Antimicrobial residues and resistance against critically important antimicrobials in non-typhoidal *Salmonella* from meat sold at wet markets and supermarkets in Vietnam

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ABSTRACT

Excessive antimicrobial usage and deficiencies in hygiene in meat production systems may result in undesirable human health hazards, such as the presence of antimicrobial drug residues and non-typhoidal *Salmonella* (NTS), including antimicrobial resistant (AMR) NTS. Recently, Vietnam has witnessed the emergence of integrated intensive animal production systems, coexisting with more traditional, locally-sourced wet markets. To date no systematic studies have been carried out to compare health hazards in beef, pork and chicken in different production systems. We aimed to: (1) estimate the prevalence of antimicrobial residues in beef, pork and chicken meat; (2) investigate the prevalence and levels of NTS contamination; and (3) investigate serovar distribution and AMR against critically important antimicrobials by animal species and type of retail (wet market vs. supermarket) in Vietnam. Fresh pork, beef and chicken meat samples ($N = 357$) sourced from wet markets and supermarkets in Ho Chi Minh City (HCMC), Hanoi and Dong Thap were screened for antimicrobial residues by PremiTest, and were further investigated by Charm II. Samples from HCMC ($N = 113$) were cultured using ISO 6579:2002/Amd 1:2007. NTS bacteria were quantified using a minimum probable number (MPN) technique. NTS isolates were assigned to serovar by Multilocus Sequence Typing (MLST), and were investigated for their phenotypic susceptibility against 32 antimicrobials. A total of 26 (7.3%) samples tested positive by PremiTest (9.5% beef, 4.1% pork and 8.4% chicken meat). Sulfonamides, tetracyclines and macrolides were detected by Charm in 3.1%, 2.8% and 2.0% samples, respectively. Overall, meat samples from wet markets had a higher prevalence of residues than those from supermarkets (9.6% vs. 2.6%) ($p = 0.016$). NTS were isolated from 68.4% samples from HCMC. Chicken samples from wet markets had by far the highest NTS counts (median 3.2 log MPN/g). NTS isolates displayed high levels of resistance against quinolones (52.2%) and β-lactams (49.6%), but low levels against 3rd generation cephalosporins (4.4%) and aminoglycosides (0.8%). The highest adjusted prevalence of multidrug resistance (MDR) corresponded to isolates from chicken meat and pork (OR 8.3 and 1.8, respectively) (baseline = beef). *S.* Kentucky was the most common serovar identified (11 from chicken, 1 from beef) and 91.7% isolates was MDR. 11/12 isolates corresponded to ST198, a worldwide-disseminated multi-resistant NTS clone. We recommend stepping up policy measures to promote responsible antimicrobial use in animal production, as well as awareness about withdrawal periods to limit the hazard of residues in animal products, and improving slaughtering/hygiene procedures to limit cross-contamination with NTS, particularly in poultry wet markets.

1. Introduction

In Vietnam, like in other low- and middle-income countries, large amounts of antimicrobials are used in animal farming to treat and prevent animal infections, as well as to increase productivity (Carrique-Mas et al., 2014; Van Boeckel et al., 2015; Van Cuong et al., 2016). The

inappropriate use of antimicrobials in farming may result not only in the generation of antimicrobial resistance (AMR) on farms, but also in the transmission of resistant bacteria (including zoonotic pathogens) and genes, and the presence of antimicrobial drug residues in meat and eggs. Antimicrobial residues in animal food products are of concern, since they may cause several adverse health effects to consumers, such as allergic/toxic reactions, disruption of intestinal flora, chronic toxic effects occurring with prolonged exposure to low levels of residues and the potential emergence of resistance in host flora or subsequent spread (Doyle, 2006; Lees and Toutain, 2012).

A number of studies have investigated antimicrobial residues in Vietnamese meat products. A study of 290 pork samples from Hanoi reported 5.5% positivity for tetracycline residues (Van Nhiem et al., 2006). A study (2009–2010) screened 180 chicken and pork samples from several provinces in the Red River using a bacteriological method, and found a positive residue prevalence of 13% and 39% for chicken and pork samples, respectively. A high fraction (27%) of positive samples contained tetracyclines and/or fluoroquinolones (Dang et al., 2013a). A subsequent study (2012 – 2013) investigated 395 chicken, pork, and beef samples in HCMC and Nha Trang (central Vietnam) for residues of 21 antimicrobials. The percentage of positive samples with detectable residues of at least one antimicrobial were 17.3%, 8.8%, and 7.4% for chicken, pork, and beef samples, respectively. Sulfaclozine and fluoroquinolones were mainly detected in chicken meat, and sulfamethazine was mainly detected in pork. Tilmicosin residues were detected in chicken samples (Yamaguchi et al., 2015). A further study (2014–2015) investigated 100 pork samples from HCMC, and found that 18% tested positive for residues using Premitest, but after further testing by LC-MS, an additional 12% positive samples were identified (Do et al., 2016).

The presence of food pathogens such as non-typhoidal *Salmonella* (NTS) in meat is also a concern, since this organism is a major cause of food-borne gastroenteritis worldwide (Majowicz et al., 2010). There is anecdotal evidence of an increase in cases of food-borne outbreaks in Vietnam, and a recent case-control study demonstrated that NTS was responsible for a large outbreak of gastroenteritis in adults (Vo et al., 2014). Hospital data suggests that NTS are also increasingly involved in invasive infection in Vietnam (Nga et al., 2012).

Several studies have investigated NTS in meat samples in Vietnam (Phan et al., 2005; Ta et al., 2014, 2012; Thai et al., 2012b; Thai and Yamaguchi, 2012; Van et al., 2007), with a median per sample prevalence of 43.4% (range 35.5–61.0%). The probability of developing disease after ingestion of NTS-contaminated products is thought to be dose-dependent (Malorny et al., 2008). Therefore enumeration of NTS in food samples is important to conduct a microbial risk assessment to predict the risk associated with specific food products. It is not known whether certain meat types have higher numbers of NTS.

Most Vietnamese people procure their fresh produce from traditional 'wet' markets, which are a feature of most southeast Asian countries. Wet markets typically sell fresh meat and other animal (and vegetable) food products. While some wet markets are informal and provisional (i.e. on a side road), others are well organised and are located a dedicated area of the village/town. Meat cuts/carcasses are typically displayed on tables/stalls at ambient temperature, in contrast with meat for sale in supermarkets, which is always kept refrigerated. Vietnam is currently experiencing rapid intensification of its animal production systems in parallel with increased living standards (FAO, 2004), with a concomitant increase of the role of supermarkets in the supply chain of meat and other food commodities (USDA, 2017). It is not clear whether meat purchased in supermarkets, which is typically sourced from large-scale, integrated companies, represents a different hazard of residues/NTS compared with meat purchased from tradiitional wet markets. A study from Hanoi reported a four-fold higher prevalence of tetracycline residues in pork samples from suburban markets, compared with 'urban shops' (Van Nhiem et al., 2006).

The aims of this study are: (1) to estimate the prevalence of

antimicrobial residues in meat; (2) to investigate the prevalence of NTS and antimicrobial resistance NTS, with a focus on antimicrobials of critical importance as defined by WHO; and (3) to quantify the levels of NTS in pork, beef and chicken meat sold in representative markets in Vietnam. The sampling frame represented the two major urban centers in Vietnam (HCMC, Hanoi), as well as the Mekong Delta province of Dong Thap.

