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Data Article

Solid-phase extraction and high performance liquid chromatography with diode array detection method for the determination of antibiotic residues in poultry tissues

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ABSTRACT

This article presents information on high performance liquid chromatography with diode array detection (HPLC-DAD) method for the simultaneous determination of six antibiotic residues (enrofloxacin, sulfadimethoxine, sulfamerazine, sulfamethoxazole, sulfamoxole, and tylosin) in three poultry tissues. The target antibiotic residues were extracted from raw poultry samples following concentration, clean-up through solid phase extraction. The data describe the extraction, determination and screening procedures of these common antibiotic residues in 111 samples of fresh and frozen poultry meats. The limit of detection (LOD) ranged from 5.37–55.4 ng/g, while the quantification limit (LOQ) was in the range of 17.9–184 ng/g, respectively, with minimal matrix effect. The calibration curves obtained exhibited a good linear response with the coefficient of determination, $R^2 > 0.996$. Some concentrations exceeded their maximum residue limits in most samples. These findings indicated elevated levels of antibiotic residues in tissues of locally produced and illegally imported poultry meat samples.

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Specifications Table

Subject	Chemistry
Specific subject area	Analytical Chemistry, Food Chemistry
Type of data	Table, Figure, Chromatogram

(*continued on next page*)

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How data were acquired	Agilent 1220 High Performance Liquid Chromatography with Diode Array Detection HPLC-DAD (Agilent Technologies, Waldbronn, Germany) system consisting of a binary high-pressure pump, autosampler, a thermostat column compartment, a fluorescence detector, and refractive index detector. Data was acquired and processed using ChemStation (version 1.9.0) software (Agilent Technologies, Waldbronn, Germany). Analytical column was an XTerra MS C18 column (Agilent Technologies, Waldbronn, Germany) was used (4.6 × 100 mm, 3.5 µm particle size). Mobile phase solvent A (Ultra-pure water) and B (acetonitrile) contained 0.1% formic acid. Column temperature was maintained at 40 °C, and separation done under gradient elution with the organic phase increasing linearly from 5 to 30% in 6 min, and further increasing to 70% within 12 min. The mobile phase was pumped at a flow rate of 1.2 mL/min and the detection wavelength was 275 nm, with a post-run time of 1 min before the next injection to equilibrate the column.
Data format	Raw, Analysed
Parameters for data collection	111 samples of live and frozen poultry meats were collected. Antibiotic residues analysed were enrofloxacin, sulfadimethoxine, sulfamerazine, sulfamethoxazole, sulfamoxole, and tylosin.
Description of data collection	Poultry tissues were extracted using SPE method with water/methanol (4:1, v/v) mixture, and the compounds eluted with 2 mL of 10% ammonium hydroxide/methanol (1:19, v/v). The eluate collected was dried under N_2 with heating at 40 °C and reconstituted in 1 mL of phosphate buffer that was filtered with a 0.45 µm Acrodisc® syringe filters before injection into the HPLC system. Analyses of extracts for antibiotic residues were carried out using HPLC-DAD.
Data source location	Ogun State/Nigeria
Data accessibility	Data are included in this article.
Related research article	A.O. Oyedeji, T.A.M. Msagati, A.B. Williams, N.U. Benson, Detection and Quantification of Multiclass Antibiotic Residues in Poultry Products using Solid Phase Extraction and High Performance Liquid Chromatography with Diode Array detection, (2019), International Journal of Food Contamination, (Under review) [1]. A.O. Oyedeji, T.A.M. Msagati, A.B. Williams, N.U. Benson, Determination of Antibiotic Residues in Frozen Poultry by a Solid-Phase Dispersion Method Using Liquid Chromatography-Triple Quadrupole Mass Spectrometry, Toxicology Reports, 6, (2019) 951–956, doi: https://doi.org/10.1016/j.toxrep.2019.09.005 [2].

1. Rationale

Veterinary antibiotics are chemically synthesized antimicrobial drugs consistently used over the years in animal production for the prevention and treatment of infectious diseases. However, in recent years, the use of antibiotics in controlled quantities has been extended from animal therapeutic purposes to growth promoters and prophylactics in feed additives [2–5]. Over the past three decades, poultry production has increased as part of human's quest for alternate sources of protein for consumption. Thus, veterinary pharmaceuticals such as amphenicols, fluoroquinolones, beta lactams, tetracyclines, aminoglycosides, macrolides, among others. are widely used for growth promotion, prophylactic and therapeutic purposes [2,6,7].

