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Preliminary Evaluation of Antimicrobial Residue Levels in Marketed Pork and Chicken Meat in the Red River Delta Region of Vietnam

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Abstract The presence of antibacterial in 97 pork and 83 chicken meat samples, randomly collected from 3 different representative provinces (Hanoi, Hai Duong and Thai Binh) of the Red River Delta, was determined by a screening step using in parallel 2 microbiological methods (Premi®-test and New Two Plate Test). In total, 27% of all samples displayed a positive response in at least one of both tests, from which 11 (13% of chicken samples) are chicken samples and 38 (39% of pork samples) are pork samples. The 33 samples from the Thai Binh which were screened positive were then submitted to post-screening tests specific for tetracyclines and (fluoro) quinolones (Tetrasensor® dipstick for tetracyclines and an ELISA for quinolones), two groups of antibiotics widely used in animal production in this region, and confirmed by liquid chromatography coupled to mass spectrometry. Tetracyclines and (fluoro)quinolones residues were found, using a post screening test, in 23 and 5 samples, respectively. Ten (all pork) and 4 samples (1 pork, 3 chicken) were confirmed containing tetracyclines (chlortetracycline, oxytetracycline, tetracycline, doxycycline) and (fluoro) quinolones (nalidixic acid, enrofloxacin and ciprofloxacin) respectively, from which 1 and 3 pork samples were found to contain enrofloxacin and tetracycline residues , respectively, with a concentration higher than their respective MRLs. This study shows the good performance of the proposed strategy to identify non-compliant meat samples (microbiological screening, tetracyclines and quinolones targeted post-screening and confirmation), which allows to obtain conclusive results in 82% of the cases.

Keywords Antibiotic Residue, Chicken Meat, Pork Meat, Red River Delta Region, Vietnam

1. Introduction

In Vietnam, chicken and pig, two staples products of livestock production play an important role to satisfy the increasing demand of meat for domestic markets of over 85 million inhabitants. However, the low level of hygiene in livestock, the inadequacy of husbandry zone planning and the lack of state management and development strategies result in some new problems such as environment pollution, as well as frequently occurring and uncontrolled epidemic diseases[1-3]. To overcome some of these, farmers consider antibiotics as one of the solutions to fight against livestock diseases and to improve the animal productivity. Consequently, the use of veterinary drugs and in particular antibiotics in animal production has increased in Vietnam, resulting in the fact that antibiotics are the most common registered drugs (70 percent of all veterinary drugs) used in animals[4].

The results of a survey on 628 pig and poultry farms in Binh Duong province from September 2001 to February 2002 showed that irrational use of antibiotics was recorded in 17.1% of the farms. The most used antibiotics were chloramphenicol (15.4%) and norfloxacin (10%). Furthermore, 40.1% of the farms were found not respecting the withdrawal time[5].

Another survey was performed from July 2009 to March 2010 on 270 animal production entities representing three different systems (farm household, semi-industrial and industrial) in Red River Delta (RRD). At least 48 antibiotics of more than 10 different groups were largely and arbitrary used in all pig and chicken production systems in this region[6]. This use in a unmethodical manner, without any veterinary prescription and supervision lead to the presence

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of residues in animal products. This issue causes bad impacts on public health and bad influences on environment[7] and therapeutic sciences[8-9] and contribute to the presence of antibiotic-resistant human pathogens in the food chain [10-14]. These alerts have caused warnings to authorities and the alarming of consumers.

Since Vietnam joined the World Trade Organization, regulation of antibiotic use in animals has strengthened and certain antibiotics have been banned. Recently, Ministry of Agriculture and Rural Development (MARD) issued the updated list of drugs, antibiotics which are banned or restricted on using in animal[15].

In addition, Vietnam as well as developed countries like EU, USA, has also promulgated a regulation to fix maximum residue limits (MRL) of many antimicrobial residues in animal products, control and monitoring strategy to protect consumer safety[16-21].

However, surveillance for antibiotic residues in meat reveals breaches of regulations regarding the use of antibiotics. In fact, most studies and national surveillance programs have looked at residue in animal products for export. Meanwhile, there is paucity of information on antibiotic residues in meat for domestic market. Therefore, to obtain information for an assessment of meat-borne exposure of consumers to antibiotic residue, a study of the occurrence of the residues of antibiotics widely used in pig and chicken production in RRD is necessary. The aim of this study was to assess antimicrobial residues in pork and chicken meat sold in the markets of the RRD, in Vietnam.