2. Methods

2.1. Sample collection

Portions (100–150 g) of pork, beef and chicken meat were purchased from different wet markets and supermarkets in Hanoi, HCMC and Dong Thap province from October 2016 to March 2017. The study aimed at 360 samples, consisting of 20 samples of each of the following combinations: (1) Province (HCMC, Hanoi, Dong Thap); (2) Meat type (beef, pork, chicken); and (3) Type of retail (supermarket, wet market). Since representative sampling is not possible, sampling was designed to maximize diversity of meat product sources. From each location, samples of the same type were collected from as many different wet markets as possible. Samples from supermarkets always represented different source companies.

2.2. Screening of antimicrobial residues using a bacteriological inhibition test

Meat samples were first screened using a microbial inhibition test (PremiTest, R-Biopharm AG, Germany). This test is based on the growth inhibition of *Bacillus stearothermophilus*, a sensitive strain to most antimicrobial agents (Gaudin et al., 2008), which is evidenced by a change in color compared with a negative control. Meat juice (100 μL) was extracted from each sample (2 g) using a meat press and introduced into *B. stearothermophilus*- containing ampoules. After pre-diffusion at room temperature for 20 min, the meat juice was removed by washing the ampoules twice with distilled water, and incubated at 64 °C until the negative control changed color from purple to yellow (approximately 3 h).

2.3. Detection of antimicrobial residues by radio-immuno assay

All samples showing positive results to the PremiTest were further investigated for residues of compounds belonging to macrolides, tetracyclines, amphenicols, β-lactams and sulfonamides antimicrobial classes, as well as for the presence of chloramphenicol, streptomycin and gentamicin/neomycin using a Charm II 7600 analyzer (Charm Sciences, USA). The test works on meat juice extracted from 10 g meat portions following the manufacturer's directions. The supernatant obtained from the extraction process was incubated alongside a binding agent and a tracer (a ^{3}H- or ^{14}C-labeled antimicrobial). Any antimicrobial residues present in the sample compete with the tracer for the receptors on the binding agent. A scintillation counter was used to measure the amount of tracer on the binding agent, and was compared with control point previously determined following the manufacturer's instruction (Kwon et al., 2011).

2.4. Isolation of NTS and characterization by MLST

On the day of collection, meat portions (25 g) from HCMC markets were investigated for the presence of NTS following a modification of ISO 6579:2002/Amendment 1: 2007. The steps were: (1) pre-enrichment in 225 mL BPW at 37 °C for 18 h; (2) plating on modified semi-solid Rappaport–Vassiliadis (MSRV, Oxoid, UK) at 42 °C for 24 h; and (3) plating on Rambach agar (CHROMagar, France) at 37 °C for 24 h as described elsewhere (Carrique-Mas et al., 2009). Isolates (up to 3 per sample) were further tested by Polyvalent Salmonella O (PSO) and

Polyvalent Salmonella H (PSH) and classified as either group B, C, D, E or 'other', according to the Kauffmann-White scheme (Grimont and Weill, 2007). All NTS suspected isolates were further confirmed using MALDI-TOF (Bruker, Germany). From each meat sample, one isolate of each of the identified serogroup was investigated by Multilocus Sequence Typing (MLST) to establish its serovar identity (Tu et al., 2015). Briefly, seven MLST loci (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*) were amplified and sequenced using the Big Dye Cycle Sequencing kit (Applied Biosystems, USA) on an ABI 3770 automatic sequence. After generating a sequence type, strain was inferred to serotype according to the data available on the MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/).

2.5. Enumeration of NTS in meat samples

Quantitative NTS cultures were performed on randomly selected samples (2–6 for each combination defined by species/type of retail) from HCMC by the most probable number (MPN) technique (Pavic et al., 2010). Briefly, from each meat portion, a 25 g sample was homogenized in 225 mL of BPW. From each homogenate a total of 10 mL were placed into three empty sterile tubes (10^{-1} dilution) to obtain three replicates per sample. Each of these tubes was serially diluted in BPW down to 10^{-4}. All tubes were incubated at 37 °C for 24 h before plating of 50 μL from each tube onto one third of MSRV plate. All MSRV plates were incubated for 24 h and 48 h at 42 °C. A loopful of media from the leading edge of white zone was taken and streaked onto Rambach agar which was incubated at 37 °C for 24 h. The estimated MPN per gram of matrix meat was determined according to a published FDA table (Blodgett, 2006), and then log transformed to approximate normality. An average of three replicates of each sample was used for analysis.

2.6. Antimicrobial susceptibility testing

Colonies selected for MLST characterization were investigated for their susceptibility against a panel of 32 antimicrobials by Vitek (Biomerieux, Marcy l'Etoile, France). Of those, 22 are classified of critical importance for human by WHO. The reference strain *S.* Typhimurium ATCC 14028 was used for quality control purposes. Interpretation of the susceptibility status of tested strains was done based on MIC breakpoint guidelines provided by the Clinical and Laboratory Standards Institute (Document No. M100–S24) (CLSI, 2014). Multidrug resistance (MDR) was defined fully resistant to at least three antimicrobial classes. Potential production of extended-spectrum β-lactamase (ESBL), as indicated by resistance to 3rd generation cephalosporin antimicrobials, was confirmed by the double disk diffusion test (Nhung et al., 2015). The presence of plasmid mediated *mcr*-1 gene among phenotypic colistin-resistant isolates was investigated by PCR as described previously (Nguyen et al., 2016).

2.7. Statistical analyses

The relationship between AMR and serovar, species and type of retail was investigated by building mixed multivariable logistic models. The outcome was 'prevalence of resistance against any antimicrobial belonging to each of the following classes': (1) aminoglycosides, (2) penems, (3) 3rd and 4th generation cephalosporins, (4) penicillins, (5) polymyxins, (6) monobactams, (7) quinolones; (8) nitrofurans, (9) folate pathway inhibitors, (10) tetracyclines, (11) phenicols and (12) MDR. The variables 'type of retail' (wet market/supermarket), 'type of meat' (chicken, pork, beef) were forced as fixed effects, and the serovar identity was modelled as random effects. The outcome of the models was used to investigate the intra-cluster correlation coefficient (ICC) to measure the degree of resistance explained by the random effects variable (serovar identity). All statistical analyses were done using R software (www.r-project.org), and logistic mixed models were built

Table 1
Detection of antimicrobial residues in meat samples from wet markets and supermarkets in Vietnam. The figures indicated the number positive out of the number tested. In brackets the specific residues found in individual samples are presented.

		Chicken	Pork	Beef	Total
Wet markets	Hanoi	0/20	1/20 (T)	0/20	1/60
	HCMC	2/20 (T, M)	0/22	1/22 (S)	3/64
	Dong Thap	6/39 (2TS, 2MS, 2 TMS)	3/38 (2S, 1 T)	10/39 (1MS, 1 T, 8 UNK)	19/116
Total wet markets		8/79	4/80	11/81	23/240
Supermarkets	Hanoi	1/20 (T)	0/21	0/19	1/60
	HCMC	1/19 (TMS)	1/19 (GB)	0/15	2/53
	Dong Thap	0/1	0/2	0/1	0/4
Total supermarket		2/40	1/42	0/35	3/117
Total by species		10/119	5/122	11/116	26/357

using the 'lmer4' package.

3. Results

3.1. Antimicrobial residues

A total of 357 (119 chicken, 122 pork and 116 beef) samples were collected from Hanoi (N = 120, from 18 wet markets and 16 supermarkets), HCMC (N = 117, from 21 wet markets and 16 supermarkets), and Dong Thap (N = 120, from 24 wet markets and 2 supermarkets).

