 5 °C. The supernatant was transferred into a glass test tube and dried using nitrogen gas at 50 °C. The dried residues were reconstituted in 500 µl of 0.2% formic acid. A 500 µl of heptane was added to remove the fat. The tube was vortex-mixed for 30 seconds. The solution was transferred into 1.5 ml microcentrifuge tube and centrifuged at 13,000 rpm for 5 minutes. The lower layer was collected and transferred to an injection vial and 15 µl was injected into the LC system.
Quinolone (MKAV/C032)	A 1 g of each sample was weighed in 50 ml centrifuge tubes. A volume of 50 µl of 5 µg/ml D5-enrofloxacin was added as an internal standard. The tubes were shaken vigorously for 5 minutes at room temperature, after which they were allowed to sit for 15 minutes to allow the drug to penetrate into the homogenized tissue. A 10 ml 1:1 glycine/hydrochloric acid solution was added to the tube. The tissue was homogenized using Ultra-Turrax T5 at the speed of 1000 rpm for 20 seconds and mixed with the end-over-end rotator for 5 minutes for maximum extraction. The homogenized extract was centrifuged at 4000 rpm for 10 minutes at 4 °C. The liquid phase was separated from the pellet and filtered through a filter paper to facilitate the SPE extraction. SPE sample clean-up was automated by using a Gilson Aspec XL4 involving an HLB SPE cartridges. The SPE cartridges were conditioned by 2 ml methanol and 2 ml of water. After the application of the extract solutions, the cartridges were cleaned with 3 ml of water and air dried by aspiration. The analytes were eluted with 2 ml of methanol in a 3 ml glass tube. The eluates were evaporated to dryness under a nitrogen stream at 50 °C. The dried residues were reconstituted in 0.5 ml of 0.1% formic acid. The tube was vortex-mixed for 30 seconds. The supernatant was transferred to an injection vial and 15 µl was injected into the LC system.

correlation coefficient (r^2) for the calibration curve was equal or greater than 0.99. The limit of detection (LOD) is the lowest point of the calibration curve at 10 µg/kg to 50 µg/kg, depending on the type of analysis. All samples were analysed in triplicates.

RESULTS AND DISCUSSION

Screening test

Out of 640 samples, three samples from two slaughterhouses in Perak and Negeri Sembilan were not analysed due to sample rejection. The number of samples suspected to be containing antibiotic residues based on the screening assay was presented in Table 5. Out of the 637 chicken samples screened, 17 (2.7%) samples were suspected positive for veterinary drug residues. Kedah and Pulau Pinang have the highest number of positive samples with the percentage of 6.8% and 6.5%, respectively, followed by two suspected positive samples detected from Perlis and one sample in Perak. Three suspected positive samples from Kedah and one from Pulau Pinang were detected with tetracycline residues, while another two samples from Pulau Pinang were detected with a mixture of tetracyclines and quinolones. Two samples from Perlis were also suspected to contain a mixture of veterinary drugs which were tetracyclines, sulfonamides and quinolones. One sample from Perak was tested to contain quinolone residues only. In the central zone, among 86 chicken samples tested for Selangor, only 2 (2.3%) samples were suspected to contain tetracycline residues. No veterinary drug residues were detected in the samples collected from other states. The distribution of suspected positive samples in the east zone showed Kelantan as having the highest number of positive samples followed by Terengganu. A total of 3 (5.8%) samples from Kelantan were suspected to contain veterinary drug residues were two of them were a mixture of tetracycline and sulfonamide residues and another sample with tetracycline residues only. Out of 52 chicken meat samples from Terengganu tested, only 2 (3.8%) samples were suspected to contain tetracycline residues. In the south zone, of the 160 chicken meat samples collected in Johor, only 1 (0.6%) sample was suspected positive for tetracycline residues. Generally, the percentage of suspected positive samples by zone are highest in the north (5.7%), followed by east (3.1%), central (1.3%) and south (0.6%). The majority of positives samples were suspected to contain tetracycline residues, followed by quinolone and sulfonamide.

Confirmatory test

The distributions of veterinary drug residues in the 17 chicken meat samples are as shown in Table 6. Of the 17 (2.7%) suspected positive chicken meat samples, the presence of tetracyclines residues were confirmed in 10 (1.6%) samples, and 1 (0.2%) sample was confirmed as non-violated positive for residues of quinolones. There were 6 (0.9%) samples violating the established MRLs for tetracyclines groups, with 3 samples containing oxytetracycline (OTC) and doxycycline (DC) residues respectively. Those samples are from Pulau Pinang, Terengganu and Kelantan. Among non-violated positives,

the main analytes found for tetracyclines and quinolones were chlortetracycline (CTC), mixture of chlortetracycline (CTC) and oxytetracycline (OTC), and enrofloxacin. None of the samples were found to contain sulfonamides residues.