2. Experimental

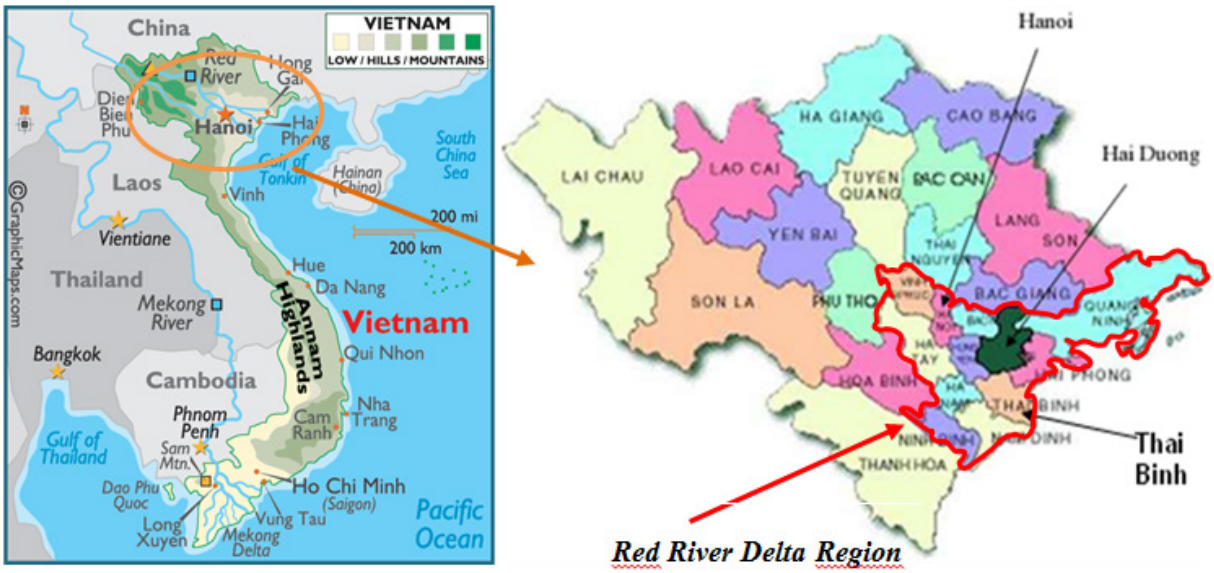
2.1. Sampling Area

With a population of about 19.5 million inhabitants in 15,000 km^2 of superficies, the RRD region was known as an agriculturally rich area and densely populated in the north of Vietnam (1225 persons/km^2, 4.8 times higher than the average population density of Vietnam). It includes the capital, Hanoi, and 10 others surrounding provinces (Fig. 1). The pig and poultry production of this region are the most developed of Vietnam (about 50% of the whole country production) with 7.0 million pigs and 66.5 million poultry in 2008[22].

Three representative provinces were selected: Hanoi (3344 km^2), Hai Duong (1661 km^2) and Thai Binh (1542 km^2). The population density of Hanoi, Hai Duong and Thai Binh are 1943; 1030 and 1155 persons/km^2, respectively. The herd of pig and poultry is the largest in Hanoi (1.2 10^6 pigs and 15.7 10^6 poultry), followed by Hai Duong (0.6 10^6 pigs and 6.9 10^6 poultry) and Thai Binh (1.0 10^6 pig and 7.9 10^6 poultry)[22].

2.2. Sample Collection

A total of 180 meat samples (83 chicken and 97 pork samples) were randomly collected from markets of 3 districts (Dong Anh, Cam Giang and Quynh Phu) in the 3 selected provinces. Chicken and pork meat samples were taken twice a month, during 5 months from July 2009 to March 2010. At each sampling time, 3 pork and 3 chicken meat samples were taken randomly from 3 markets of each district. In the Thai Binh province, the 60 meat samples (23 chicken and 37 pork samples) were collected from July to November 2010 (just after the "blue ear" or Porcine Reproductive and Respiratory disease (PRRS) epidemic). Each sample (300 g of pork meat or breast muscle of chicken) was collected and frozen at –80°C in separate plastic bags until the analysis.