A total of 26/357 samples (7.3%; 95% CI 4.6–10.0%) tested positive by PremiTest. The highest proportion corresponded to beef 11/116 (9.5%; 95% CI 4.2–14.8%), followed by chicken 10/119 (8.4%; 95% CI 3.5–13.7%) and pork 5/122 (4.1%; 95% CI 0.6–7.6%) samples. However results for beef meat were largely driven by Dong Thap wet markets, where 10/39 beef samples (25.6%) tested positive. Overall, meat samples purchased in wet markets had a higher prevalence of residues than those from supermarkets (9.6% vs. 2.6%) (Fisher's p-value = 0.016) (Table 1).

B = β-lactams; G = gentamicin/neomycin; M = macrolides; S = sulfonamides; T = tetracyclines; UNK = unknown.

Sulfonamides, tetracyclines, and macrolides were detected in 11, 10 and 7 PremiTest-positive samples, respectively (3.1%, 2.8% and 2.0% of all samples). A total of 9/26 samples contained more than one antimicrobial. All six positive chicken samples from Dong Thap contained residues of 2–3 antimicrobial classes. In addition, one pork sample contained both gentamicin/neomycin and β-lactams. Most (8/10) beef samples collected from Dong Thap contained antimicrobials that were not identified by Charm II.

3.2. Prevalence of NTS in meat samples

Overall, chicken meat samples had the highest prevalence of NTS (71.8%, 95%CI 57.7–85.9%), and beef samples had the lowest (62.2%, 95%CI 46.5–77.8%) (χ^2 = 0.421; p = 0.516). Chicken samples from wet markets had the overall highest (90.0%), and chicken samples from supermarkets had the lowest prevalence (52.6%) (Fisher's exact test p = 0.013) (Table 2).

MLST was performed on 113 isolates from 80 meat NTS-positive samples. A total of 24 serovars were identified. Two different serovars were identified in 43.5%, 41.4% and 32.1% of positive beef, pork and chicken meat samples, respectively; all remaining samples contained one serovar each. The most common serovars among 37 chicken isolates were Kentucky (29.7% isolates), Corvallis (16.2%); Agona and ST2024 (10.8% each); among 40 pork isolates, Rissen (22.5%), Anatum (17.5%) and London (12.5%); and among 36 beef isolates, Lexington (16.7%), Give (13.9%), Meleagridis and Derby (8.3%) (Table 3). ST198

Table 2
Contamination of meat samples from HCMC with NTS.

	Chicken	Pork	Beef	Total
Wet markets	18/20 (90.0%)	16/22 (72.7%)	12/22 (54.5%)	46/64 (71.9%)
Supermarkets	10/19 (52.6%)	13/19 (68.4%)	11/15 (73.3%)	34/53 (64.1%)
Total	28/39 (71.8%)	29/41 (70.7%)	23/37 (62.2%)	80/117 (68.4%)

Table 3
Serovar identity of the 113 NTS isolates from HCMC meat samples. The numbers within brackets indicate the total number of isolates; figures within square brackets indicate the number of isolates from meat purchased in wet markets, followed by the number of isolates from meat purchased in supermarkets.

	Chicken	Pork	Beef
All isolates	(37) [26|11]	(40) [25|15]	(36) [18|18]
Group B	Agona (4) [3|1]	Derby (4) [3|1]	Derby (3) [1|2]
	ST2040 (4) [4|0]	Typhimurium (4) [3|1]	Agona (1) [1|0]
	Saintpaul (2) [0|2]	Stanley (2) [1|1]	Stanley (1) [1|0]
	Typhimurium (1) [0|1]		
	Derby (1) [1|0]		
Group C	Kentucky (11) [9|2]	Rissen (9) [6|3]	Kottbus (2) [0|2]
	Corvallis (6) [6|0]		Braenderup (1) [0|1]
	Newport (3) [0|3]		Newport (1) [0|1]
	Braenderup (1) [1|0]		Kentucky (1) [0|1]
			Bareilly (1) [1|0]
Group D	Enteritidis (1) [1|0]		ST1547 (1) [1|0]
Group E	Give (1) [1|0]	Anatum (7) [4|3]	Lexington (6) [5|1]
		London (5) [5|0]	Give (5) [2|3]
		Weltevreden (3) [2|1]	Weltevreden (4) [2|2]
		Meleagridis (2) [1|1]	Anatum (2) [1|1]
		Give (2) [0|2]	Meleagridis (3) [2|1]
			London (2) [1|1]
Group G			Kedougou (1) [0|1]
Group H		Bahrenfeld (1) [0|1]	Bahrenfeld (1) [0|1]
other	ST1546 (2) [0|2]	ST1546 (1) [0|1]	

were the most common clone among Kentucky isolates (11/12 isolates).

3.3. Quantification of NTS in meat products

A total of 21 samples from HCMC were investigated by the MPN technique. The counts of NTS bacteria per gram (log MPN/g) are presented in the Fig. 1. The overall median log MPN/g counts were 2.4 (range 0.9–4.9), 2.4 (0.6–2.7), and 1.6 (1.4–2.6) for chicken, pork and beef samples, respectively. Chicken meat sourced from wet markets had the highest NTS counts (median 3.2 log MPN/g), whereas the lowest corresponded to pork purchased from supermarkets (median 0.9 log MPN/g).

There was statistical higher levels of NTS contamination in chicken meat from wet markets (median of 3.2 log MPN/g) than from supermarkets (1.3 log MPN/g) (Wilcoxon, p = 0.049). Similarly, pork samples from wet markets (median of 2.4 log MPN/g) had a borderline statistically higher NTS count than pork from supermarkets (median of 0.9 log MPN/g) (Wilcoxon, p = 0.057). In contrast, beef from supermarkets had higher NTS counts compared to those from wet markets, but the difference was not significant.

3.4. Antimicrobial resistance of NTS isolates

Among antimicrobials of critical importance, the highest overall
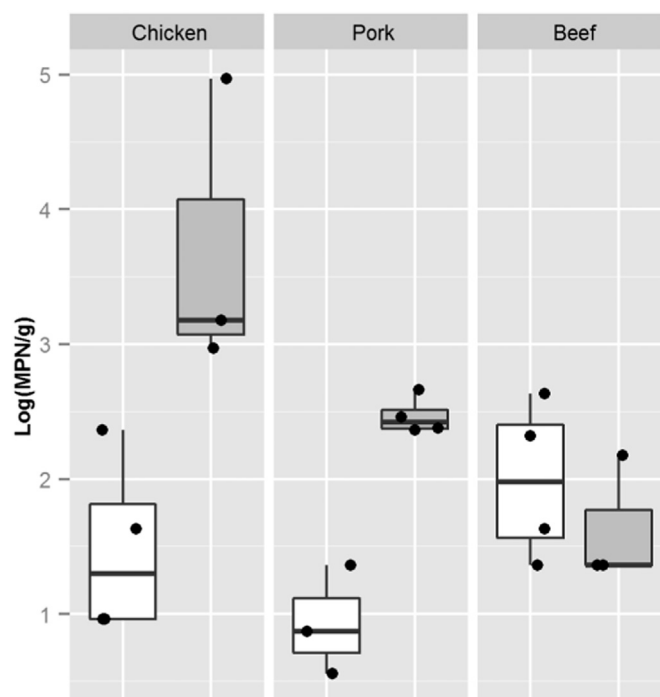
Fig. 1. NTS counts (log MPN/g) in 21 meat samples from markets in HCMC. White and grey bars indicate samples collected from supermarkets and wet markets, respectively.

prevalence of resistance corresponded to quinolones (nalidixic acid, ciprofloxacin, ofloxacin, levofloxacin) (20.3–45.1% by antimicrobial), followed by penicillins (ampicillin, ampicillin/sulbactam, amoxicillin/ clavulanic acid, ticarcillin, piperacillin, piperacillin/tazobactam) (0–49.6%). Levels of resistance against aminoglycosides, 3rd and 4th generation cephalosporins, penems, monobactams, and penicillins were < 4.5% in all cases (Table 4).

