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RESEARCH ARTICLE

Antimicrobial residues in meat from chickens in Northeast Vietnam: analytical validation and pilot study for sampling optimisation

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Abstract

Antimicrobials used in chicken farming for therapeutic and/or prophylactic purposes may result in unacceptable levels of edible residues, if withdrawal periods are not respected. To evaluate the risk in Vietnam, we validated an analytical method to detect antimicrobial residues from chicken meat samples and carried out a pilot cross-sectional study to identify optimal sampling strategies. A total of 45 raw meat samples were collected from 4 markets, 1 slaughterhouse and 4 farms (5 per site) in Northern Vietnam, between March and April 2021. Farmers were asked about antimicrobials used during sampled production cycles (5 chickens sampled per batch). Samples were analysed using liquid chromatography-tandem mass spectrometry for the presence of 68 antimicrobials at a pre-defined validation concentration. 7 compounds were identified from 4 classes (tetracyclines, sulphonamides, macrolides, and fluoroquinolones). In markets, where the source of sampled chickens was unknown, a diverse pool of residual antimicrobials was detected in 20% (4/20) of the meat samples. No residues were detected in samples from the slaughterhouse. No residues were detected in chickens from the one farm that reported using antimicrobials, whereas sulfadimethoxine, doxycycline and tilmicosin residues were identified from the other 3 farms reporting no antimicrobial use. The probability of detecting antimicrobial residues present in a flock based on sampling a single chicken was estimated at 0.93 (highest density interval 0.735–0.997). The preliminary results suggest a disparity between farmers' reports on antimicrobial drug use and actual usage, and that the analysis of a single sample per farm has a high probability of detecting antimicrobial residues, if present.

Keywords Poultry market · Chicken farm · Antibiotic residues · Maximal residue limit · Withdrawal period · LC–MS/MS

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1 Introduction

An increase in the global demand for protein from animal sources has been paralleled by the intensification of livestock production, particularly within the poultry sector. This has resulted in an increased use of antimicrobial drugs (AMDs) for the prevention (prophylaxis) and (therapeutic) treatment of disease, and, on occasions, for use as growth promoters (Page and Gautier 2012; Kim et al. 2013; Chattopadhyay 2014; Carrique-Mas et al. 2015; Nhung et al. 2016). Antimicrobial drug use in livestock poses a threat to disease control globally and causes concerns for human, animal, and environmental health due to its contribution to antimicrobial resistance (AMR) (Donoghue 2003). The issues surrounding AMR and AMD usage in livestock have been highlighted in recent strategy reports including a Global Action Plan by the WHO that lists certain AMDs (including gentamycin, erythromycin, and colistin) as critical for human health and calls for their restricted use in livestock and agriculture (World Health Organization 2015; Collignon et al. 2016; World Organisation for Animal Health 2016). In poultry, antimicrobial drugs are commonly administered at high levels around the world with different approaches towards monitoring AMD use and evaluation of the prevalence of resistant micro-organisms (Roth et al. 2019). In Vietnam, systems of poultry production frequently differ from those of high-income countries. Around 90% of Vietnamese households keep poultry, of which the production contributes to 19% of households' income (Desvaux et al. 2008). AMDs are administered to poultry flocks in Vietnam primarily via feed (Van Cuong et al. 2016), with a recent study highlighting the wide range of antimicrobial classes administered within a flock (Nhung et al. 2016). These commonly reported AMDs include penicillins, fluoroquinolones, tetracyclines, aminoglycosides, macrolides, polymyxins, trimethoprim, and amphenicols. The use of antimicrobials as growth promoters has been prohibited in Vietnam since the beginning of 2018 (Ministry of Agriculture and Rural Development in Vietnam 2016).

To ensure the safety of poultry products, maximum residue limits (MRL) are defined as the maximum levels of antimicrobial residues within meat samples that are deemed safe for human consumption. In Europe, MRLs for allowed substances are enforced by Commission Regulation (EU) No 37/2010 (Commission Regulation (EU) 2009). The Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) determines MRLs, together with the FAO/WHO Joint Expert Committee on Food Additives (JECFA) which evaluates and recommends levels of Acceptable Daily Intake (Lees et al. 2021). Typically, these levels can be met by adhering to the source-specific withdrawal periods (Landoni and Albarellos 2015), although rules may differ from country to country. In Vietnam, the list of products with MRLs includes fewer medicines than in the EU and the MRL sometimes differs for the same compound between regulators (Ministry of Health in Vietnam 2013). Progressive and consistent implementation of the residue monitoring scheme for the internal market is desirable to ensure consumer safety.

The UKRI GCRF One Health Poultry Hub (OHPH) is an international interdisciplinary research project established in 2019 that evaluates zoonotic health risks associated with the intensification of poultry production. Pilot studies were necessary to optimise the design of larger-scale field studies that aim to gather data on poultry husbandry practices across participating study sites in Bangladesh, India, and Vietnam.

The objectives of this pilot study were to: (1) transfer the multianalyte liquid chromatography-mass spectrometry method for the detection of AMD residues in chicken meat from the EU reference laboratory (ANSES, France) into the analytical laboratory at the Nanyang Technical University, Singapore, (2) assess the types of AMD residues detected in meat samples from farms, markets, and slaughterhouses in Vietnam, and (3) to determine the optimal number of chicken carcasses to sample on a farm to detect antimicrobial residues present in the farmed chickens.

2 Material and methods

Ethical approvals were obtained from the National Institute of Veterinary Research (NIVR) (020-433/DD-YTCC) and the Royal Veterinary College (RVC) Ethics and Welfare Committee (URN: 2020 1983-3).