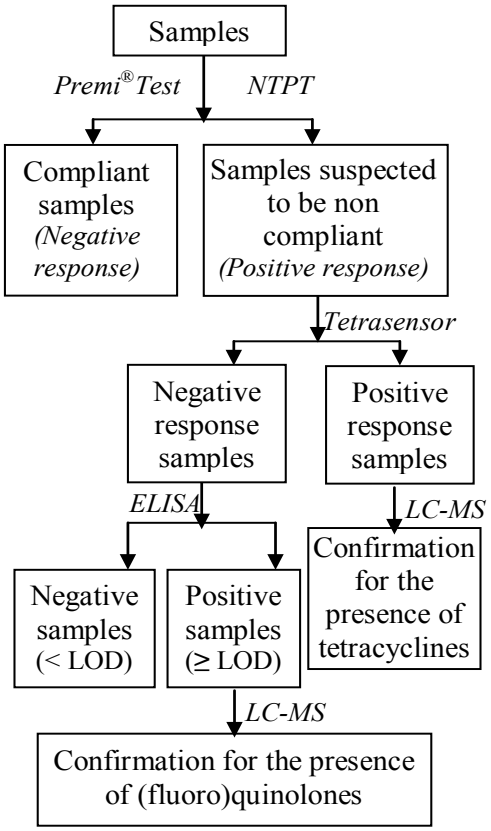
Source: http://www.worldatlas.com/webimage/countrys/asia/vn.htm
http://www.mangdulich.com/dialyvietnam/images/vietnammap/vietnammap_nordmap.gif

Figure 1. Map of Red River Delta region indicating the three representative provinces where the samples were collected (Hai Duong, Thai Binh, Ha Noi)

2.3. Strategy for Residue Analysis

A screening step was applied by using a microbiological test to rapidly detect the samples suspected to be non-compliant. These samples were then further tested using a post-screening step in order to identify the antibiotic group responsible for the positive response at the screening stage. A confirmation analysis was performed to identify and quantify the molecule(s) possibly present in the samples giving a positive response at the post-screening stage.
All samples were analyzed in parallel using two microbiological screening tests, the New Two Plate Test (NTPT) and the Premi®Test.

The (fluoro) quinolones and tetracyclines are two antibiotic groups which are often found to be used in chicken and pig production in RRD[6]. For this reason, in the framework of this study, samples which displayed a positive response in one of both screening tests were analyzed using two different specific post-screening tests (the Tetrasensor® Test was used to detect tetracyclines and an ELISA to detect (fluoro)quinolones, only in samples giving a negative response after the Tetra-Sensor® analysis). Samples giving a positive response at the post-screening stage were analyzed by liquid chromatography coupled to mass spectrometry (LC-MS) (Fig. 2). The whole strategy was applied to the 60 samples from the Thai Binh province.



LOD: Limit of Detection of the ELISA test

Figure 2. Strategy of antibiotic residues analysis in pork and chicken meat marketed in the Thai Binh province, in the RRD

2.4. New Two Plate Test (Screening test)

The NTPT is a "home made" assay based on the growth inhibition of *Bacillus subtilis*. It has been recently optimized for shrimp and meat by Dang and co-workers [24-25]. Meat samples were extracted and analyzed as previously described[25].

2.5. Premi® Test (Screening step)

Premi®Test is another kind of microbiological assay using *Bacillus stearothermophilus*. Test kits were purchased from DSM (DSM Food Specialities R&D, Delft, Netherlands). A multi-residue extraction step, as described by Stead and co-workers was applied to chicken and pork meat samples [23].

2.6. Group Specific Tests (Post screening steps)

Tetrasensor® Test provided by Unisensor, S.A (Wandre, Liège, Belgium) is a receptor-based dipstick assay for a rapid screening of the presence of all the main tetracyclines in animal tissue (limit of detection ≤ 20 µg Kg^{-1} of tetracycline equivalents). The tetracyclines potentially present in 5 grams of sample (weighed in stomacher bags) were extracted with 15 ml of the buffer provided in the kit. After homogenizing tissue and buffer for 2 minutes with a stomacher, 2 mL of the fluid were centrifuged at 20 000 g for 1 minute. A total of 200 ml of the supernatant was transferred into the vial containing the receptor and the contents were mixed gently until the dried pellet was dissolved completely. The strip was then dipped into the vial and the whole was incubated for 10 minutes at room temperature. Meanwhile, the fluid mounts the strip and passes the two green capture lines on it, turning their color into red. The first test line binds the remaining free receptor, while the second control line binds the excess receptor. The result was read by visual inspection by comparing the color intensities of the first and the second line.

The ELISA used here is a direct competitive enzyme-linked immunosorbent assay (ELISA) for the quantitative analysis of a broad range of (fluoro)quinolones residues in various matrices (using an anti-sarafloxacin antibody, a norfloxacin-peroxydase conjuguate, and a sarafloxacin calibration curve) (EIA Fluoroquinolones 2 hours E.G.3), which was provided by CER (Marloie, Belgium). The sample preparation and test procedure were performed as prescribed by the manufacturer. Five grams of homogenized sample were extracted using a simple and rapid extraction carried out with a 1:1 mixture of methanol and phosphate-buffered saline adjusted to pH 7.4. After vortexing for 30 seconds and shaking for 30 minutes, the sample was centrifuged at 4000 rpm for 10 minutes; the supernatant was transferred into a new tube. The extract (50 µl) is directly analyzed after a second centrifugation at 3000 rpm for 10 minutes, and a 10 times dilution with the dilution buffer provided with the kit.