For other antimicrobial classes, the highest resistance corresponded to tetracyclines (66.4–69.0%), followed by phenicols (chloramphenicol) (47.8%) and folate pathway inhibitors (30.1–34.5%). The prevalence of resistance against 1st/2nd generation cephalosporins and nitrofurans were < 10%. There were high levels of intermediate resistance against ciprofloxacin (78.8%), ofloxacin (53.1%), levofloxacin (38.9%) and ampicillin-sulbactam (40.7%).

The overall prevalence of MDR among NTS isolates was 52.2% (95%CI 43.0–61.4%) with the highest level corresponding to isolates from chicken meat (67.6%, 95%CI 52.5–82.6%), followed by pork (55.0%, 95%CI 35.8–74.2%) and beef (33.3%, 95%CI 17.9–48.7%). Of the nine serovars of which there were > 5 isolates, MDR was most common in *S.* Kentucky (91.7% isolates), followed by Derby (87.5%), London (71.4%), and lowest among Weltevreden and Lexington isolates (0% in both) (Table 5). *S.* Kentucky isolates were resistant to a median of 12 antimicrobials. A *S.* London isolate from pork, which was resistant against colistin (MIC = 8 µg/mL) was confirmed to harbour the *mcr*-1 gene by PCR.

All five isolates (2 *S.* Kentucky, 2 *S.* Newport, 1 *S.* Agona) that were resistant against 3rd cephalosporins came from chicken meat, and were all negative to ESBL by the double disk diffusion test. These strains were resistant to almost tested antimicrobials, except penems, aminoglycosides and colistin. Among them, one strain (*S.* Newport) was fully resistant to cefepime (a 4th generation cephalosporin) with an MIC ≥ 64 µg/mL, and another *S.* Newport strain was intermediate resistant to this antimicrobial (MIC 4 µg/mL). The AMR patterns (ignoring intermediate resistance) by serovar are presented in Table S1.

Table 4
Phenotypic antimicrobial susceptibility results for 113 NTS isolates from meat samples purchased in HCMC. The figures presented in columns 2–5 correspond to the number of intermediate resistant strains, followed by the number of resistant strains.

Class and antimicrobial	All species (n = 113)	Chicken (n = 37)	Pork (n = 40)	Beef (n = 36)	Total intermediate resistant (%)	Total fully resistant (%)	MIC breakpoints (µg/mL)	
							Sensitive	Resistant
Critically important antimicrobials								
Aminoglycosides								
Gentamicin	0|0	0|0	0|0	0|0	0 (0%)	0 (0%)	≤ 4	≥ 16
Amikacin	0|0	0|0	0|0	0|0	0(0%)	0 (0%)	≤ 16	≥ 64
Tobramycin	6|1	4|1	1|0	1|0	6 (5.3%)	1 (0.9%)	≤ 4	≥ 16
3rd and 4th generation cephalosporins								
Cefotaxime	0|5	0|5	0|0	0|0	0 (0%)	5 (4.4%)	≤ 1	≥ 4
Ceftazidime	0|5	0|5	0|0	0|0	0 (0%)	5 (4.4%)	≤ 4	≥ 16
Cefixime	0|5	0|5	0|0	0|0	0 (0%)	5 (4.4%)	≤ 1	≥ 4
Ceftriaxone	0|5	0|5	0|0	0|0	0 (0%)	5 (4.4%)	≤ 1	≥ 4
Cefepime	1|1	1|1	0|0	0|0	1 (0.9%)	1 (0.9%)	≤ 2	≥ 16
Penems								
Ertapenem	0|0	0|0	0|0	0|0	0 (0%)	0 (0%)	≤ 0.5	≥ 2
Imipenem	0|0	0|0	0|0	0|0	0 (0%)	0 (0%)	≤ 1	≥ 4
Meropenem	0|0	0|0	0|0	0|0	0 (0%)	0 (0%)	≤ 1	≥ 4
Monobactams								
Aztreonam	0|5	0|5	0|0	0|0	0 (0%)	5(4.4%)	≤ 4	≥ 16
Penicillins								
Ampicillin	56|0	0|22	0|23	0|11	0 (0%)	56 (49.6%)	≤ 8	≥ 32
Ampicillin/sulbactam	46|9	14|8	22|0	10|1	46 (40.7%)	9 (8.0%)	≤ 8/4	≥ 32/16
Amoxicillin/clavulanic acid	5|3	4|3	0|0	1|0	5(4.4%)	3 (2.6%)	≤ 8/4	≥ 32/16
Ticarcillin	155	1|21	0|23	0|11	1(0.9%)	55 (48.7%)	≤ 16	≥ 128
Piperacillin	0|56	0|22	0|23	0|11	0 (0%)	56 (49.6%)	≤ 16	≥ 128
Piperacillin/tazobactam	6|0	5|0	0|0	1|0	6 (5.3%)	0 (0%)	≤ 16/4	≥ 128/4
Polymyxins								
Colistin	0|1	0|0	0|1	0|0	0 (0%)	1 (0.9%)	≤ 2	> 2
Quinolones								
Nalidixic acid	0|28	0|20	0|2	0|6	0 (0%)	28 (24.8%)	≤ 16	≥ 32
Ciprofloxacin	89|24	19|18	38|2	32|4	89 (78.8%)	24 (21.2%)	≤ 0.06	≥ 1
Ofloxacin	60|51	10|26	26|14	24|11	60 (53.1%)	51 (45.1%)	≤ 0.12	≥ 2
Levofloxacin	44|23	18|17	12|3	14|3	44 (38.9%)	23 (20.3%)	≤ 2	≥ 8
Other antimicrobial classes								
1st generation cephalosporins								
Cefalotin	0|10	0|9	0|0	0|1	0 (0%)	10 (8.8%)	≤ 8	≥ 32
2nd generation cephalosporins								
Cefoxitin	0|5	0|5	0|0	0|0	0 (0%)	5 (4.4%)	≤ 8	≥ 32
Cefuroxime	15|6	3|6	9|0	3|0	15 (13.3%)	6 (5.3%)	≤ 8	≥ 32
Nitrofurans								
Nitrofurantoin	8|0	7|0	1|0	0|0	8 (7.1%)	0 (0%)	≤ 32	≥ 128
Folate pathway inhibitors								
Trimethoprim	0|34	0|10	0|18	0|6	0 (0%)	34 (30.1%)	≤ 8	≥ 16
Co-trimoxazole	0|39	0|11	0|20	0|8	0 (0%)	39 (34.5%)	≤ 2/38	≥ 4/76
Tetracyclines								
Tetracycline	0|75	0|35	0|28	0|12	0 (0%)	75 (66.4%)	≤ 4	≥ 16
Minocycline	5|78	2|34	2|30	1|14	5 (4.4%)	78 (69.0%)	≤ 4	≥ 16
Phenicols								
Chloramphenicol	4|54	3|21	0|21	1|12	4 (3.5%)	54 (47.8%)	≤ 8	≥ 32

3.5. Association between AMR, serovar, host species and place of sampling

The prevalence of resistance against aminoglycosides, penems, 3rd and 4th generation cephalosporins, polymyxins, monobactams, nitrofurans could not be investigated by modelling because of the low levels of resistance. Six models giving adequate fit are presented in Table 6. Compared with beef isolates, isolates from chicken meat had significantly higher levels of quinolones, penicillins and tetracyclines resistance, and MDR (ORs from 8.34 to 65.3). In contrast, the highest prevalence of resistance against folate pathway inhibitors corresponded to pork isolates (OR = 3.6) (baseline = beef). Isolates from chicken meat and pork had higher levels of chloramphenicol resistance (OR = 3.8 and 1.6, respectively) compared to beef but the differences

were not significant. The impact of sampling locations (wet market vs. supermarket) on AMR was not significant.