2.1 Sample collection

The pilot study ran between March and April 2021 as part of a larger project that involved sampling chickens on markets, slaughterhouses, and their supplying farms in Northern Vietnam. The study was conducted in Bâc Giang province, Vietnam, with 1 additional farm site located in Thái Nguyên province (Fig. 1). The Thái Nguyên site raised exotic *white* broiler breeds (e.g., Ross, Cobb, Arbor Acres). The other 3 farm sites in Bâc Giang raised hybrid *coloured* broiler chickens, which are the crossbred progeny of a mating between indigenous males (e.g., Ri, Choi, Ho, Dong Tao) and exotic hens (e.g., Luong Phuong breed from China). The size of the farms varied, with the smallest having 400–500 chickens (Farm 3) and the largest having 4000–5000 chickens (Farm 2).

The markets were categorised according to their trading practices, with markets 1 and 2 defined as "retail" and

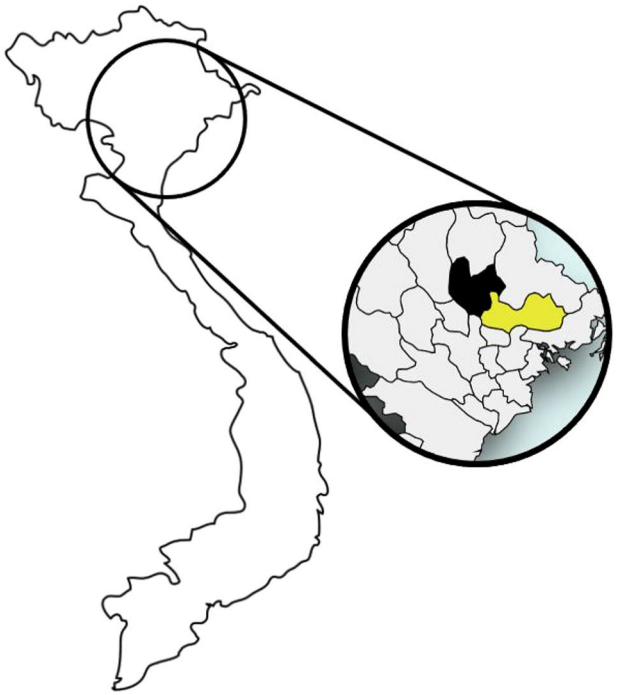

Fig. 1 An outline of Vietnam showing the two sampled provinces: Thái Nguyên (black) and Bâc Giang (yellow) (color figure online)

markets 3 and 4 defined as "wholesale". Wholesale markets sold more poultry (50–150,000) than retail (between 20 and 150/day) with all markets comprising more than one stall selling poultry products. 3 markets sold coloured broilers alongside other bird species (e.g., ducks, pigeons, geese), while the remaining market sold only coloured broilers. The single slaughterhouse in the study slaughtered approximately 1500 white broilers per day. Chickens were sampled from randomly selected stalls within a market or randomly appointed sections within the slaughterhouse.

On farms, chickens were sampled towards the end of their production period (i.e. ≥ 35 day-old for white broilers and ≥ 70 day-old for hybrid coloured broilers) but before any chickens of the same batch were traded, and therefore likely before the completion of withdrawal periods. Table 1 provides a summary of the location, age, and type of birds sold at each site.

2.2 Sample collection and storage

Each selected chicken was culled using ethically approved methods (e.g., cervical dislocation) and by trained staff only and the samples were prepared immediately, one at a time. The pectoralis muscles (P) were removed from the sternum, exposing the supracoracoideus muscle (Fig. 2). The pectoralis muscle was detached, and samples were cut to include a minimum of 20 g of chicken breast meat (equivalent to 2.6 cm^3). The final prepared samples were sealed in a plastic bag, frozen at − 20 °C immediately after collection and during transportation and then stored at − 80 °C until preparation for analysis. This process was repeated until 5 chickens had been culled and sampled per site. All samples were shipped on dry ice to Nanyang Technological University and arrived frozen. Sample analysis took place between 3 and 4 months after sampling.

2.3 Questionnaire data on farms

Questionnaires were completed during meat sampling on farms to assess husbandry practices, especially the use of drugs during the production cycle and, where relevant, which drugs were used. Confirmation included photographic evidence of the AMD product(s) including supplier details. Reasons for drug administration were reported. On market, there is no information available about previous administration as sellers ignore what treatment may have been given in the farm the chicken came from or during the time of transportation to the market (Fig. 3).

2.4 LC–MS/MS for antimicrobial residue detection

The bioanalytical method of the EU reference laboratory (Dubreil et al. 2017) was transferred to the Singapore Phenome Centre (SPC) at Nanyang Technological University (Singapore). The method was validated for sample analysis according to the requirements of Council Directive (EC) 96/23, 29 April 1996 (Council of the European Union 1996) (now repealed by Regulation (EU) 2017/625 by 14 December 2019 and its annexes I, II, III and IV by 14 December 2022).

2.4.1 Analytical standards

Analytical standards of drug/residue markers from the EU reference method were purchased in analytical standard grade from Sigma-Aldrich, TRC, Dr. Ehrenstorfer LGC (Singapore), or received as a donation from Eco Animal Health. 5 analytes of the original method (n = 73) were not included as not validated in the original method (sulfanilamine), unavailable (8-α-hydroxy mutilin), irrelevant (baquiloprim, pirlimycin) or no longer used in the detection of residues (thulathromycin marker). Individual standard stock solutions were prepared at 0.5 mg/mL in appropriate solvents and kept according to validated stability conditions. 2 pools of spiking solutions were prepared: pool A contained 37 analytes from betalactam, sulphonamide, and tetracyclines families and pool B contained 31 analytes from the quinolone, fenicol, macrolide, and pleuromutilin families, as well as miscellaneous antibiotics, including trimethoprim (Appendix 1B). An intermediate internal standard of