In this assay, the anti-sarafloxacin antibody is able to bind several (fluoro)quinolones, with the cross-reactivity indicated into brackets: sarafloxacin (100%), norfloxacin

(105%), difloxacin (64%), ciprofloxacin (17%), pefloxacin (30%), ofloxacin (55%), flumequine (4%), cinoxacin (1%), oxolinic acid (4%), danofloxacin (88%), enrofloxacin (66%), marbofloxacin (45%), lomefloxacin (24%), enoxacin (27%) and nalidixic acid (14%). The limit of detection (LOD) is 0.5 μg Kg^{-1} of sarafloxacin equivalents.

2.5. Quantification of (fluoro) Quinolones (Confirmation step)

The sample extraction procedure was adapted from the method described in the papers of Toussaint and co-workers [26-27]. Briefly, 1 g of tissue was spiked with 100 μL of lomefloxacin and 2-phenyl-4-quinoline carboxylic acid (Cincophen), both from Sigma–Aldrich (3 μg mL−1 in methanol), used as an internal standards. The extraction step was performed using 10 mL acetonitrile. A defatting step of the sample was realized by adding 3 mL hexane. The sample was vortexed, centrifuged (4000 rpm, 5 min) and then the hexane phase was discarded. The sample extract was evaporated to dryness and ammonium acetate buffer (5 mM, pH 4) was then added to obtain a final volume of 2 mL. The purification step was performed using SPE cartridges (SDB-RPS, 3 M Empore). Analytes were eluted with 4 mL of a mixture of methanol and ammonium hydroxide 1 M (75:25, v/v), the eluate was then evaporated to dryness and reconstituted with 300 μL of water/formic acid (pH 2.5). A calibration curve containing 13 (fluoro)quinolones standards (norfloxacin, ofloxacin, cinoxacin, flumequine, enoxacin, oxolinic acid, nalidixic acid, enrofloxacin, and danofloxacin mesylate from Sigma, (St Louis, MO, USA) and marbofloxacin from Vetoquinol (Belgium) was prepared with the same procedure than for the samples, using blank meat fortified at 5 different concentrations around the EU MRL[17], for each antibiotic. For antibiotics with no MRL in pork or chicken (sarafloxacin, norfloxacin, ofloxacin, cinoxacin, nalidixic acid, enoxacin), a reference concentration of 100 μg kg^{-1} was chosen.

Identification and quantification were performed by LC–MS/MS on a 2690 Alliance separation Modules integrated autosampler, solvent delivery system and column heater coupled to a Quattro Ultima Platinum triple-quadrupole mass spectrometer (Micromass, Manchester, UK). The mass spectrometer was equipped with an electrospray ionization (ESI) interface. The LC column used was a Polaris C18A 3 μm (150 × 2.0 mm) with a Chromsep guard column SS (10 × 2.0 mm) both from Varian.

The limit of quantification was 12.5 μg kg^{-1} for enrofloxacin and ciprofloxacin, 25 μg kg^{-1} for enoxacin, marbofloxacin, norfloxacin, ofloxacin, danofloxacin, cinoxacin, oxolinic acid and nalidixic acid, 37.5 μg kg^{-1} for sarafloxacin, 50 μg kg^{-1} for flumequine and 75 μg kg^{-1} for difloxacin.

2.6. Quantification of Tetracyclines (Confirmation step)

Five grams of tissue were spiked with methacycline (from Dr. Ehrenstorfer, Augsburg, Germany) used as internal standard. The sample was extracted two times by blending 25 mL of succinate buffer 0.05 M pH 4 containing 20 mg of EDTA and 15 mL of hexane, to remove fat. After shaking vigorously, the samples were centrifuged; hexane was removed and the aqueous phase was transferred into a new tube. This extraction was reproduced without hexane. Both pooled supernatants were applied on a pre-conditionned SPE column (OASIS hydrophobic lipophilic-balanced (HBL), 6 mL, 200 mg, Waters Corp, Milford, MA, USA). The column was washed with 20 mL of water/methanol (95/5, v/v) and degreased with 5 mL of hexane. The tetracyclines were eluted with 5 mL methanol. The extracts were evaporated to dryness at 45°C under a flow of nitrogen. The dry residue was dissolved in 1 mL of water/methanol (70/30, v/v).