ICC values were high to very high in most models (0.28–0.84) in most of models indicating that NTS serovar identity was a significant explanatory factor of AMR.

MDR = Multidrug resistance; ICC = Intra-cluster correlation coefficient.

4. Discussion

Using a simple bacteriological inhibition assay (PremiTest), and a sampling strategy designed to maximize diversity of sources, we demonstrated antimicrobial residues in 7.3% samples (8.4%, 4.1% and

Table 5
Phenotypic resistance (number and % of isolates) against antimicrobial classes among serovars.

Serovar	No. isolates	Aminoglycosides	3rd and 4th generation cephalosporins	Penems	Monobactams	Penicillins	Polymyxin	Quinolones	1st generation cephalosporins	2nd generation cephalosporins	Nitrofurans	Folate pathway inhibitors	Tetracyclines	Phenicols	MDR (%)
Kentucky	12	0	2	0	2	9	0	11	7	3	0	2	11	10	11 (91.7%)
Anatum	9	0	0	0	0	1	0	0	0	0	0	6	9	7	5 (55.6%)
Rissen	9	0	0	0	0	9	0	3	0	0	0	5	9	3	5 (55.6%)
Derby	8	0	0	0	0	7	0	7	0	0	0	4	7	7	7 (87.5%)
Give	8	0	0	0	0	4	0	8	0	0	0	4	4	4	4 (50.0%)
London	7	0	0	0	0	5	1	3	0	0	0	5	4	5	5 (71.4%)
Weltevreden	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0%)
Corvallis	6	0	0	0	0	0	0	6	0	0	0	0	6	1	1 (16.7%)
Lexington	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0%)
Agona	5	0	1	0	1	5	0	5	1	1	0	4	5	4	5 (100%)
Meleagridis	5	0	0	0	0	3	0	1	0	0	0	3	5	3	3 (60%)
Typhimurium	5	0	0	0	0	4	0	3	0	0	0	2	5	4	4 (80%)
Newport	4	0	2	0	2	2	0	2	2	2	0	2	2	2	2 (50%)
ST2040	4	0	0	0	0	0	0	1	0	0	0	0	4	0	0 (0%)
ST1546	3	0	0	0	0	2	0	2	0	0	0	0	2	0	2 (66.7%)
Stanley	3	0	0	0	0	1	0	0	0	0	0	1	1	1	1 (33.3%)
Bahrenfeld	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0%)
Braenderup	2	0	0	0	0	0	0	2	0	0	0	0	1	0	0 (0%)
Kottbus	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0%)
Saintpaul	2	1	0	0	0	2	0	2	0	0	0	2	2	2	2 (100%)
Bareilly	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0 (0%)
Enteritidis	1	0	0	0	0	1	0	1	0	0	0	0	1	0	1 (100%)
Kedougou	1	0	0	0	0	1	1	1	0	0	0	0	1	1	1 (100%)
ST1547	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0%)
Total	113	1	5	0	5	56	1	59	10	6	0	40	80	54	59 (52.2%)

Table 6
Mixed multivariable models investigating association between AMR meat type and type of retail.

Outcome	Index	Meat type (baseline = beef)		Type of retail (baseline = supermarket)
		Chicken	Pork	Wet market
MDR (ICC = 0.84)	OR (95%CI)	8.3 (1.4–48.9)	1.8 (0.5–6.7)	0.6 (0.2–1.9)
	p_{value}	0.02	0.38	0.43
Penicillins (ICC = 0.57)	OR (95%CI)	6.4 (0.9–43.3)	1.7 (0.4–7.5)	1.3 (0.4–4.4)
	p_{value}	0.06	0.48	0.69
Quinolones (ICC = 0.28)	OR (95%CI)	32.9 (3.1–351.2)	1.19 (0.2–6.1)	1.1 (0.3–4.1)
	p_{value}	0.004	0.83	0.93
Tetracyclines (ICC = 0.51)	OR (95%CI)	65.3 (4.8–895.4)	3.3 (0.7–17.0)	1.0 (0.2–4.6)
	p_{value}	0.01	0.13	0.99
Folate pathway inhibitors (ICC = 0.81)	OR (95%CI)	2.2 (0.5–9.8)	3.6 (1.1–12.5)	1.2 (0.4–3.2)
	p_{value}	0.29	0.04	0.72
Phenicols (ICC = 0.74)	OR (95%CI)	3.8 (0.7–19.6)	1.6 (0.4–6.1)	1.5 (0.5–4.5)
	p_{value}	0.11	0.48	0.42

9.5% in chicken, pork and beef samples, respectively). These results appear to be generally lower compared with previous studies in the country (Dang et al., 2013a; Do et al., 2016; Van Nhiem et al., 2006; Yamaguchi et al., 2015). Our results however probably under-estimated the true prevalence, since bacteriological inhibition assays are known to have limited sensitivity of detection for certain antimicrobial residues (Chafer-Pericas et al., 2010; Do et al., 2016). The main advantage of such methods is their affordability and ease of use. Although there are number of more sophisticated techniques available (Do et al., 2016; Gaudin et al., 2008), the huge range of antimicrobials used, and the high testing costs is a limiting factor in a developing country setting such as Vietnam. In descending order, we found that sulfonamides, tetracyclines and macrolides were the most commonly detected antimicrobials in meat samples. All these antimicrobial classes are used intensively in pig and poultry production in Vietnam (Carrique-Mas et al., 2014; Dang et al., 2013b; Van Cuong et al., 2016).

Our results indicate higher overall prevalence of antimicrobial residues in meat samples sourced from wet markets (9.6%) compared with supermarkets (2.6%). Positive chicken samples were found in a larger variety of retail type/provinces (4/6), compared with pork (3/6) and beef (2/6). However on sample level, the highest proportion of positive samples corresponded to beef, due to the high prevalence of this type of beef from Dong Thap wet markets (10/39 positive). We found evidence of residues of more than one antimicrobial in 7/10 positive chicken samples, probably reflecting higher usage levels of antimicrobials in this species. High levels of antimicrobial use have been shown in chicken production systems in the Mekong Delta, often consisting of products containing more than one antimicrobial (Carrique-Mas et al., 2014).

The prevalence of NTS contamination of meat samples of a similar scale, if somehow higher than in previous studies (Ta et al., 2014; Thai et al., 2012a, 2012b; Van et al., 2007), and not too dissimilar between species. However this prevalence (62.7–71.8%) is extremely high when compared with 2014 surveillance data from the European Union, with a per 25 g sample prevalence of 2.26%, 0.62% 0.23% for chicken, pork and beef respectively (EFSA, 2014). In our study, chicken and pork sourced from wet markets had considerably higher NTS counts than their equivalent samples from supermarkets. Chicken meat from wet markets had both the highest prevalence of NTS (90%) and the highest bacterial counts (3.2 log MPN/g). A previous study on chicken carcasses collected from retail markets in Vietnam showed that NTS counts ranged from 1.0 to 3.8 log MPN per carcass (Ta et al., 2014). The different sampling methodologies present a challenge to the interpretation and comparability across studies. It could be argued that those results indicate a lower count "per gram of meat" than in our study, since most of the NTS contamination occurs on the surface of the meat. A study on six pork samples from northern Vietnam also indicated a lower number of NTS counts (< 0.18 log MPN/g) compared to 2.4 log MPN/g meat in

our study (Yokozawa et al., 2016). Since most of the meat sold in wet markets is stored at ambient temperatures, and meat sold in supermarkets is normally stored refrigerated (2–8 °C), it is not possible to conclusively determine whether this difference is due to differences in storage conditions or to more hygienic slaughtering and handling conditions of chickens and pigs in the supermarket value chain.