Table 7 shows the concentration of tetracycline and quinolone compounds from different states. Residues of tetracycline detected in the chicken samples ranged from 28.48 µg/kg to 520.06 µg/kg. In comparison among states, chicken meat samples from Kelantan (520.06 µg/kg) showed the highest concentration of doxycycline (DC) at more than 5 times MRL, followed by Pulau Pinang (201.55 µg/kg) and Terengganu (145.64 µg/kg), also in DC and oxytetracycline (OTC), respectively. Chlortetracycline (CTC) was found in one sample each from Johor and Selangor, but with non-violated values. Two samples from Kedah were detected with a mixture of TCs compound which are OTC and CTC, but the sum of both concentrations (58.22 µg/kg and 66.24 µg/kg) was below the maximum limit. One sample from Pulau Pinang showed non-violated quinolones residues at the concentration of 22.10 µg/kg.

Generally, all tetracyclines drug types were detected in a low frequency ranging from 0.47% doxycycline (DC) to 0.78% oxytetracycline (OTC) and none for tetracycline (TTC). Considering the standard limit of tetracyclines (TCs) residues in chicken meat defined by MRL from Malaysian Food Regulation 1985 (2015), the average concentration of the four examined drugs among the positive samples (560.77 µg/kg) and in the whole sample population (51.48 µg/kg) were approximately 6 times greater and 0.5 times lower than the standard level, respectively. Doxycycline (DC) in particular had the highest share in this contamination (73.81%) and its mean concentration in the positive samples (413.9 µg/kg) was 4 times greater than the mentioned permitted limit, respectively (Table 8).

An efficient screening method should low cost with high throughput, and should be able to effectively identify positive samples from a large set of negative samples (Pikkemaat, 2008). Microbiological screening method or also known as microbiological inhibition assays is the earliest method on detection of antibiotic residues and this method is still widely used in almost every laboratory in Europe to detect antibiotic residues in food of animal origins (KirbiŠ, 2007; Pikkemaat, 2009). This method is appropriate for the detection of antimicrobial residues as it is less expensive than immunochemical and chromatographic methods and is able to screen a large number of samples at minimal cost (Pikkemaat, 2009). Ferrini *et al*. (2006) claimed that, in their experience, the proposed combination of six (6) plates method gives its best performance when used for meat tissues screening for indicated antibacteria.

Studies in other countries have also shown positive results upon screening of antibiotic residues in poultry. According to Kabir *et al.* (2004), positive results from screening of slaughtered chicken for antimicrobial substances were detected in 82 (21.8%) samples which were higher compared to this study. Results from antibiotic residues monitoring programme from 2008 and 2009 in Brazil showed 364 (25%) and 644 (42%) samples were positive

Table 5. Results of antibiotic residues screening in samples collected from small and medium scale chicken slaughterhouses by states

Zone	State	No. of samples tested	Percentage of Suspected Positive Samples (No.)	Veterinary Drug Residues Detected
North	Perlis	18	11.1 (2)	Tetracycline, sulfonamide and quinolone[a]
	Kedah	44	6.8 (3)	Tetracycline
	P. Pinang	46	6.5 (3)	Tetracycline; Quinolone[b]
	Perak	51	2.0 (1)	Quinolone
Total		159	5.7 (9)	
Central	Selangor	86	2.3 (2)	Tetracycline
	W.P. K.L.	2	0.0	-
	Melaka	24	0.0	-
	N. Sembilan	46	0.0	-
Total		158	1.3 (2)	
East	Pahang	56	0.0	-
	Terengganu	52	3.8 (2)	Tetracycline
	Kelantan	52	5.8 (3)	Tetracycline; Tetracycline and Sulfonamide[c]
Total		160	3.1 (5)	
South	Johor	160	0.6 (1)	Tetracycline
Grand Total		**637**	**2.7 (17)**	

a Sample was suspected to contain a mixture of drugs
b One sample detected with tetracycline, others with quinolone
c One sample detected with tetracycline, others with mixture of drugs

Table 6. The distribution of positive samples and confirmed (for one or more analytes) by states

State	Suspected Positive	Positives (No.)	Tetracyclines (TCs)	Quinolones (Quins)
Perlis	2	-	-	-
Kedah	3	2	2	-
Pulau Pinang	3	2	1[b]	1
Perak	1	-	-	-
Selangor	2	1	1	-
Terengganu	2	2	2[a]	-
Kelantan	3	3	3[ab]	-
Johor	1	1	1	-
Total	17	11	10	1

a Violation samples for oxytetracycline (OTC) b Violation samples for doxycycline (DC)

Table 7. Tetracyclines and quinolones residues concentration in chicken meat samples from different states of Peninsular Malaysia