The method used for quantification of tetracyclines was based on the method described by Xu *et al.* in 2008[28]. In short, the final separation and detection were performed by LC–MS/MS using a Quattro Ultima tandem mass spectrometer coupled to a HPLC 2690 separation module system and integrated autosampler (Micromass, Manchester, UK). The tetracyclines were detected in positive electrospray ionization (ESI) in multiple reaction monitoring (MRM) acquisition mode. A Sunfire C18 column (150 mm × 2.1 mm, 5.0 μm particle size) (Waters, Milford, MA, USA) was used for the chromatographic separation. The limit of quantification was 25 μg kg^{-1} for the sum of tetracyclines residues.

3. Results and Discussion

3.1. Screening Antibiotic in the Meat Samples

From a total of 180 meat samples (83 chicken and 97 pork samples) collected on local markets of the RRD, 49 samples (27% of all samples) displayed a positive response in at least one of both screening tests (named “suspect samples”), from which 11 (13% of chicken samples) are chicken meat samples and 38 (39% of pork samples) are pork samples (Table 1).

In the samples collected in the province of Hanoi, all the chicken meat was negative (a negative response was recorded in both screening tests) and only 7 pork samples (23% of the pork meat sampled in the province of Hanoi) were suspected to contain antibiotic residues. In Hai Duong, 17% of the pork meat samples and 13% of the chicken meat samples were screened positive. From the 49 samples screened positive (from all the 3 provinces), 33 are from the province of Thai Binh and only 16 from the other two provinces. These 16 positive results out of the 120 chicken and pork samples taken on the markets of the province of Hanoi and Hai Duong were already discussed in a previous study[25]. In the rest of this paper, we will discuss only of the 60 samples from the province of Thai Binh, to which the analytical strategy proposed in Figure 2 was applied.

Table 1. Results of antibiotic residues screening in samples collected from local markets of the Red River Delta region in Vietnam

Areas sample collection	Result of screening	Pork	Chicken	Total sample by area
Hanoi	Number of suspect samples/Number of samples analyzed	7/30	0/30	7/60
	Percent (%)	*23*	*0*	*12*
Hai Duong	Number of suspect samples/Number of samples analyzed	5/30	4/30	9/60
	Percent (%)	*17*	*13*	*15*
Thai Binh	Number of suspect samples/Number of samples analyzed	26/37	7/23	33/60
	Percent (%)	*70*	*30*	*55*
Total by type of sample	**Number of suspect samples/Number of samples analyzed**	**38/97**	**11/83**	**49/180**
	Percent (%)	***39***	***13***	***27***

3.2. Post-screening Analysis and Confirmation of Tetracycline and (fluoro) Quinolone Residues

The 60 samples taken from the Thai Binh province (presenting the higher rate of positive results) were used to evaluate the detection capacity of the screening tests used here as well as to evaluate our 3 steps analysis strategy, based on a screening, followed by a post-screening, and finally, a confirmation analysis. If we compare the results of the two microbiological screening tests (NTPT and Premi®-Test), the results (Table 2) showed that, from the 33 suspect samples, the NTPT detected 26 samples and the Premi®-Test 22 samples. Fifteen samples were detected by both tests, 11 samples displayed a positive response only with the NTPT and 7 samples only with the Premi®-test (Table 2). The interpretation results of the NTPT, according to Dang et al.[25].indicated that most of the NTPT positive samples are suspected to contain residues of both tetracycline and quinolone groups (Tables 3 and 4). These results confirm our recent survey results[6] showing that these two antibiotic groups are the most commonly used in pig and chicken production in the RRD region.

Table 2. Results of the screening and post-screening analysis of meat samples collected from the Thai Binh province

Results of screening tests		Number of samples (n=60)	Results of post-screening tests (number of positive results)	
NTPT	Premi®-Test		Tetra-Sensor(n = 60)	(fluoro)quinolones ELISA (n=29)
Negative	Negative	27	5	1 (n = 14)
	Positive	7	0	2 (n=6)
Positive	Negative	11	7	0 (n=4)
	Positive	15	11	2 (n=5)
Number of positives samples		**33**	**23**	**5**

According to our strategy (Figure 2), the 33 samples screened positive in at least one of the two screening tests were first analyzed using the Tetrasensor® kit, and the samples giving a negative response in the Tetrasensor® assay were analyzed using an ELISA specific for (fluoro) quinolones.

This study also analyzed the 27 samples screened negative with both post-screening assays (all the samples with the Tetra®sensor kit and only 13 samples with the ELISA), in order to check for possible false negative results of the screening tests.