S. Kentucky ST198 was the most common serovar found in chicken meat. This serovar has been identified in poultry products in the USA for decades (Shah et al., 2017). Increasing occurrence of human cases of *S.* Kentucky infections related to consumption of chicken and turkey meat have been reported in Europe and USA (Antunes et al., 2016; CDC, 2012). *S.* Kentucky ST198 has been associated with wide range of livestock reservoirs in Europe, Africa, Middle East and Asia (Le Hello et al., 2013). The spread of *S.* Kentucky ST198 is suspected to be linked to increased globalization of travel and food/animal trade, with poultry being a major suspect (Le Hello et al., 2011). This serovar seems to be associated with high level ciprofloxacin resistance (due to *gyr*A and *par*C mutations) and can carry rare β-lactam genes (Wasyl et al., 2015). In our study, 11/12 (91.7%) of *S.* Kentucky strains were fully resistance against all tested quinolones.

S. Rissen was the predominant serovar in pork (9/40 isolates). This serotype has previously been found in pig production in several regions of Vietnam (Lettini et al., 2016; Thai et al., 2012b). This serotype was among the most common serovars found in human patients with treatment of diarrhea in Thailand (Angkititrakul et al., 2005). Serovars Lexington, Give and Weltevreden were the most frequent isolates identified in beef. This finding is consistent with a previous study in southern of Vietnam, where *S.* Weltevreden and *S.* Lexington were identified as dominant serovars in cattle (Vo et al., 2006).

Five chicken isolates (4.4%) were fully resistant against all tested 3rd generation cephalosporins, one of them being fully resistant against 4th generation cephalosporin. Recently, a *S.* Albany strain isolated from chicken meat in Vietnam was found to be resistant against ceftriaxone (3rd generation cephalosporin) but not cefepime (4th generation cephalosporin) (Ta et al., 2014). A recent study on NTS isolates from pork in China has shown a relatively high prevalence of resistance (15.8%) against 3rd generation cephalosporins (ceftiofur, cefotaxime, ceftriaxone and ceftazidime) (Yang et al., 2017).

We found moderate to high rates of quinolone resistance including levofloxacin (20.3%), ciprofloxacin (21.2%), nalidixic acid (24.8%), and ofloxacin (45.1%). This is a concern since the prevalence of resistance against ciprofloxacin and ofloxacin has ranged from 0% to 5% in previous studies (Ta et al., 2014; Thai et al., 2012a, 2012b; Van et al., 2007). However, high level of ciprofloxacin resistance among NTS isolates from poultry (52.2%) and swine farms (62.7%) have been reported in Central Vietnam (Lettini et al., 2016). Reduction in quinolone susceptibility is reflecting the frequent use of these antimicrobials in animal husbandry (Carrique-Mas et al., 2014; Nhung et al., 2016), that

is therefore considered an enormous challenge in the treatment of *Salmonella* infections in humans and animals (Thai et al., 2012b).

In Vietnam, *mcr*-1 plasmid-mediated colistin resistance has previously been identified in *E. coli* isolated from pig and chickens (Malhotra-Kumar et al., 2016; Nguyen et al., 2016; Trung et al., 2017). This is the first report confirming *mcr*-1 in NTS from meat (pork), although phenotypic colistin resistance has been recently reported among *S.* Enteritidis isolates in poultry farms (Lettini et al., 2016). Although presence in a small proportion of isolates, compared with other countries (i.e. *coli*stin resistance has been reported in 14% in Portugal livestock NTS strains) (Figueiredo et al., 2016) there is a concern of potential dissemination of *mcr*-1, since *mcr*-1 can be transmitted horizontally (Snesrud et al., 2016; Trung et al., 2017), and colistin is often used in animal production in Vietnam (Carrique-Mas et al., 2014; Nguyen et al., 2016; Van Cuong et al., 2016).

In summary, we report considerably high levels of contamination with antimicrobial residues in meat products sold at markets in Vietnam. The presence of residues appears to be more common in meat sourced from wet markets, whereas resistance among NTS was more common in the chicken species, regardless of the type of retail. Unsurprisingly sulfonamides, tetracyclines and macrolides, all antimicrobials extensively used in pig and poultry production, were the most frequently detected residues. We also report high levels of AMR among NTS isolates against quinolones and penicillins, all considered of critical importance for human medicine. To ensure the safety of animal food for human consumption appropriate withdrawal periods should be strictly enforced after the administration of antimicrobials. We recommend a review of slaughtering/hygiene procedures to limit cross-contamination with NTS, with particular attention to poultry wet markets. The study also highlights the diversity of NTS serovars in the animal reservoir, and the need to better characterize clinical infections due to NTS in humans in Vietnam. At present little is known about zoonotic foodborne sources of human enteric infection with NTS in the country, because of limitations in the medical care system and the costs associated with isolate typing. The quantification of the NTS disease burden, including the characterization of serovars in Vietnam patients and animals should provide an impetus for better control of NTS in the animal reservoir.

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References

Angkititrakul, S., Chomvarin, C., Chaita, T., Kanistanon, K., Waethewutajarn, S., 2005. Epidemiology of antimicrobial resistance in salmonella isolated from pork, chicken meat and humans in Thailand. Southeast Asian J. Trop. Med. Public Health 36, 1510–1515.

Antunes, P., Mourao, J., Campos, J., Peixe, L., 2016. Salmonellosis: the role of poultry meat. Clin. Microbiol. Infect. 22, 110–121. http://dx.doi.org/10.1016/j.cmi.2015.12.004.

Blodgett, R., 2006. Bacteriological analytical manual online, appendix 2. In: Most Probable Number Determination from Serial Dilutions.

Carrique-Mas, J.J., Barnes, S., McLaren, I., Davies, R., 2009. Comparison of three plating media for the isolation of Salmonella from poultry environmental samples in Great Britain using ISO 6579:2002 (Annex D). J. Appl. Microbiol. 107, 1976–1983. http://dx.doi.org/10.1111/j.1365-2672.2009.04386.x.

Carrique-Mas, J., Trung, N.V., Hoa, N.T., Mai, H.H., Thanh, T.T., Campbell, J., Wagenaar, J., Hardon, A., Hieu, T.Q., Schultsz, C., 2014. Antimicrobial usage in chicken production in the Mekong delta of Vietnam. Zoonoses Public Health 61 (Suppl. 2), 1–9.

CDC, 2012. National Enteric Disease Surveillance: Salmonella Annual Report. www.cdc.gov/ncezid/dfwed/pdfs/salmonella-annual-report-2012-2508c.pdf (accessed on 13 August 2017).

Chafer-Pericas, C., Maquieira, A., Puchades, R., Miralles, J., Moreno, A., 2010. Fast screening immunoassay of sulfonamides in commercial fish samples. Anal. Bioanal. Chem. 396, 911–921. http://dx.doi.org/10.1007/s00216-009-3229-3.

CLSI, 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement (CLSI/NCCLS M100–S24). Clinical and Laboratory Standards Institute, Wayne, PA.

Dang, P.K., Degand, G., Douny, C., Pierret, G., Delahaut, P., Ton, V.D., Granier, B., Scippo, M.L., 2013a. Preliminary evaluation of antimicrobial residue levels in marketed pork and chicken meat in the Red River Delta Region of Vietnam. Food Public Health 3, 267–276. http://dx.doi.org/10.5923/j.fph.20130306.02.