Antibiotic residues have been reported in trace concentrations in edible parts of poultry meats [2,8]. These residues may consist of the parent compounds as well as their conjugates, and possibly all could be present together resulting in direct toxic effects on consumers and allergic reactions in hypersensitive individuals, and above all antimicrobial resistance. The World Health Organisation (WHO) has warned about an imminent antimicrobial resistance crisis, thus putting the antibiotic era at risk if urgent remedial actions are not taken to reduce antibiotic usage in human and veterinary medicine [9]. To safeguard public health and ensure some level of consumer protection, there is, therefore, a need to establish surveillance systems that allow for the collection of reliable data on antibiotic usage and residues.

Antibiotic residues in foods could pose serious indirect and direct long-term health hazards, and therefore present a significant research interest due to their extensive use as well as their persistence and prevalence in animal tissues, because they portend undesirable consequences such as treatment failure and disease severity to the consumer [2,6,10]. Consequently, it is necessary to provide reliable analytical data on multiresidues of different classes of antibiotics in poultry products and, in particular, those imported into Nigeria to ensure that there is no risk of secondary exposure to the consumer.

2. Procedure

2.1. Collection of samples

Smuggled frozen poultry products and fresh chicken (poultry meat) consisting of layers, broilers and cockerels were purchased directly from major farms and markets in the study area between May and September 2017.

2.2. Sample preparation and extraction

Using the modified method of Zhao et al. [11], the drugs residues were extracted from 2 g of blended tissues of poultry by weighing and transferring into a 50 mL previously washed stainless centrifuge tube. 10 mL of phosphate buffer solution (0.01 M adjusted to pH 7.0) was added to the samples. Each sample was allowed to stand for 15 mins at room temperature, and then vortex mixed for about 20 s before centrifuging for 5 mins at 3500 rpm. The supernatant was transferred to

Table 1
Distribution and concentration (ng/g) of antibiotic residues in frozen turkey muscle tissues ($n = 14$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
FTM-1	–	–	–	209.26	–	–
FTM-2	–	30.04	–	176.46	–	–
FTM-3	186.48	–	–	–	–	–
FTM-4	186.48	–	486.84	28.92	–	–
FTM-5	–	–	–	–	–	–
FTM-6	–	–	–	–	–	–
FTM-7	–	10.04	–	53.52	31.54	70.70
FTM-8	–	30.04	–	45.32	42.46	36.98
FTM-9	83.9	–	–	94.50	–	59.46
FTM-10	18,955.7	–	9223.68	151.88	53.38	3.28
FTM-12	20,340.3	–	10,065.80	–	–	–
FTM-13	135.18	50.04	–	37.12	20.60	25.76
FTM-14	–	–	–	28.92	–	–
MRL	100	100	100	100	100	100

–:Below limit of detection (LOD); MRL: Maximum residue limit.

Table 2
Distribution and concentration (ng/g) of antibiotic residues in frozen turkey gizzards ($n = 8$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
FTG-1	20.04	–	53.52	–	–	–
FTG-2	30.04	–	–	9.68	–	–
FTG-3	90.04	–	12.54	9.68	–	32.62
FTG-4	0.04	–	102.70	–	3.28	–
FTG-5	–	–	–	–	–	–
FTG-6	–	–	955.60	–	–	–
FTG-7	–	–	4.34	–	–	–
FTG-8	190.04	–	389.58	42.46	–	–
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

another flask, and the extraction process repeated two more times. 10 mL of the combined extracts were passed through an SPE (Supelclean^{TM}) column previously conditioned with 2 mL of methanol and HPLC grade water, respectively. Later, it was washed with 3 mL water/methanol (4:1, v/v) mixture, and the compounds eluted with 2 mL of 10% ammonium hydroxide/methanol (1:19, v/v). The eluate collected was dried under N_2 with heating at 40 °C and reconstituted in 1 mL of phosphate buffer that was filtered with a 0.45 µm Acrodisc® syringe filters before injection into the HPLC system. To enhance instrument response, sample extracts were spiked with 50 µg/mL mixed standards of the drugs.