Our willing was to analyze all the 60 samples with all the techniques, but due to various reasons (limited amount of samples in some cases or limitation of budget), only some of the 60 samples were analyzed using the ELISA and the LC-MS techniques.

The complete results are presented in Tables 3 and 4, for pork and chicken meat, respectively, and are summarized, for the results of the screening and post-screening tests, in Table 2.

First, if we compare screening and post-screening results (Table 2), we see that 18 samples (more than half of the 33 samples screened positive) give a positive response in the Tetrasensor® assay, meaning that they probably contain tetracycline residues. Five of the 27 negative samples gave also a positive response when using the Tetrasensor® kit. Only one of the samples screened negative gave a low detectable response in ELISA (this sample was negative in the Tetrasensor® test), and 4 of the samples screened positive and post-screened negative using the Tetrasensor®, gave a response higher than the LOD (0.5 μg kg^{-1}) after ELISA analysis.

The results of tetracycline and quinolone quantification in 20 samples positive at the post-screening stage indicated that 10 (all pork) and 4 samples (1 pork and 3 chicken) (Tables 3b and 4) were confirmed containing residues from tetracyclines (chlortetracycline, oxytetracycline, tetracycline,

doxycycline) and (fluoro)quinolones (nalidixic acid, enrofloxacin and ciprofloxacin), respectively. Four pork samples were found non-compliant because containing residues (3 samples containing tetracyclines and one other containing enrofloxacin residues) with a concentration higher than their respective maximum residue limits (Table 3b). The MRLs fixed for muscle by the Codex[16] are 200 µg Kg^{-1} for all tetracyclines (parent drug singly or in combination) and there is no MRL in the Codex for enrofloxacin and ciprofloxacin. In EU, the respective MRL are 100 µg Kg^{-1} in pork and poultry muscle, for tetracyclines (sum of parent drug and its 4-epimer), for enrofloxacin (sum of enrofloxacin and ciprofloxacin), as well as for doxycline [17].

If we look at the performance of the NTPT assay that we have recently developed[24-25], we see that 34 samples were screened negative (21 from chicken and 13 from pork) (Tables 3a and 4). From these 34 negative samples, only 7 samples were analyzed using a confirmatory technique resulting in 7 compliant samples, and thus no false negative result was recorded. But the amount of data is too small to calculate a rate of false negative results.

From these 7 samples, 1 pork and 2 chicken samples, respectively, were Tetrasensor® false positive samples (TB-51, TB-31, TB-34) (Tables 3a and 4), due the limit of sensitivity (20 µg Kg^{-1}) of the Tetrasensor® kit which was chosen in this study. Let's note that another Tetrasensor® kit, with a limit of detection of 100 µg Kg^{-1}, is available. Two chicken samples (TB-8 and TB-33) (Table 4), declared compliant, appear to be Premi®-Test "false positive", but contained indeed traces of residues, as it was also shown by their ELISA results.

If we look at all the 25 NTPT positive samples, 22 were analyzed using a confirmatory technique.

Four samples were confirmed non-compliant, 8 were declared compliant but were shown to contain residues (7 pork samples, Table 3b, and 1 chicken, Table 4) and 6 were declared compliant, with no residues detected in it by LC-MS, but displaying a post-screening positive response (6 pork samples, Table 3b).

From the 22 samples analyzed using the whole strategy proposed in Figure 2, only 4 samples remained "suspected to be compliant, to be confirmed" (pork samples TB-48, TB-50, TB-56 and TB-47, Table 3b) because the post-screening methods as well as the confirmatory technique didn't detect any residue. We cannot exclude that other antibiotics are responsible of the NTPT screening. This shows that the proposed strategy seems to work well; with a good rate of conclusive results (82% of the sample submitted to the whole strategy were elucidated).

The fact that fluoroquinolone and tetracycline residues are found in a lot of meat samples is not surprising. The (fluoro)quinolones and tetracyclines are broad-spectrum antibacterial agents which are often found to be used in animal production. Tetracyclines are active against gram positive and gram negative bacteria, and also act against some others pathogenic agents unaffected by other antibiotics[29]. For this reason, as in other countries, this group is one of the most commonly used groups of antibiotics in livestock in Vietnam. While (fluoro)quinolones are only used to treat and prevent diseases, tetracyclines are also used for disease prevention and for growth promotion in both chicken and pig production[6].