Dang, P.K., Saegerman, C., Douny, C., Ton, V.D., Bo, H.X., Binh, D.V., Ngan, P.H., Scippo, M.L., 2013b. First survey on the use of antibiotics in pig and poultry production in the Red River Delta Region of Vietnam. Food Public Health 3, 247–256.

Do, M.H., Yamaguchi, T., Okihashi, M., Harada, K., Konishi, Y., Uchida, K., Bui, L.T., Nguyen, T.D., Phan, H.B., Bui, H.D., Nguyen, P.D., Kajimura, K., Kumeda, Y., Dang, C.V., Hirata, K., Yamamoto, Y., 2016. Screening of antibiotic residues in pork meat in Ho Chi Minh City, Vietnam, using a microbiological test kit and liquid chromatography/tandem mass spectrometry. Food Control 69, 262–266.

Doyle, M.E., 2006. Veterinary Drug Residues in Processed Meats — Potential Health Risk. A Review of the Scientific Literature. Food Res. Institute, Univ. Wisconsin–Madison.

EFSA, 2014. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/zoonoses-trends-sources-EU-summary-report-2014.pdf (accessed on 15 August 2017).

FAO, 2004. Review of the livestocks sector in the Mekong countries. http://www.fao.org/ag/againfo/resources/en/publications/sector_reports/lsr_mekong.pdf, Accessed date: 8 January 2017.

Figueiredo, R., Card, R.M., Nunez, J., Pomba, C., Mendonca, N., Anjum, M.F., Da Silva, G.J., 2016. Detection of an mcr-1-encoding plasmid mediating colistin resistance in Salmonella enterica from retail meat in Portugal. J. Antimicrob. Chemother. 71, 2338–2340. http://dx.doi.org/10.1093/jac/dkw240.

Gaudin, V., Juhel-Gaugain, M., Moretain, J.P., Sanders, P., 2008. AFNOR validation of Premi Test, a microbiological-based screening tube-test for the detection of antimicrobial residues in animal muscle tissue. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 25, 1451–1464. http://dx.doi.org/10.1080/02652030802429088.

Grimont, P.A.D., Weill, F.-X., 2007. Antigenic Formulae of the Salmonella Serovars, 9th Edition. WHO Collab. Cent. Ref. Res (Salmonella). http://www.pasteur.fr/sante/clre/cadrecnr/salmoms/WKLM_2007.pdf, Accessed date: 8 March 2017.

Kwon, S.I., Owens, G., Ok, Y.S., Lee, D.B., Jeon, W.T., Kim, J.G., Kim, K.R., 2011. Applicability of the Charm II system for monitoring antibiotic residues in manure-based composts. Waste Manag. 31, 39–44. http://dx.doi.org/10.1016/j.wasman.2010.08.018.

Le Hello, S., Hendriksen, R.S., Doublet, B., Fisher, I., Nielsen, E.M., Whichard, J.M., Bouchrif, B., Fashae, K., Granier, S.A., Jourdan-Da Silva, N., Cloeckaert, A., Threlfall, E.J., Angulo, F.J., Aarestrup, F.M., Wain, J., Weill, F.X., 2011. International spread of an epidemic population of Salmonella enterica serotype Kentucky ST198 resistant to ciprofloxacin. J. Infect. Dis. 204, 675–684. http://dx.doi.org/10.1093/infdis/jir409.

Le Hello, S., Bekhit, A., Granier, S.A., Barua, H., Beutlich, J., Zajac, M., Munch, S., Sintchenko, V., Bouchrif, B., Fashae, K., Pinsard, J.L., Sontag, L., Fabre, L., Garnier, M., Guibert, V., Howard, P., Hendriksen, R.S., Christensen, J.P., Biswas, P.K., Cloeckaert, A., Rabsch, W., Wasyl, D., Doublet, B., Weill, F.X., 2013. The global establishment of a highly-fluoroquinolone resistant salmonella enterica serotype Kentucky ST198 strain. Front. Microbiol. 4 (395). http://dx.doi.org/10.3389/fmicb.2013.00395.

Lees, P., Toutain, P.L., 2012. Pharmacokenitics, Distribution, Bioavailability, and Relationship to Antibiotic Residues. Wiley Series on Mass Spectrometry. Wiley.

Lettini, A.A., Vo Than, T., Marafin, E., Longo, A., Antonello, K., Zavagnin, P., Barco, L., Mancin, M., Cibin, V., Morini, M., Dang Thi Sao, M., Nguyen Thi, T., Pham Trung, H., Le, L., Nguyen Duc, T., Ricci, A., 2016. Distribution of Salmonella serovars and antimicrobial susceptibility from poultry and swine farms in Central Vietnam. Zoonoses Public Health 63, 569–576. http://dx.doi.org/10.1111/zph.12265.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., Hoekstra, R.M., International Collaboration on Enteric Disease' Burden of Illness, S, 2010. The global burden of nontyphoidal salmonella gastroenteritis. Clin. Infect. Dis. 50, 882–889. http://dx.doi.org/10.1086/650733.

Malhotra-Kumar, S., Xavier, B.B., Das, A.J., Lammens, C., Hoang, H.T., Pham, N.T., Goossens, H., 2016. Colistin-resistant Escherichia coli harbouring mcr-1 isolated from food animals in Hanoi, Vietnam. Lancet Infect. Dis. 16, 286–287. http://dx.doi.org/10.1016/S1473-3099(16)00014-1.

Malorny, B., Lofstrom, C., Wagner, M., Kramer, N., Hoorfar, J., 2008. Enumeration of salmonella bacteria in food and feed samples by real-time PCR for quantitative microbial risk assessment. Appl. Environ. Microbiol. 74, 1299–1304. http://dx.doi.org/10.1128/AEM.02489-07.

Nga, T.V., Parry, C.M., Le, T., Lan, N.P., Diep, T.S., Campbell, J.I., Hoang, N.V., Dung le, T., Wain, J., Dolecek, C., Farrar, J.J., Chau, N.V., Hien, T.T., Day, J.N., Baker, S., 2012. The decline of typhoid and the rise of non-typhoid salmonellae and fungal infections in a changing HIV landscape: bloodstream infection trends over 15 years in southern Vietnam. Trans. R. Soc. Trop. Med. Hyg. 106, 26–34. http://dx.doi.org/10.1016/j.trstmh.2011.10.004.

Nguyen, N.T., Nguyen, H.M., Nguyen, C.V., Nguyen, T.V., Nguyen, M.T., Thai, H.Q., Ho,

M.H., Thwaites, G., Ngo, H.T., Baker, S., Carrique-Mas, J., 2016. Use of colistin and other critical antimicrobials on pig and chicken farms in Southern Vietnam and its association with resistance in commensal *Escherichia coli* bacteria. Appl. Environ. Microbiol. 82, 3727–3735. http://dx.doi.org/10.1128/AEM.00337-16.

Nhung, N.T., Cuong, N.V., Campbell, J., Hoa, N.T., Bryant, J.E., Truc, V.N., Kiet, B.T., Jombart, T., Trung, N.V., Hien, V.B., Thwaites, G., Baker, S., Carrique-Mas, J., 2015. High levels of antimicrobial resistance among *Escherichia coli* isolates from livestock farms and synanthropic rats and shrews in the Mekong Delta of Vietnam. Appl. Environ. Microbiol. 81, 812–820. http://dx.doi.org/10.1128/AEM.03366-14.

Nhung, N.T., Cuong, N.V., Thwaites, G., Carrique-Mas, J., 2016. Antimicrobial usage and antimicrobial resistance in animal production in Southeast Asia: a review. Antibiotics (Basel) 5. http://dx.doi.org/10.3390/antibiotics5040037.