2.3. HPLC-DAD analysis

Sample extracts were analysed using an Agilent 1220 High Performance Liquid Chromatography coupled with diode-array detector (HPLC-DAD) (Agilent Technologies, Waldbronn, Germany) system comprising a binary high-pressure pump, autosampler, a fluorescence detector, a thermostat column compartment, and refractive index detector. The analytical column used was an XTerra MS C18 column (4.6 × 100 mm, 3.5 µm particle size) (Agilent Technologies, Waldbronn, Germany). Mobile phase solvent A (ultra-pure water) and B (acetonitrile) contained 0.1% formic acid. Column temperature was maintained at 40 °C, and separation done under gradient elution with the organic phase increasing linearly from 5 to 30% in 6 min, and further increasing to 70% within 12 min. The mobile phase injection flow rate was 1.2 mL/min, and the detection λ was 275 nm, with a post-run time of 1 min before the next injection to equilibrate the column. Data acquisition and processing was carried out using ChemStation (version 1.9.0) software (Agilent Technologies, Waldbronn, Germany).

The antibiotics were eluted singly from the column after optimising the chromatographic parameters and their retention time obtained. Standards mix of the different antibiotics were prepared with a concentration range of 0–1000 ng/mL. The six antibiotics including sulfamozole, sulfamerazine, sulfadimethoxine, enrofloxacin, tylosin and sulfamethoxazole in that order were eluted as shown in the chromatogram. A 15-point calibration curve was prepared using the standard's retention time and the integrated peak area to obtain related linear equations. To enhance analyte signal and prominent peaks, sample extracts were spiked with 50 ng/mL standard mix. Analytes were quantified using their peak areas from linear equations and the spiked value subsequently subtracted from the concentration value upon evaluation.

3. Data, value and validation

Tables 1, 2 and 3 show the detected concentrations of antibiotic residues (enrofloxacin, sulfadimethoxine, sulfamerazine, sulfamethoxazole, sulfamoxole, and tylosin) in frozen turkey muscle, gizzard, and chicken muscle tissues, respectively. The

Table 3
Distribution and concentration of antibiotic residues in broiler muscle tissues ($n = 11$, ng/g).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
FCM-1	–	–	–	–	–	–
FCM-2	40.04	–	20.74	–	3.28	–
FCM-3	70.04	–	28.92	–	3.28	186.46
FCM-4	–	–	266.64	–	–	–
FCM-5	–	–	143.68	–	–	–
FCM-6	10.04	–	28.92	–	–	–
FCM-7	–	–	53.52	20.60	25.76	–
FCM-8	–	–	1184.66	–	36.98	–
FCM-9	40.04	–	12.54	–	–	–
FCM-10	20.04	–	78.10	–	–	83.90
FCM-11	–	1644.74	37.12	20.60	–	1006.98
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

Table 4
Distribution and concentration (ng/g) of antibiotic residues in layer muscle tissues ($n = 8$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
LCM-1	–	–	28.92	–	–	–
LCM-2	–	–	–	–	–	–
LCM-3	–	–	–	–	–	–
LCM-4	340.04	–	78.10	–	–	391.60
LCM-5	–	–	–	–	–	289.02
LCM-6	280.04	592.10	–	–	722.38	4186.46
LCM-7	240.04	1644.74	594.50	490.54	–	10,186.46
LCM-8	–	–	–	20.60	14.52	–
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

Table 5
Distribution and concentration (ng/g) of antibiotic residues in layer liver tissues ($n = 13$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
LCL-1	200.04	1223.68	–	20.60	–	–
LCL-2	110.04	3855.26	176.46	–	3756.10	7512.20
LCL-3	30.04	–	61.72	–	36.98	73.96
LCL-4	210.04	1223.68	–	–	3666.20	7332.40
LCL-5	730.04	14,381.58	301.62	1211.86	2138.12	4276.24
LCL-6	1170.04	276.32	569.92	774.70	1632.50	3265.00
LCL-7	–	–	–	7703.66	–	–
LCL-8	–	–	–	359.40	–	–
LCL-9	–	–	–	–	–	–
LCL-10	–	–	–	–	–	–
LCL-11	410.04	1539.48	–	–	–	–
LCL-12	–	–	–	–	–	–
LCL-13	110.04	–	–	–	–	–
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