Table 1 Summary of antimicrobial residues detected in meat collected from chickens sampled on farms, at markets or in a slaughterhouse in Vietnam compared with farmer-declared antimicrobial use

Site	Size of Premises (Sold/ Slaughtered per day or flock size)	Broiler Type	Age (days)	Bird ID	AM Residues Detected in Meat							AMU Questionnaire	
					SULF.		TET.		MACRO.		FQ.	SULF.	Other
					Sulfaclozine	Sulfadimethoxine	Doxycycline	Oxytetracycline	Azithromycin	Tilmicosin	Enrofloxacin	Sulfamonomethoxine	Trimethoprim
Market 1 (Bâc Giang, Retail market)	20–60/day	Coloured	-	1				++					
				2									
				3			++						
				4									
				5									
Market 2 (Bâc Giang, Retail market)	60–150/day	Coloured	-	1									
				2									
				3									
				4									
				5			t						
Market 3 (Bâc Giang, wholesale markets)	6,000–15,000/day	Coloured	-	1					++	+/-	t		
				2									
				3									
				4									
				5									
Market 4 (Bâc Giang, wholesale markets)	50–200/day	Coloured	-	1									
				2	++	t	++						
				3									
				4									
				5									
Slaughter-house (Bâc Giang, semi-industrial)	1,500/day	White	-	1									
				2									
				3									
				4									
				5									
Farm 1 (Bâc Giang)	2,000	Coloured	120	1		+++							
				2		+/-							
				3		++							
				4		++							
				5		++							
Farm 2 (Bâc Giang)	5,000	Coloured	Mixed	1								✓	✓
				2								✓	✓
				3								✓	✓
				4								✓	✓
				5								✓	✓
Farm 3 (Bâc Giang)	3,000	Coloured	107	1			+++						
				2			+++						
				3			t						
				4			+++						
				5			+++						
Farm 4 (Thái Nguyên)	16,000	White	43	1			+/-			+/-			
				2			+/-			t			
				3			+/-			+/-			
				4			+/-			+/-			
				5			+/-			t			

For antimicrobial residues detected above 10×MRL cells are filled black with '+++', for detection > 1×MRL cells are grey with '++'. Where antimicrobial residues were detected below MRL '±' indicates a concentration between 0.5 and 1×MRL, and cells containing 't' indicate trace levels (between 0.1 and 0.5×MRL). Cells containing a '✓' indicate where a drug was declared during interviews carried out on farm sites only (only declared in Farm 2)

SULF sulfonamides, *TET* tetracyclines, *MACRO* macrolides, *FQ* fluoroquinolones, *AMU* antimicrobial use

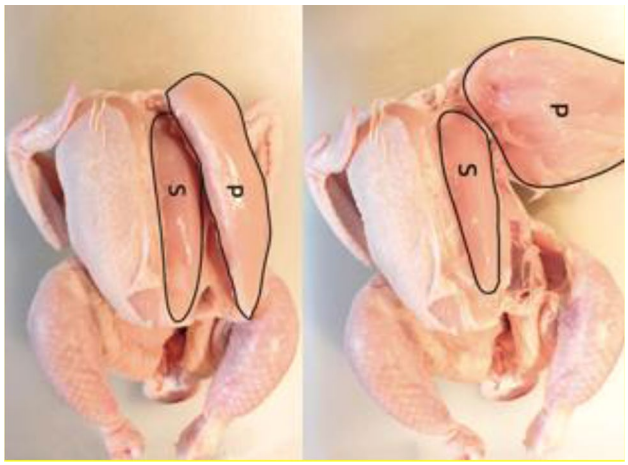

Fig. 2 Location of breast meat samples collected for antimicrobial residue testing showing the pectoralis (P) and supracoracoideus (S) muscles. Pictures reproduced with the permission of Edmond Hui (https://www.scienceinschool.org/article/2017/how-do-birds-fly-hands-demonstration/)

sulfaphenazole at 20 µg/mL was kept for up to 6 months at a temperature of ≤ − 18 °C from which the working internal standard solution at 1 µg/mL was prepared.

2.4.2 Preparation of meat samples and spiked controls

Each chicken sample (minimum 20 g) was minced in a food chopper (La Moulinette, Tefal). A volume of 200 µL of the internal standard sulfaphenazole (1 µg/mL; Sigma-Aldrich) was added to tubes containing 2 g of the minced meat samples. The tubes were shaken vigorously before and after adding 800 µL of ultra-pure water (Optima LC–MS Grade, Fisher) to the samples and the blank control, and 600 µL of water to the spiked controls. Samples were then left for 10 min in a dark place before adding 8 mL of acetonitrile (Optima LC–MS Grade, Fisher Scientific), vortexed for 2 min for homogenisation and shaken for 10 min in a rotary shaker at 100 rpm. After centrifugation (5 min, 14,000×*g*, room temperature) 6 mL of the supernatant was transferred into another tube and left to evaporate with a nitrogen stream at 30 °C. The residue was diluted in 0.6 mL of water:acetonitrile (50:50; v/v) and vortexed for 2 min to re-dissolve all the residues. The mixture was then centrifuged for 10 min at 14,000×*g* at room temperature and the supernatant was filtered using 0.45 µm filters. Spiked control samples with both pool A or pool B analytes (QCA and QCB) were prepared to the targeted concentration level [Cval (0.5 × MRL)] in advance and frozen to at least -18 °C, for a validated maximum storage duration of 6 months.