Table 3a. Results of the screening, post-screening and confirmation analysis of pork meat marketed in the RRD region, for the samples screened negative in the NTPT assay

ID of sample	NTPT	Premi®-Test	Tetrasensor®	ELISA to detect quinolones	LC-MS Confirmation		Conclusion
					Tetracyclines	Quinolones	
TB-3	-	-	-	< LOD			C
TB-10	-	-	-	<LOD			C
TB-14	-	-	-	<LOD			C
TB-26	-	-	-				C
TB-36	-	-	-				C
TB-51	-	-	+		ND		C
TB-53	-	-	-			ND	C
TB-55	-	-	-	<LOD			C
TB-57	-	-	-	0.6 µg Kg^{-1}		ND	C
TB-59	-	-	-				C
TB-60	-	-	-				C
TB-5	-	+	-				SNC
TB-38	-	+	-	<LOD			SNC

NTPT: New Two Plate Test; -: negative response; +: positive response; ND: not detected
LOD: Limit of Detection; C: compliant, SNC: suspected to be non compliant (to be confirmed)

Table 3b. Results of the screening, post-screening and confirmation analysis of pork meat marketed in the RRD region, for the samples screened positive in the NTPT assay *(numbers in brackets are concentrations expressed in µg kg^{-1})*

ID of sample	NTPT	Suspiscion of	Premi® Test	Tetrasensor®	ELISA to detect quinolones	LC-MS Confirmation		Conclusion
						Tetracyclines	Quinolones	
TB-9	+	Quinolones, Aminoglycosides, Macrolides, Florfenicol	-	+		chlortetracycline & 4 epi-chlortetracycline <MRL oxytetracycline (812) 4 epi-oxytetracycline (165)		NC
TB-12	+	Quinolones, Aminoglycosides, Macrolides, Florfenicol	-	+		ND		C
TB-13	+	Tetracyclines, Oxolinic acid, Flumequine	-	+		ND		C
TB-16	+	Tetracyclines, Oxolinic acid, Flumequine	-	+		chlortetracycline & 4 epi-chlortetracycline <MRL		C
TB-37	+	Tetracyclines, Oxolinic acid, Flumequine	-	+		chlortetracycline & 4 epi-chlortetracycline <MRL		Ct
TB-40	+	Tetracyclines, Oxolinic acid, Flumequine	-	+	<LOD	ND		C
TB-46	+	Tetracyclines, Oxolinic acid, Flumequine	-	+		chlortetracycline & 4 epi-chlortetracycline <MRL		C
TB-48	+	Tetracyclines, Oxolinic acid, Flumequine	-	-	<LOD		ND	SNC
TB-50	+	Tetracyclines, Oxolinic acid, Flumequine	-	-	<LOD		ND	SNC
TB-56	+	Tetracyclines, Oxolinic acid, Flumequine	-	-	<LOD		ND	SNC
TB-1	+	Tetracyclines, Oxolinic acid, Flumequine	+	+		chlortetracycline & 4 epi-chlortetracycline <MRL		C
TB-2	+	Sulfonamides	+	+		ND		SNC
TB-4	+	Tetracyclines, Oxolinic acid, Flumequine	+	+		chlortetracycline & 4 epi-chlortetracyckine <MRL		C
TB-6	+	Tetracyclines, Oxolinic acid, Flumequine	+	+		ND		C
TB-11	+	Quinolones, Aminoglycosides, Macrolides, Florfenicol	+	+				SNC
TB-15	+	Quinolones, Aminoglycosides, Macrolides, Florfenicol	+	+		tetracycline & epi-tetracycline < MRL chlortetracycline (180) 4epi-chlortetracycline (113)		NC
TB-17	+	Quinolones, Aminoglycosides, Macrolides, Florfenicol	+	+	<LOD			SNC
TB-18	+	Tetracyclines, Oxolinic acid, Flumequine	+	-	<LOD			C
TB-39	+	Tetracyclines, Oxolinic acid, Flumequine	+	+		oxytetracycline (89) 4 epi-oxytetracyckine (33) doxycycline (372)		NC
TB-47	+	Tetracyclines, Oxolinic acid, Flumequine	+	-	<LOD		ND	SNC

NTPT: New Two Plate Test; -: negative response; +: positive response; ND: non detected; MRL: Maximum Residue Limit
LOD: Limit of Detection; LOQ: Limit of Quantification; C: compliant, SNC: suspected to be non compliant (to be confirmed)

Table 3b. *Cont.*

ID of sample	NTPT	Suspiscion of	Premi®-Test	Tetrasensor	ELISA to detect quinolones	LC-MS Confirmation		Conclusion
						Tetracyclines	Quinolones	
TB-49	+	Tetracyclines, Oxolinic acid, Flumequine	+	+		ND		C
TB-52	+	Quinolones, Aminoglycosides, Macrolides, Florfenicol	+	+		chlortetracycline & 4 epi-chlortetracyckine< MRL		C
TB-54	+	Quinolones, Aminoglycosides, Macrolides, Florfenicol	+	-	147.5		enrofloxacine (1473) ciprofloxacine (2042)	NC
TB-58	+	Tetracyclines, Oxolinic acid, Flumequine	+	+		chlortetracycline & 4 epi-chlortetracyckine <MRL		C