antibiotic residues concentration varied according to each sample tissue analysed. The distribution and concentration of antibiotic residues in muscle, liver and gizzard tissues obtained from laying chickens are presented in Tables 4, 5 and 6, respectively. Also presented are the respective levels of enrofloxacin, sulfadimethoxine, sulfamerazine, sulfamethoxazole, sulfamoxole, and tylosin in muscle (Table 7), liver (Table 8), and gizzard (Table 9) tissue samples collected from broilers (*Gallus gallus domesticus*) raised mainly for meat consumption. Tables 10, 11, and 12 show the distribution and concentrations of antibiotic residues in muscle, liver, and gizzard samples of cockerels. Antibiotic residues were not detected in some tissue samples analysed whereas concentrations above EU Commission maximum residue limits (MRL) were observed in most samples. Fig. 1 shows the representative HPLC-DAD chromatogram of samples analysed in this study. The human exposure risks assessment was calculated using Estimated Daily Exposure Dose and the Hazard index, and the data are presented in Table 13.

The dataset provides an insight into the distribution of six (6) antibiotics including a fluoroquinolone, macrolide and four sulfonamides that could serve as primary data for drug residues in food chain originating from poultry meat. These dataset are useful for further toxicological and safety investigations into antibiotics levels in investigated foodstuffs and human health risk assessment associated with exposure to antibiotic residues. Details of extraction, analysis and characterisation