2.4.3 LC–MS/MS conditions

An HPLC machine (ACQUITY UPLC I-Class PLUS Waters Pacific Pte Ltd) was connected to a triple quadrupole mass spectrometer (Xevo TQ-S, Waters, Singapore). The chromatography column (BEH C18 Column, 130 Å, 1.7 µm, 2.1 mm × 150 mm, ACQUITY) was protected by a pre-Column (VANGUARD BEH C18, 1.7 µM, ACQUITY). Mobile phases A and B were water and acetonitrile (both with 0.5 mmol/L Heptafluorobutyric acid and 4.75 mmol/L Pentafluoropropionic acid), respectively and were injected according to an optimised gradient, with a flow rate of 0.3 mL/min (column oven 25 ºC). The gradient timetable

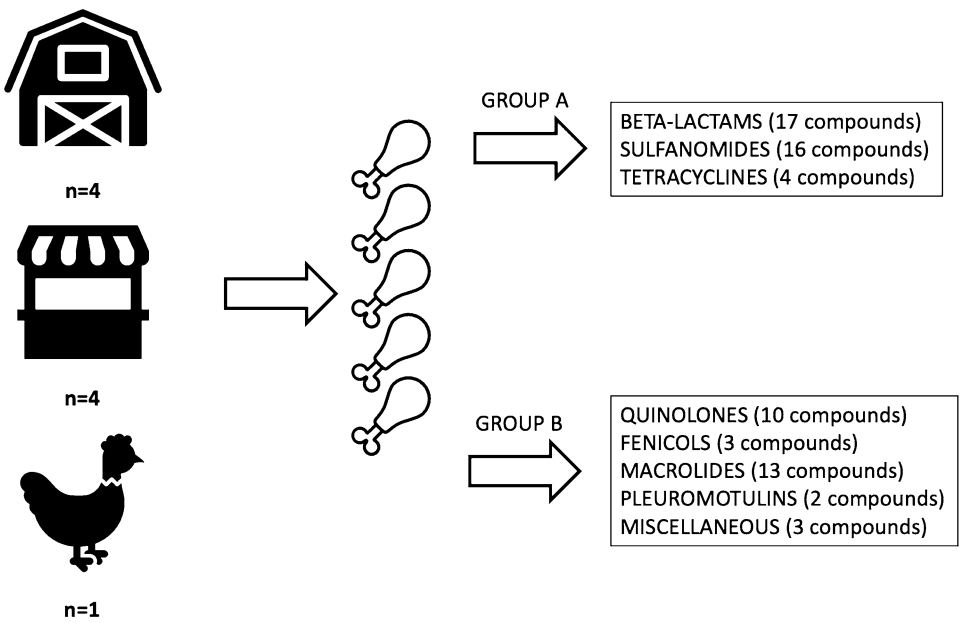

Fig. 3 A schematic diagram summarising the collection of meat samples from a farm (n = 4), market (n = 4), or slaughterhouse (n = 1) to LC–MS/MS analysis of meat residues. LC–MS/MS analysis was split into groups A and B (see Appendix 1, Supplementary Material) for 68 antimicrobial compounds. Group A contained 37 antimicrobials (betalactam, sulfonamide and tetracyclines) and group B contained 31 analytes (quinolone, fenicol, macrolide, pleuromutilin and miscellaneous 5 antibiotics)

is described in Appendix 1A (Supplementary Material). A volume of 5 µL of the extract was injected after being kept at 4 ºC. The mass spectrometer was operated in positive electrospray ionisation mode (ESI+), using multiple reaction monitoring (MRM) detections (additional settings in Appendix 1A, Supplementary Material). A blank sample control and the 2 spiked controls were run for every ~ 10 samples to detect contamination in the event of high antibiotic levels. For each analyte, we initially validated elution time and optimal source conditions for LC–MS/MS to characterise the ion signal observed for the 2 strongest transitions (see Appendix 1B, Supplementary Material, for compound transitions Tr1 and Tr2).

2.4.4 LC–MS/MS method validation

The LC–MS/MS was validated according to CRL 20/1/2010 guidelines for the validation of screening methods for residues of veterinary medicines (Community Reference Laboratories Residues 2010) to ensure that there were no more than 5% false negatives at the Cval. 21 organic chicken meat samples were purchased and verified for the absence of AMD residues. 3 batches of 7 blank and 7 matrix samples were spiked using a Cval of 0.5 × MRL and analysed per day, over 3 days (n = 21). For azithromycin, Cval was set as 50 µg/kg as no MRL has been defined. This validation design allows to assess the following performance characteristics of the method: detection capability CCb, and precision.

To estimate CCb, for each analyte, the cut-off factor (Fm) was determined using the mean and standard deviation of areas under the peak in the blank samples spiked at the predefined validation concentration Cval (Fm = mean − 1.64 SD). Analysis of the background noise for each of the chromatograms corresponding to the transitions allowed for the calculation of T, the threshold value (T = mean + 1.64 SD), related to the limit of detection of the method. Validation was successful if Fm > T for the 2 transitions, demonstrating that CCb is < Cval. Precision was assessed by measuring the intra-day and inter-day variability of the signals using peak areas measurement.

For ongoing method performance verification, the analysis of quality control samples was carried out in each series of analysis. QCs consist of matrix blanks to check the absence of contamination in the analytical procedure and of spiked control samples (QCA and QCB) to check the ongoing validity of the level of detection. A sample was positive when peaks were present for the 2 transitions at the correct retention time and the ratio of peak areas was similar to that in the spiked samples, but exact quantification was not attempted. Based on the peak area-under-curve (AUC) values from spiked controls and positive results from the analysis of samples, detectable residues were categorised as either between "> 0.5 × MRL" (between 0.5 × and 1 × MRL), "> 1 × MRL" (between 1 × and 10 × MRL) or "> 10 × MRL". When chromatograms from the 2 transitions were present with the correct ion ratio but between 0.1 × and 0.5 × MRL, the sample was qualified semi-quantitatively as "t" (= trace), i.e., > 0.1 × MRL.