Pavic, A., Groves, P.J., Bailey, G., Cox, J.M., 2010. A validated miniaturized MPN method, based on ISO 6579:2002, for the enumeration of Salmonella from poultry matrices. J. Appl. Microbiol. 109, 25–34. http://dx.doi.org/10.1111/j.1365-2672.2009.04649.x.

Phan, T.T., Khai, L.T., Ogasawara, N., Tam, N.T., Okatani, A.T., Akiba, M., Hayashidani, H., 2005. Contamination of salmonella in retail meats and shrimps in the Mekong Delta, Vietnam. J. Food Prot. 68, 1077–1080.

Shah, D.H., Paul, N.C., Sischo, W.C., Crespo, R., Guard, J., 2017. Population dynamics and antimicrobial resistance of the most prevalent poultry-associated Salmonella serotypes. Poult. Sci. 96, 687–702. http://dx.doi.org/10.3382/ps/pew342.

Snesrud, E., He, S., Chandler, M., Dekker, J.P., Hickman, A.B., McGann, P., Dyda, F., 2016. A model for transposition of the colistin resistance gene mcr-1 by ISApl1. Antimicrob. Agents Chemother. 60, 6973–6976. http://dx.doi.org/10.1128/AAC.01457-16.

Ta, Y.T., Nguyen, T.T., To, P.B., Pham da, X., Le, H.T., Alali, W.Q., Walls, I., Lo Fo Wong, D.M., Doyle, M.P., 2012. Prevalence of Salmonella on chicken carcasses from retail markets in Vietnam. J. Food Prot. 75, 1851–1854. http://dx.doi.org/10.4315/0362-028X.JFP-12-130.

Ta, Y.T., Nguyen, T.T., To, P.B., Pham da, X., Le, H.T., Thi, G.N., Alali, W.Q., Walls, I., Doyle, M.P., 2014. Quantification, serovars, and antibiotic resistance of salmonella isolated from retail raw chicken meat in Vietnam. J. Food Prot. 77, 57–66. http://dx.doi.org/10.4315/0362-028X.JFP-13-221.

Thai, T.H., Yamaguchi, R., 2012. Molecular characterization of antibiotic-resistant salmonella isolates from retail meat from markets in northern Vietnam. J. Food Prot. 75, 1709–1714. http://dx.doi.org/10.4315/0362-028X.12-101.

Thai, T.H., Hirai, T., Lan, N.T., Shimada, A., Ngoc, P.T., Yamaguchi, R., 2012a. Antimicrobial resistance of salmonella serovars isolated from beef at retail markets in the north Vietnam. J. Vet. Med. Sci. 74, 1163–1169.

Thai, T.H., Hirai, T., Lan, N.T., Yamaguchi, R., 2012b. Antibiotic resistance profiles of salmonella serovars isolated from retail pork and chicken meat in North Vietnam. Int. J. Food Microbiol. 156, 147–151. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.03.016.

Trung, N.V., Matamoros, S., Carrique-Mas, J.J., Nghia, N.H., Nhung, N.T., Chieu, T.T., Mai, H.H., van Rooijen, W., Campbell, J., Wagenaar, J.A., Hardon, A., Mai, N.T., Hieu, T.Q., Thwaites, G., de Jong, M.D., Schultsz, C., Hoa, N.T., 2017. Zoonotic transmission of mcr-1 Colistin resistance gene from small-scale poultry farms, Vietnam. Emerg. Infect. Dis. 23, 529–532. http://dx.doi.org/10.3201/eid2303.

161553.

Tu, L.P.T., Hoang, N.V., Cuong, N.V., Campbell, J., Bryant, J.E., Hoa, N.T., Kiet, B.T., Thompson, C., Duy, D.T., Phat, V.V., Hien, V.B., Thwaites, G., Baker, S., Carrique-Mas, J., 2015. High levels of contamination and antimicrobial resistant non-typhoidal Salmonella serovars on pig and poultry farms in the Mekong Delta of Vietnam. Epidemiol. Infect. 143, 3074–3086.

USDA, 2017. Vietnam retail foods. Sector report 2016. Global agricultural information network. https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Retail%20Foods_Hanoi_Vietnam_23-27-2017.pdf (accessed on 13 August 2017).

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R., 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. U. S. A. 112, 5649–5654. http://dx.doi.org/10.1073/pnas.1503141112.

Van Cuong, N., Nhung, N.T., Nghia, N.H., Mai Hoa, N.T., Trung, N.V., Thwaites, G., Carrique-Mas, J., 2016. Antimicrobial consumption in medicated feeds in Vietnamese pig and poultry production. EcoHealth 13, 490–498. http://dx.doi.org/10.1007/s10393-016-1130-z.

Van Nhiem, D., Paulsen, P., Witaya Suriyasathaporn, W., Smulders, F., Kyule, M., Baumann, M., Zessin, K., Ngan, P.H., 2006. Preliminary analysis of tetracycline residues in marketed pork in Hanoi, Vietnam. Ann. N. Y. Acad. Sci. 1081, 534–542.

Van, T.T., Moutafis, G., Istivan, T., Tran, L.T., Coloe, P.J., 2007. Detection of Salmonella spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. Appl. Environ. Microbiol. 73, 6885–6890. http://dx.doi.org/10.1128/AEM.00972-07.

Vo, A.T., van Duijkeren, E., Fluit, A.C., Heck, M.E., Verbruggen, A., Maas, H.M., Gaastra, W., 2006. Distribution of *Salmonella enterica* serovars from humans, livestock and meat in Vietnam and the dominance of *Salmonella typhimurium* phage type 90. Vet. Microbiol. 113, 153–158. http://dx.doi.org/10.1016/j.vetmic.2005.10.034.

Vo, T.H., Le, N.H., Cao, T.T., Nuorti, J.P., Minh, N.N., 2014. An outbreak of food-borne salmonellosis linked to a bread takeaway shop in Ben Tre City, Vietnam. Int. J. Infect. Dis. 26, 128–131. http://dx.doi.org/10.1016/j.ijid.2014.05.023.

Wasyl, D., Kern-Zdanowicz, I., Domanska-Blicharz, K., Zajac, M., Hoszowski, A., 2015. High-level fluoroquinolone resistant *Salmonella enterica* serovar Kentucky ST198 epidemic clone with IncA/C conjugative plasmid carrying bla(CTX-M-25) gene. Vet. Microbiol. 175, 85–91. http://dx.doi.org/10.1016/j.vetmic.2014.10.014.

Yamaguchi, T., Okihashi, M., Harada, K., Konishi, Y., Uchida, K., Do, M.H., Bui, H.D., Nguyen, T.D., Nguyen, P.D., Chau, V.V., Dao, K.T., Nguyen, H.T., Kajimura, K., Kumeda, Y., Bui, C.T., Vien, M.Q., Le, N.H., 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, 5141–5145. http://dx.doi.org/10.1021/jf505254y.

Yang, L., Li, W., Jiang, G.Z., Zhang, W.H., Ding, H.Z., Liu, Y.H., Zeng, Z.L., Jiang, H.X., 2017. Characterization of a P1-like bacteriophage carrying CTX-M-27 in salmonella spp. resistant to third generation cephalosporins isolated from pork in China. Sci. Rep. 7, 40710. http://dx.doi.org/10.1038/srep40710.

Yokozawa, T., Sinh, D.X., Hung, N.V., Lapar, L., Makita, K., 2016. Transition of Salmonella prevalence in pork value chain from pig slaughterhouse to markets in Hung Yen, Vietnam. J. Vet. Epidemiol. 20, 51–58.