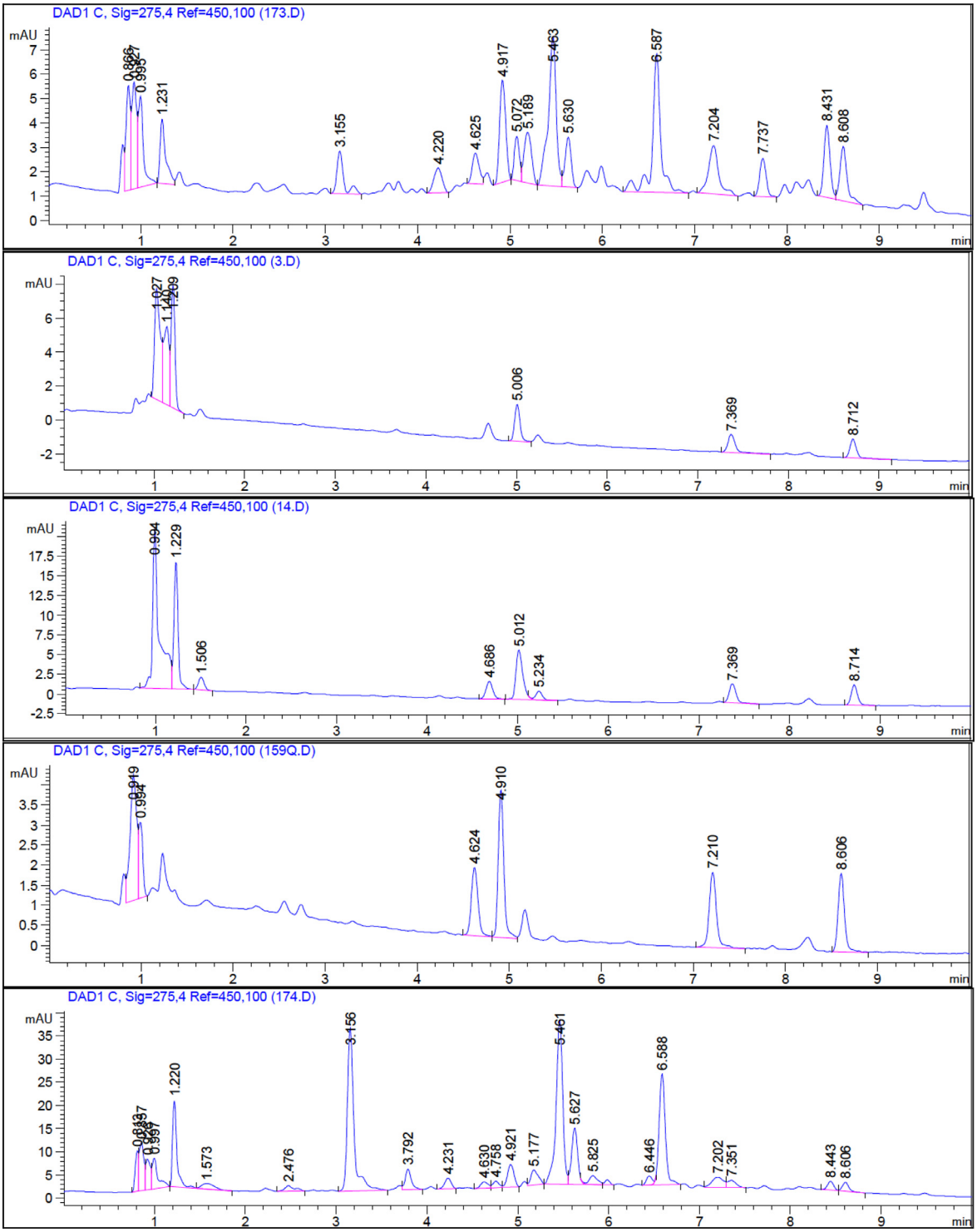

Fig. 1. Representative HPLC-DAD chromatograms of the analysed samples.

Table 6
Distribution and concentration (ng/g) of antibiotic residues in layer gizzards ($n = 18$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
LCG-1	–	276.32	119.10	–	–	–
LCG-2	–	3118.42	2397.78	–	587.56	2032.62
LCG-3	–	9118.42	1840.40	–	–	4340.30
LCG-4	–	–	37.12	–	–	–
LCG-5	–	381.58	–	–	14.52	3827.48
LCG-6	–	–	53.52	–	–	904.42
LCG-7	–	–	–	–	36.98	–
LCG-8	10.04	–	12.54	–	–	–
LCG-9	–	1644.74	184.66	–	–	–
LCG-10	–	–	–	–	–	135.18
LCG-11	100.04	486.84	–	–	–	289.02
LCG-12	110.04	–	37.12	326.62	–	–
LCG-13	70.04	–	–	42.46	–	–
LCG-14	–	3855.26	69.92	–	93.16	1981.34
LCG-15	20.04	–	–	–	–	904.42
LCG-16	30.1	–	3381.38	1244.64	–	–
LCG-17	–	–	45.32	–	–	–
LCG-18	–	–	–	–	–	904.42
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

Table 7
Distribution and concentration (ng/g) of antibiotic residues in broiler muscle tissues ($n = 6$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
BCM-1	1320.04	–	21.00	206.40	105.89	83.90
BCM-2	–	–	–	–	120.78	–
BCM-3	–	–	–	–	11.56	545.44
BCM-4	–	–	–	–	0.09	–
BCM-5	–	171.06	–	403.12	–	1263.38
BCM-6	–	–	176.46	–	–	–

–: Below limit of detection (LOD); MRL: Maximum residue limit.

Table 8
Distribution and concentration (ng/g) of antibiotic residues in broiler liver tissues ($n = 8$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
BCL-1	–	8171.06	–	–	45.67	1417.24
BCL-2	–	–	–	–	–	–
BCL-3	–	–	20.74	–	45.89	–
BCL-4	–	–	78.10	–	–	–
BCL-5	–	–	405.98	206.40	41.76	3212.10
BCL-6	1450.04	–	–	1332.08	–	17,981.34
BCL-7	–	–	–	–	–	–
BCL-8	1870.04	1539.48	–	326.62	1711.14	6904.42
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

provide information for further evaluation of antibiotic residues in poultry meat and human exposure assessment. The data yields information on the potential safety concerns associated with poultry products illegally imported into Nigeria and those produced locally with respect to antibiotic residues.

3.1. Human health risk exposure assessment

The estimated daily exposure dose of antibiotics from the different poultry products for adults (male and female) and children based on their average daily consumption was calculated using the modified formula [11]:

$$E_d = \frac{C_L \ x \ M_L}{M_B \ x \ 1000}$$

Where, E_d = estimated daily exposure dose, µg/kg/day; C_L = antibiotic content in poultry produce, µg/kg; M_L = daily adult consumption of poultry meat, g/day; M_B = average body weight, kg. The estimated poultry meat consumption in Nigeria as at 2014 stood at 1.41 metric tonnes [12] and this was projected to increase by 2% annually resulting from rapid population and economic growth [13] giving a current estimate of 1.56 million metric tonnes for 2019. Daily consumption