2.5 Probability of detecting residues on farms

We estimated the probability p of detecting residues in a single chicken on a farm in which these residues were detectable. In other words, p was the within-farm prevalence, the probability of a chicken being positive for residues if at least 1 chicken was positive in the flock. A model was formulated as follows. The number of chickens found positive on farm i followed a binomial distribution, with $n = 5$, the number of chickens sampled per farm, as the number of trials, and π_i, the probability of a chicken sampled on farm i being positive. π_i was equal (1) to p, the abovementioned within-farm prevalence, if residues could be found in farm i, or (2) to 0 otherwise (i.e. residues were not detectable on any chickens in farm i). The status of a farm followed a Bernoulli distribution with μ, the probability of a farm being positive, referred to as the farm-level prevalence, as the probability of success.

We estimated μ and p within a Bayesian Markov Chain Monte Carlo (MCMC) framework, implemented in JAGS (Plummer 2003) using the library "R2jags" (Su and Yajima 2021) in R.4.2.1 (Team 2009). 4 chains were run with different starting values. After a burn-in period of 10,000 iterations, each chain was iterated up for another 50,000 iterations. Convergence was assessed based on the Gelman and Rubin statistic and the visual inspection of the trace plots for both parameters. Effective sample sizes were also checked. The posterior mode and 95% highest density interval estimates were reported for each parameter. Weakly informative prior distributions, beta(1,1), were used for μ and p.

3 Results

3.1 Method validation

All analytes were successfully validated (full validation results in Appendix 1C, Supplementary Material), except cefalexin, penicillin V and cephapirin, although the latter could still be detected using transition 1 of its metabolite, desacetylcephapirin.

3.2 Residue detection in meat samples from farms

Chicken meat from 3 of the 4 farms tested positive for detectable levels (> 0.5 × MRL) of AMD residues (14/20). Table 1 provides a summary of the AMD residue profiles for

all the meat samples per site and the AMD reported to be used by farmers. Sulfadomethoxine was detected on 1 farm (5/5 chickens), doxycycline in 2 farms (4/5 and 5/5 chickens), and tilmicosin in 1 of the farms which was also positive for doxycycline (3/5). These compounds were detected at concentrations ranging from 0.5 × to > 10 × MRL. In 2 out of the 4 farms, the AMD residue profile was the same for all samples collected on the given farm. For the third farm the AMD residue profile was shared by 4 out of 5 of the samples and for the final farm the AMD residue profile was shared by 3 out of 5 of the samples. Only 1 farm declared the administration of antimicrobials, a sulfamonomethoxine/trimethoprim combination, but no AMD residues were found in meat sampled from chickens from this farm.

3.3 Residue detection in meat samples from endpoints (markets and slaughterhouse)

At markets, where chickens sourced from different farms are mixed, the proportion of samples containing residues from at least 1 AMD was 20% (4/20). Positive samples were collected in 3 markets, and all had unique AMD residues profiles, with apparent higher compound diversity than in farms. The presence of sulfaclozine, doxycycline, oxytetracycline, azithromycin, tilmicosin was detected at concentration levels ranging from 0.5 × to 10 × MRL. Sulfadimethoxine and enrofloxacin were seen at trace level ("t"). No AMD residues were detected in the samples from the slaughterhouse. Full residue profiles can be seen in Table 1.

Collectively across all sources, the most common class of AMD identified was tetracycline (26.7%), followed by sulphonamide (15.6%), macrolide (4.4%), and fluoroquinolone (2.2%). Across all the sites, there was no correlation between the type of bird (white/coloured) and the AMD residues detected (Table 1).

3.4 MRLs

Only 11 drugs included in our detection panel have a MRL published in Vietnam (Ministry of Health in Vietnam 2013). These local Vietnamese MRL (vMRL) are aligned on the document CX/MRL 2–2021 form the Codex Alimentarus (Food and Agriculture Organization 2021). For 4 of these antimicrobials, the MRL were comparable with the EU ones: spiramycin (200 µg/kg), tylosin (100 µg/kg), danofloxacin (200 µg/kg) and sarafloxacin (10 µg/kg). For 6 molecules, vMRLs were higher than the European MRL, including 3 tetracyclines (200 µg/kg vs 100 µg/kg, for tetracycline, oxytetracycline and chlortetracycline), lincomycin (200 µg/kg vs 100 µg/kg), tilmicosin (150 µg/kg vs 75 µg/kg) and flumequine (500 µg/kg vs 400 µg/kg). Only the vMRL for erythromycin was lower than the EU equivalent (200 µg/kg vs 100 µg/kg). Our interpretation of the results was unchanged when applying vMRL for these 11 antimicrobials instead of the EU MRL. The oxytetracycline positive samples from markets 1 would remain > 1 × vMRL. On farm 4, tilmicosin samples > 0.5 × MRL would be > 0.1 vMRL (trace).

3.5 Probability of detecting residues on farms

The probability of detecting residues in a farm in which these were detectable by sampling only 1 chicken (i.e. within-farm prevalence) was estimated to be high, with a posterior mode of 0.934 (95% HDI 0.735–0.997). This probability of detecting residues almost reached 1 when 2 chickens were sampled (posterior predictive mode = 0.998, 95% HDI 0.930–1). Note that the 95%HDI of the farm-level prevalence was wide (0.333–0.977, mode = 0.760), due to the small number of farms sampled (n = 4). Trace plots and marginal posterior distributions are shown in Appendix 2 (Supplementary Material).