NTPT: New Two Plate Test; -: negative response; +: positive response; ND: non detected; MRL: Maximum Residue Limit
LOD: Limit of Detection; LOQ: Limit of Quantification; C: compliant, SNC: suspected to be non compliant (to be confirmed)

Table 4. Results of the screening, post-screening and confirmation analysis of chicken meat marketed in the RRD region

ID of sample	NTPT	Suspiscion of	Premi®-Test	Tetrasensor®	ELISA to detect quinolones	LC-MS Confirmation		Conclusion
						Tetracyclines	quinolones	
TB-7	-		-	+		ND		C
TB-19	-		-	-	< LOD			C
TB-20	-		-	+	< LOD			SNC
TB-21	-		-	-	< LOD			C
TB-22	-		-	-				C
TB-24	-		-	-				C
TB-27	-		-	-	< LOD			C
TB-28	-		-	-				C
TB-29	-		-	-				C
TB-31	-		-	+		ND		C
TB-32	-		-	-	< LOD			C
TB-34	-		-	+		ND		C
TB-35	-		-	-	< LOD			C
TB-41	-		-	-	< LOD			C
TB-43	-		-	-	< LOD			C
TB-44	-		-	-	< LOD			C
TB-8	-		+	-	0.5		enrofloxacin (traces, < LOQ)	C
TB-25	-		+	-	< LOD			SNC
TB-33	-		+	-	0.6		enrofloxacin (traces, < LOQ)	C
TB-42	-		+	-	< LOD			SNC
TB-45	-		+	-	< LOD			SNC
TB-23	+	Tetracyclines, Oxolinic acid, Flumequine	-	-				SNC
TB-30	+	Tetracyclines, Oxolinic acid, Flumequine	+	-	0.6		enrofloxacin & nalidixic acid (traces, < LOQ)	C

NTPT: New Two Plate Test; -: negative response; +: positive response; ND: not detected
LOD: Limit of Detection; LOQ: Limit of Quantification; C: compliant, SNC: suspected to be non compliant (to be confirmed)

Pork meat is the most contaminated, probably because the sampling was realized after an epidemic of Porcine Reproductive and Respiratory Syndrome[30]. This could explain that the farmers used more antibiotics at that time, even if this disease is a viral one, showing again the irrational antibiotic use in meat production in Vietnam as in other countries, this group is one of the most commonly used groups of antibiotics in livestock in Vietnam.

The problem of veterinary drugs residues in general and of antibiotics in particular in animal products is becoming increasingly important in many developing countries[31]. In developed countries, the use of antibiotics in animal production is strictly regulated. In Europe, the proportion of non-compliant results for antibiotic residues in food is only 0.27% (out of 750 000 analyzed samples) in the 27 countries members of the European Union[32]. In Kuwait, the result of a recent study of antibiotic residues in animal products of Al Mazeedi and coworkers showed that 5% of chicken samples and 18% of milk samples were non-compliant[33], while in Egypt, the proportion of non-compliant samples for the presence of tetracyclines residues in chicken meat was more than 7%[34].

In Vietnam, a study conducted in 2003 by Thuat et al (2002)[35], on antibiotic use in animal production and their residues in pork and chicken meat, showed that twenty six different antibiotics were used in pig and chicken production, from which, the seven more frequent were chloramphenicol in 15% of farm, tylosin (15%), colistin (13%), norfloxacin (10%), gentamicin (8%), tetracycline (8%) and ampicillin (7%). In 103 analyzed samples of pork, kidney and liver and 149 samples of broiler meat and liver, more than 43 % samples had a residue level from 2 to 1,100 times higher than that issued by regulations.

In 2006, a study of 3 tetracyclines residue (tetracycline, oxytetracycline and chlortetracycline) in pork sold in the markets of Hanoi showed that 5.5% of the samples (16 of 290 samples analyzed) were contaminated with tetracyclines, including 2 samples containing tetracycline at a concentration higher than MRLs[36].

4. Conclusions

The high proportion of pork samples containing antibiotic residues is of concern. They may cause a potential hazard to public health and particularly increase the problem of drug resistance of pathogenic bacteria.

Further studies are necessary to evaluate other antibiotic residues in referred edible tissues and to estimate the risk in relation with animal product daily intakes.

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