Table 9
Distribution and concentration (ng/g) of antibiotic residues in broiler gizzards ($n = 6$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
BCG-1	–	–	–	–	–	–
BCG-2	–	697.36	–	140.82	–	–
BCG-3	275.02	13,434.22	143.68	577.98	329.12	550.04
BCG-4	–	–	102.70	–	–	–
BCG-5	680.02	17,434.22	750.24	–	–	1360.04
BCG-6	–	–	176.46	–	–	–
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

Table 10
Distribution and concentration (ng/g) of antibiotic residues in cockerel muscle tissues ($n = 8$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
CCM-1	–	–	–	–	–	2391.60
CCM-2	–	–	–	–	–	–
CCM-3	–	–	–	–	–	1212.10
CCM-4	20.04	276.32	37.12	–	–	237.74
CCM-5	–	6802.64	102.70	–	14.52	–
CCM-6	220.04	1855.26	–	851.20	–	–
CCM-7	60.04	171.06	143.68	–	452.72	904.42
CCM-8	10.04	–	69.92	–	–	442.88
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

Table 11
Distribution and concentration (ng/g) of antibiotic residues in cockerel liver tissues ($n = 4$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
CCL-1	–	–	–	–	76.99	–
CCL-2	–	–	–	9.68	67.41	–
CCL-3	280.04	381.58	324	–	–	–
CCL-4	70.04	–	225.64	–	–	3673.64
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

Table 12
Distribution and concentration (ng/g) of antibiotic residues in cockerel gizzards ($n = 7$).

Sample ID	Enrofloxacin	Sulfadimethoxine	Sulfamerazine	Sulfamethoxazole	Sulfamozole	Tylosin
CCG-1	400.04	–	–	97.10	–	–
CCG-2	180.04	–	–	–	3.28	1263.38
CCG-3	20.04	–	28.92	–	–	32.62
CCG-4	10.04	–	45.32	–	–	–
CCG-5	–	–	561.72	–	–	–
CCG-6	0.04	2802.64	–	75.24	–	545.44
CCG-7	–	–	37.12	–	14.52	83.90
MRL	100	100	100	100	100	100

–: Below limit of detection (LOD); MRL: Maximum residue limit.

estimated from this data for the present study is 21.92 g/day for a population of approximately 195 million [14]. The average body weight for adults and children (age range 6–18 years), in Nigeria, was 70 and 48 kg, respectively [15]. The hazard index (HI) was computed using [16]:

$$\mathrm{HI} = \frac{E_d}{ADI}$$

Where, ADI is the acceptable daily intake for veterinary pharmaceuticals (50 μg/kg body weight, upper bound) [16]. $\mathrm{HI} < 1$ = risk is considered acceptable; $1 \leq \mathrm{HI} \leq 10$ = risk exists but does not require immediate action; $\mathrm{HI} > 10$ = risk is at unacceptable [16].

Ethics approval

This study was approved by the Ethics Review Committee of the University of South Africa School of Science under Ethics Number 2017/SSR-ERC/012 & 2017/SSR-EC/010.

Table 13
Estimated Daily Exposure Dose (E_d) and the hazard index (HI) in different poultry tissues .

Poultry		E_d (µg/kg/day)	HI
Turkey muscle	Adult	19.18	0.384
	Children	27.92	0.558
Turkey gizzard	Adult	0.61	0.012
	Children	0.89	0.018
Chicken muscle	Adult	1.59	0.032
	Children	2.32	0.046
Layers muscle	Adult	6.31	0.126
	Children	9.19	0.184
Layers liver	Adult	22.09	0.442
	Children	32.15	0.643
Layers gizzard	Adult	8.81	0.176
	Children	12.83	0.257
Broiler muscle	Adult	1.39	0.028
	Children	2.02	0.040
Broiler liver	Adult	14.68	0.294
	Children	21.37	0.427
Broiler gizzard	Adult	11.51	0.230
	Children	16.75	0.335
Cockerel muscle	Adult	5.11	0.102
	Children	7.44	0.149
Cockerel liver	Adult	1.50	0.030
	Children	2.19	0.044
Cockerel gizzard	Adult	1.95	0.039
	Children	2.83	0.057

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Competing financial interests

The authors declare no competing financial interests.

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