4 Discussion

This pilot investigation screened chicken meat samples collected in Northern Vietnam for the presence of AMD residues at different stages of the production cycle. The LC–MS/MS method transferred to our laboratory in Singapore offered a comprehensive panel, covering 68 different AMD analytes applied to a total of 45 meat samples. Overall, the detection of AMD residues was less common in meat sampled from chickens in markets compared to farms, although the range of AMDs detected at the market level was more diverse. The small scale of this pilot study precluded statistical analysis of the significance of these differences, although a trend has been detected and will be used to develop hypotheses as this work is expanded. Validation of the protocols has been successful. Macrolides, such as azithromycin and tilmicosin, and fluoroquinolones were both detected during this study and have been listed as critically important AMDs for human health by the WHO (Collignon et al. 2016).

Antimicrobial residues were more common in meat sampled from chickens obtained from farms (70%) than from markets (20%) or the slaughterhouse (0%). It is possible that the results from the farms were influenced by the shared exposure history of the individual chickens within a production batch. The lower prevalence of AMD residues at the endpoints (market and slaughterhouse) may be due to a reduced likelihood of exposing chickens to AMDs at the end stages. The probability of detecting residues in a flock, if present, by sampling a single chicken was estimated to be 93%. Recommendations for control and surveillance protocols for AMD residue testing in meat usually stipulate sampling 5% of the production, depending on the size of the

farm, with a minimum of 2 and a maximum of 10 chickens from the same production lot (French General Directorate for Food 2019). While it is still prudent to sample 2 chickens, analysis of a single sample (archiving the second as a backup) can save resources without unduly compromising data quality for future investigations into AMD residues in resource-limited settings.

The proportion of samples positive for AMD residues at market sites was in line with previous reports from Ho Chi Minh City in 2012–2013 (17.3%) (Yamaguchi et al. 2015) and from the Tay Ninh Province in 2017 (27.4%) (Huong-Anh et al. 2020), although these studies used less extensive LC–MS detection panels. Chicken meat collected from market stalls are likely to be sourced from farms across provinces and not just the area the market is located. Despite a lower occurrence of AMDs in market samples than farm samples, there is a greater diversity in AMD class. Hybrid-coloured broilers were sampled most frequently in our study (7/9 sites), with exotic-white broilers sampled at 1 farm and the slaughterhouse. While the sample size was small, there was no clear difference in AMD residue patterns between the different types of chickens.

The total yearly antimicrobial use (AMU) in chickens for Vietnam was recently estimated at 185 tonnes (Carrique-Mas et al. 2020), however reporting per antibiotic family was not available. Such high definition data on declared use at country level are scarce. A recent study gathered the AMU from 42 countries to provide a worldwide picture (Mulchandani et al. 2023) and reported that data source frequently provided pooled estimates by World Organisation for Animal Health (WOAH) region or data pooled for all animal production (attribution to chicken production is frequently lacking). Nevertheless, Asian meat production sector received the majority of antimicrobials in 2020 (58,377 tonnes), for which China accounted for 32,776 tonnes (Mulchandani et al. 2023). The yearly AMU in Thailand of 2,567 tonnes total was comparable to Vietnam (2,751 tonnes total), but the proportion attributed to chicken production was higher in Thailand (711 tonnes vs 185 tonnes in Vietnam) and no data from other neighbouring countries was available.

Withdrawal periods are determined from sequential meat residue measurements from healthy birds treated with globally accepted licensed dosage regimen and characterised when tissue concentrations fall below MRL (Landoni and Albarellos 2015). Of the drugs detected in this study, the sulfonamides have the shortest withdrawal periods in edible tissue (5 days; sulfaclozine, sulfadimethoxine). For tetracyclines, the withdrawal periods of doxycycline hyclate in poultry meat varies with dose (licensed 20–25 mg/kg/day, in drinking water) between 5 and 9 days. Oxytetracycline dihydrate and hydrochloride formulations (20–50 mg/kg/day in feed or water) have a withdrawal period of 7–8 days. For enrofloxacin (licensed as 10 mg/kg/day for 3–5 consecutive days in drinking water), the withdrawal period in chicken meat is 7 days. Finally, tilmicosin (licensed at a dose of 15–20 mg/kg/day for 3 days in drinking water) has the longest withdrawal period for broiler meat of 12 days. There is no clear association between the withdrawal periods of the detected drugs (5–12 days) and the prevalence with which they are found within this small pilot study. A complete review of the literature on antimicrobial residues in poultry meat is available elsewhere (Patel et al. 2018). It is important to flag that some diseases can result in a slower elimination rate of medicinal products, especially in the case of liver and kidney impairment as they aid drug elimination (Landoni and Albarellos 2015). Other hypotheses for positive residue results include the use of AMDs at higher doses, accidental contamination of feed, drinking water or the environment, but recent treatment leading to an inadequate withdrawal period remains the most likely cause. Traders at markets may also give AMDs to chickens on route or upon arrival.

Only 1 farm declared AMD use during the production cycle, the others claimed no usage. This is relatively low compared to other studies on AMU in Vietnam [59.1% (Carrique-Mas et al. 2015); 87.9% (Luu et al. 2021)]. From the data, this low level of declared use did not match with the high occurrence of AMD residues seen from the analysis of meat sampled from farms, with all samples taken at the end of the production cycle. This could be due to several factors: knowingly not reporting AMD use, not recalling AMD administration, or unknowingly administering AMDs for example through the presence of AMD in animal feed. This highlights a limitation of questionnaire-based studies and emphasises the importance of direct AMD analysis to compliment qualitative research. This misalignment strongly supports the further application of methods for AMD detection such as analysis of feather samples to complement results from meat AMD residue analysis (Jansen et al. 2017).