State	Concentrations (µg/kg)			
	Tetracycline (TCs) (MRL 100 µg/kg)			Quinolone (MRL 30 µg/kg)
	Oxytetracycline (OTC)	Chlortetracycline (CTC)	Doxycycline (DC)	Enrofloxacin
Kedah	28.48; 29.26	29.74; 36.98	-	-
Pulau Pinang	-	-	201.55	22.10
Selangor	-	30.55	-	-
Terengganu	144.53; 145.64	-	-	-
Kelantan	178.34	-	520.06; 520.06	-
Johor	-	69.25	-	-

Table 8. Tetracycline residues (µg/kg) in chicken meat samples (n=637)

Types of TCs	Number of non-violated positive samples[a]	Number of violated positives samples[b]	Minimum concentration (µg/kg)	Maximum concentration (µg/kg)	Mean[c]	Each drug ratio to the total TCs concentration (µg/kg)
Chlortetracycline	4	-	29.74	69.25	41.63	7.42
Doxycycline	-	3	201.55	520.06	413.89	73.81
Oxytetracycline	2	3	28.48	178.34	105.25	18.77
Tetracycline	-	-	-	-	-	-
Total residues in positives samples		10[d]	259.77	767.65	560.77	100
Total residues in total samples		637			51.48[e]	

a Concentration in the sample < MRLs
b Concentration in the sample ≥ MRLs
c The average concentration (µg/kg) in the positive samples
d The sum of a and b
e Negative samples were replaced by limit of detection (LOD)

by screening using microbiological method and liquid chromatography analysis (Nonaka *et al.*, 2011), which is almost 10 to 16 times higher than this study.

The concentrations detected in the chicken meat samples were considered acceptable if they did not exceed MRL of 100 µg/kg for tetracycline and 30 µg/kg for quinolone adopted by Malaysia (Malaysian Food Regulation 1985, 2015). Among the antibiotics investigated in the present study, tetracyclines were mostly detected which agrees with KirbiŠ (2007), who reported that tetracycline residues were the most common detected residues in chicken meat sample. Findings from Malaysian monitoring residue programme of certified poultry processing plants from 2010 to 2011 reported that 16 (2%) out of 771 chicken samples were incompliant for tetracycline, 0.6% for quinolone and none for sulphonamide residue (Marzura *et al.,* 2012). The reported findings are in agreement with the current study as the percentage of contamination with tetracycline and quinolone were about the same values. In Egypt, the contamination rate of TCs in chicken samples was reported high at 44%. Among four tetracycline analytes, the highest incidence rate was observed in OTC (21.3%), followed by DC (12.67%), CTC (8.67%) and TTC (1.33%) (Salama *et al.*, 2011). Samples collected from an inspection audit in Saudi Arabia showed that OTC, TTC, CTC and DC were positively detected in 58.1%, 20.5%, 25.6% and 7.7% of the investigated chicken meat samples, respectively (Al-Ghamdi *et al.,* 2000). Samples of poultry meat surveyed in Turkey tested compliant positive in five out of 60 samples with the concentration levels ranging from 19.9 to 35.6 µg/kg for DC in four samples and 17.2 µg/kg for TTC in one sample (Cetinkaya *et al.,* 2012). In comparison to those reported in other countries, in this study, tetracyclines detected in chicken meat samples from Peninsular Malaysia is considered low.

The acquired results reflect the widespread use of multiple tetracyclines agents in chicken husbandry practices. However, in the present study, there are two samples detected with mixed tetracycline drugs, indicating that they were used in the poultry farm. Multi-tetracycline usage is not clinically justifiable as these drugs are effective against the same microbial spectrum and share the same mechanism of action. Thus, this practice may contribute significantly to the development of microbial drug resistance (Al-Ghamdi *et al.,* 2000).

CONCLUSION

Results from this study showed that 98.3% of chicken meat samples from small and medium scale chicken slaughterhouses were in compliance with Malaysian legislation. Among 637 samples tested, only six samples (0.9%) contain veterinary drug residues above the MRL. The major veterinary drug found was tetracycline (TCs). From these results, generally, it can be concluded that veterinary drugs are being used correctly and safely according to the veterinary husbandry practices recommended. However, further investigation should be done for samples 5 times above MRL value for tetracycline residues as found in Kelantan. As a recommendation, more samples should be tested for the presence of veterinary

drug residues in order to have an overview of its status in Kelantan and to trace back to the source. The investigation is necessary because the main source of chicken for small and medium scale slaughterhouses usually originates from small or semi commercial poultry farming which uses minimal or no veterinary medicines, supposedly allowing a more "natural" output and resulting in lesser residues compared to industrial poultry farming. Furthermore, the estimated dietary intake of veterinary drug residues from chicken meat consumption can also be conducted to determine the potential health risk to consumers.

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