Bacteria can acquire AMR to antibiotics through 4 different mechanisms: limiting uptake of a drug, modifying a drug target, inactivating a drug, or active drug efflux (Reygaert 2018). The mechanism of action can be determined by the class of AMD and the type of bacteria. The AMDs detected most frequently in this study, sulphonamides and tetracyclines, typically inhibit metabolic pathways and protein synthesis respectively. Other commonly reported antibiotics used in Vietnam are polypeptides (colistin), aminoglycosides, phenicols, lincosamides, and pleuromutilins (Carrique-Mas et al. 2015), and although not detected in meat residues in this pilot study they have previously been shown to contribute to the occurrence of resistance genes in the enteric microbiome of poultry (Davis et al. 2018; Ahmed et al. 2020; Laconi et al. 2022). The class of AMD strongly influences the emergence of AMR and the mechanism of action by which the bacteria become resistant. Antimicrobial residues pose a great threat to human and animal health

which has led to a global call for action by leaders in governments and science. By understanding the current farming practices and levels of drug residues in meat, improved policies, and motivations to reduce the use of antimicrobials in agriculture can be formed.

5 Conclusion

The methods applied in this pilot study have allowed for the validation and capacity to detect AMD residues from chicken meat samples in Vietnam. A total 65 analytes were validated in a single analytical screening method. The results from the sample analysis suggest a disparity between farmer reports on AMD use and actual AMD residue detection in meat sampled from chicken farms in Vietnam, and that analysing meat from a single chicken per flock may be representative of a flock. The work also indicates that, although a reduction of overall prevalence of positive samples is observed between farm and market, some AMD residues persist in retail meat samples and enter the human food chain.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Ahmed S, Das T, Islam MZ, Herrero-Fresno A, Biswas PK, Olsen JE (2020) High prevalence of mcr-1-encoded colistin resistance in commensal *Escherichia coli* from broiler chicken in Bangladesh. Sci Rep 10(1):18637. https://doi.org/10.1038/s41598-020-75608-2

Carrique-Mas JJ, Trung NV, Hoa NT, Mai HH, Thanh TH, Campbell JI, Wagenaar JA, Hardon A, Hieu TQ, Schultsz C (2015) Antimicrobial usage in chicken production in the Mekong Delta of Vietnam. Zoonoses Public Health 62 (Suppl 1):70–78. https://doi.org/10.1111/zph.12165

Carrique-Mas JJ, Choisy M, Van Cuong N, Thwaites G, Baker S (2020) An estimation of total antimicrobial usage in humans and animals in Vietnam. Antimicrob Resist Infect Control 9(1):16. https://doi.org/10.1186/s13756-019-0671-7

Chattopadhyay MK (2014) Use of antibiotics as feed additives: a burning question. Front Microbiol 5:334. https://doi.org/10.3389/fmicb.2014.00334

Collignon PC, Conly JM, Andremont A, McEwen SA, Aidara-Kane A, For the World Health Organization Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR) (2016) World Health Organization Ranking of Antimicrobials According to Their Importance in Human Medicine: A Critical Step for Developing Risk Management Strategies to Control Antimicrobial Resistance From Food Animal Production. Clin Infect Dis 63(8):1087–1093. https://doi.org/10.1093/cid/ciw475

Commission Regulation (EU) (2009) Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:015:0001:0072:en:PDF. Accessed 27 Sept 2023

Community Reference Laboratories Residues (2010) Guidelines for the validation of screening methods (initial validation and transfer), COMMUNITY REFERENCE LABORATORIES RESIDUES. https://food.ec.europa.eu/system/files/2016-10/cs_vet-med-residues_guideline_validation_screening_en.pdf. Accessed 27 Sept 2023

Council of the European Union (1996) Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A31996L0023. Accessed 27 Sept 2023

Cuong NV, Nhung NT, Nghia NH, Hoa NTM, Trung NV, Thwaites G, Carrique-Mas J (2016) Antimicrobial consumption in medicated feeds in Vietnamese Pig and Poultry production. EcoHealth 13(3):490–498. https://doi.org/10.1007/s10393-016-1130-z

Davis GS, Waits K, Nordstrom L, Grande H, Weaver B, Papp K, Horwinski J, Koch B, Hungate BA, Liu CM, Price LB (2018) Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. BMC Microbiol 18(1):174. https://doi.org/10.1186/s12866-018-1322-5

Desvaux S, Ton VD, Thang PD, Hoa PTT (2008) A general review and a description of the poultry production in Vietnam. https://scholar.google.com.vn/scholar?oi=bibs&cluster=4718040865508406419&btnI=1&hl=en. Accessed 27 Sept 2023

Donoghue DJ (2003) Antibiotic residues in poultry tissues and eggs: human health concerns? Poultry Sci 82(4):618–621. https://doi.org/10.1093/ps/82.4.618

Dubreil E, Gautier S, Fourmond MP, Bessiral M, Gaugain M, Verdon E, Pessel D (2017) Validation approach for a fast and simple targeted screening method for 75 antibiotics in meat and aquaculture products using LC-MS/MS. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 34(4):453–468. https://doi.org/10.1080/19440049.2016.1230278

Food and Agriculture Organization (2021). Maximal residue limits (MRLs) and risk management recommendations (RMRs) for residues of veterinary drugs in foods (CX/MRL 2–2021). https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXM%2B2%252FMRL2e.pdf. Accessed 27 Sept 2023

French General Directorate for Food (2019) Instruction technique DGAL/SDSPA/2019-49 du 18/01/2019. https://info.agriculture.gouv.fr/gedei/site/bo-agri/instruction-2019-2049. Accessed 27 Sept 2023

Huong-Anh NT, Chinh DV, Tuyet-Hanh TT (2020) Antibiotic residues in Chickens and Farmers' knowledge of their use in Tay Ninh Province, Vietnam, in 2017. Asia Pac J Public Health 32(2–3):126–132. https://doi.org/10.1177/1010539520909942

Jansen LJM, Bolck YJC, Rademaker J, Zuidema T, Berendsen BJA (2017) The analysis of tetracyclines, quinolones, macrolides, lincosamides, pleuromutilins, and sulfonamides in chicken feathers using UHPLC-MS/MS in order to monitor antibiotic use in the poultry sector. Anal Bioanal Chem 409(21):4927–4941. https://doi.org/10.1007/s00216-017-0445-0

Kim DP, Saegerman C, Douny C, Dinh TV, Xuan BH, Vu BD, Hong NP, Scippo ML (2013) First survey on the use of antibiotics in pig and poultry production in the Red River Delta Region of Vietnam. Food Public Health 3(5):9. https://doi.org/10.5923/j.fph.20130305.03

Laconi A, Tolosi R, Mughini-Gras L, Cuccato M, Cannizzo FT, Piccirillo A (2022) Amoxicillin and thiamphenicol treatments may influence the co-selection of resistance genes in the chicken gut microbiota. Sci Rep 12(1):20413. https://doi.org/10.1038/s41598-022-24927-7

Landoni MF, Albarellos G (2015) The use of antimicrobial agents in broiler chickens. Vet J 205(1):21–27. https://doi.org/10.1016/j.tvjl.2015.04.016

Lees P, Pelligand L, Giraud E, Toutain PL (2021) A history of antimicrobial drugs in animals: evolution and revolution. J Vet Pharmacol Ther 44(2):137–171. https://doi.org/10.1111/jvp.12895

Luu QH, Nguyen TLH, Pham TN, Vo NGV, Padungtod P (2021) Antimicrobial use in household, semi-industrialized, and industrialized pig and poultry farms in Viet Nam. Prev Vet Med 189:105292. https://doi.org/10.1016/j.prevetmed.2021.105292

Ministry of Agriculture and Rural Development in Vietnam (2016) Circular No. 06/2016/TT-BNNPTNT dated May 31, 2016 of the Ministry of Agriculture and Rural development on the Lists of permissible antibiotics as growth stimulants in livestock and poultry feeds in Vietnam and contents thereof. Hanoi, Vietnam. https://english.luatvietnam.vn/circular-no-06-2016-tt-bnnptnt-dated-may-2031-2016-of-the-ministry-of-agriculture-and-rural-development-on-the-lists-of-permissible-antibiotics-as-gro-105650-doc105651.html. Accessed 27 Sept 2023

Ministry of Health in Vietnam (2013) Circular No. 24/2013/TT-BYT dated August 14, 2013 of the Ministry of Health issuing the Regulation on the maximum limits for veterinary medicine residue in foods. https://english.luatvietnam.vn/circular-no-24-2013-tt-byt-dated-august-2014-2013-of-the-ministry-of-health-issuing-the-regulation-on-the-maximum-limits-for-veterinary-medicine-resid-80644-doc80641.html. Accessed 27 Sept 2023

Mulchandani R, Wang Y, Gilbert M, Van Boeckel TP (2023) Global trends in antimicrobial use in food-producing animals: 2020 to 2030. PLOS Glob Public Health 3(2):e0001305. https://doi.org/10.1371/journal.pgph.0001305

Nhung NT, Cuong NV, Thwaites G, Carrique-Mas J (2016) Antimicrobial usage and antimicrobial resistance in animal production in Southeast Asia: a review. Antibiotics (basel). https://doi.org/10.3390/antibiotics5040037

Page SW, Gautier P (2012) Use of antimicrobial agents in livestock. Revue Sci Tech 31(1):145–188. https://doi.org/10.20506/rst.31.1.2106

Patel T, Marmulak T, Gehring R, Pitesky M, Clapham MO, Tell LA (2018) Drug residues in poultry meat: a literature review of commonly used veterinary antibacterials and anthelmintics used in poultry. J Vet Pharmacol Ther 41(6):761–789. https://doi.org/10.1111/jvp.12700

Plummer M (2003) JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. Proceedings of the 3rd international workshop on distributed statistical computing, Vienna, Austria. http://www.r-project.org/conferences/DSC-2003/. Accessed 27 Sept 2023

R Core Team (2009) A language and environment for statistical computing. https://www.R-project.org/. Accessed 27 Sept 2023

Reygaert WC (2018) An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol 4(3):482–501. https://doi.org/10.3934/microbiol.2018.3.482

Roth N, Kasbohrer A, Mayrhofer S, Zitz U, Hofacre C, Domig KJ (2019) The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: a global overview. Poult Sci 98(4):1791–1804. https://doi.org/10.3382/ps/pey539

Su YS, Yajima M (2021) R2jags: using R to Run 'JAGS'. R package version 0.6-1; 2020. https://cran.r-project.org/package=R2jags. Accessed 27 Sept 2023

World Health Organization (2015) Global action plan on antimicrobial resistance. from https://apps.who.int/iris/handle/10665/193736. Accessed 27 Sept 2023

World Organisation for Animal Health (2016) The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials. https://www.woah.org/fileadmin/Home/eng/Media_Center/docs/pdf/PortailAMR/EN_OIE-AMRstrategy.pdf. Accessed 27 Sept 2023

Yamaguchi T, Okihashi M, Harada K, Konishi Y, Uchida K, Do MHN, Bui HDT, Nguyen TD, Nguyen PD, Chau VV, Dao KT, Nguyen HT, Kajimura K, Kumeda Y, Bui CT, Vien MQ, Le NH, Hirata K, Yamamoto Y (2015) Antibiotic residue monitoring results for pork, chicken, and beef samples in Vietnam in 2012–2013. J Agric Food Chem 63(21):5141–5145. https://doi.org/10.1021/jf505254y