RESEARCH ARTICLE

INTERNATIONAL MICROBIOLOGY
OPEN ACCESS

INTERNATIONAL MICROBIOLOGY 20(2):85-93 (2017)
doi:10.2436/20.1501.01.288. ISSN (print): 1139-6709. e-ISSN: 1618-1095
www.im.microbios.org

Antimicrobial resistance gene expression associated with multidrug resistant *Salmonella* spp. isolated from retail meat in Hanoi, Vietnam

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Received 4 April 2017 · Accepted 8 May 2017

Summary. The purpose of this study was to further characterize the multi-antimicrobial resistance and antibiotic resistance gene expression associated with multi-drug resistance (MDR) in *Salmonella* spp. isolates from retail meats in Hanoi, Vietnam. A total of 14 *Salmonella* spp. belonging to 9 serotypes (e.g., Warragul, London, Derby, Indiana, Meleagridis, Give, Rissen, Assine, and Typhimurium) were tested for sensitivity to 8 antibiotics. Resistance to at least one antibiotic was shown in 13 strains (92.85%). The multiple antimicrobial resistances accounted for 64.29% of isolates (9/14). One hundred percent of MDR isolates possessed antibiotic resistant genes, in which 17, 16 and 11 genes were found in *Salmonella* (Salm) Typhimurium S360, S384, S181 respectively; 12 genes in each strain as Indiana, Warragul, and Meleagridis; 11 genes in Give, 8 genes in Derby and 6 genes in Rissen. Three antibiotic resistance genes (*ssaQ*, *aadA*, and *gyrB*) were present in all isolates, whereas Cephalosporin-resistant gene (e.g., *CTX-M3-like*) was not detected in any isolates. The results suggest that retail meats could constitute a source of human exposure to multi-drug resistant *Salmonella* and future research should focus on the impact of these MDR source on the human genome. [**Int Microbiol** 20(2): 85-93 (2017)]

Keywords: *Salmonella* spp. · multidrug resistance · retail meat

Introduction

The increasing human population around the world places a huge demand on food in order to ensure the survival of mankind. It exerts pressure on a number of food industries as an effort to satisfy the increasing food demand. At the same time, food poisoning with the cause of contaminating bacteria is also increasing and greatly affecting human health. Salmonellosis is one of the most major causes of foodborne infections in the world and it is still one of the most widespread foodborne bacterial illnesses in humans [26]. The presence of *Salmonella* in retail meat and its related products has often caused them to be unsafe for human consumption [6]. *Salmonella* is associated with approximately 2500 serovars. Serovars are generally found in animal origin products include Salm. Enteriditis, Salm.Typhimurium, Salm. Gallinarum,

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Salm. Weltevreden and Salm. Infantis. These vehicles that cause infection appear to be more prevalent in poultry than in any other food animal [15].

The worldwide increase of foodborne infections associated with antibiotic resistant pathogens and the spread of antibiotic resistance is one of major concern in both developed and developing countries [10]. The use of antibiotics in any venue, including disease treatment and growth promotion in domestic livestock, can potentially lead to widespread dissemination of antibiotic resistant bacteria [17,39]. Increasing evidence demonstrates that antimicrobial usage in animals promotes the emergence of a wide range of resistant zoonotic pathogens such as *Salmonella*, which compromises the effectiveness of antibiotic treatments used in humans when an infection occurs [19]. Therefore, surveillance of multiple antimicrobial resistance and resistance genes expression associated with multi-drug resistance in pathogenic bacteria such as *Salmonella* is crucial for providing information on the magnitude and tendency of resistance in foodborne pathogens in each country [2].

Salmonella strains resistant to antimicrobial drugs are now increasing because of selection from the use of antimicrobial drugs [34,40]. Various studies have focused on investigating the prevalence of antimicrobial resistance and resistance genes in *Salmonella* such as Thailand and Laos [32], the United States [16], Japan [31], China [24], Vietnam [36], the UK [26], and Mexico [29]. In recent years, contamination and antibiotic resistance of *Salmonella* isolates from foodstuffs and animals in Vietnam are increasing due to the use disorder of antibiotics in disease treatment and domestic livestock [36,35]. Thus, assessing the distribution of antimicrobial resistance genes in *Salmonella* will represent a more detailed and potentially useful tool for improving our understanding of antimicrobial resistance epidemiology, particularly in Hanoi, where such information is also disorderliness.

Table 1. *Salmonella* strains used in this study

ID	Description	Source of sample (Year)
Salm 1	*Salmonella* Warragul	Chicken (2016)
Salm 2	*Salmonella* London	Pork (2016)
Salm 3	*Salmonella* Derby	Pork (2016)
Salm 4	*Salmonella* Indiana	Chicken (2016)
Salm 5	*Salmonella* Derby	Pork (2016)
Salm 5.1	*Salmonella* Meleagridis	Pork (2016)
Salm 6	*Salmonella* Derby	Pork (2016)
Salm 7	*Salmonella* Give	Pork (2016)
Salm 7.1	*Salmonella* Rissen	Chicken (2016)
Salm 8	Salmonella Typhimurium S360	Beef (2016)
Salm 9	*Salmonella* Derby	Pork (2016)
Salm 10	*Salmonella* Assine	Chicken (2016)
Salm 11	*Salmonella* Typhimurium S384	Pork (2016)
Salm 12	*Salmonella* Typhimurium S181	Pork (2016)

Material and methods

Bacterium strains. A total of 14 strains were serotyped and received from laboratory in Institute of Genome Research (Hanoi, Vietnam) (listed in Table 1) including 1 Warragul, 1 London, 4 Derby, 1 Indiana, 1 Meleagridis, 1 Rissen, 1 Give, 3 Typhimurium and 1 Assine. The originated strains from pork, beef and chicken meat at retail markets in Hanoi, Vietnam.

Antibiotic susceptibility testing. The antimicrobial susceptibility test was performed according to the Clinical and Laboratory Standards Institute (CLSI-2015) [9] and used the disk diffusion method as Kirby-Bauer's description. The isolated strains were grown overnight in Brain Heart Broth Infusion (Biolife-Italia) and prepared in a lawn on Mueller-Hinton agar [20]. The antibiotic disks were placed aseptically on it and incubated at 37°C for 16–18 hours. The results were recorded by measuring the inhibition zones and scored as sensitive, intermediate, and resistant according to guide in CLSI-2015. The eight tested antimicrobials were often used in husbandry and treatment of animal farms as well as human diseases in Vietnam, namely, ampicillin (AM) 10 µg, ceftazidime (CAZ) 30 µg, gentamicin (GN) 10 µg, streptomycin (S) 10 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (C) 30 µg, tetracycline (TE) 30 µg, and trimethoprim/sulfamethoxazole (SXT) 1.25/23.75 µg (BD Diagnostics).

Total RNA extraction and cDNA synthesis. Total RNA was extracted from one milliliter of Brain Heart Broth Infusion contained 10^8 Salm bacteria as the manufacturer's protocol (TRIzol Reagent, Life Technologies Inc.). The RNA was treated with RNase-free DNase to remove contaminating genomic DNA. RNA quality and quantity were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop® Technologies, Thermo Scientific) by measuring the absorbance at 260 nm and the ratio of A260/A280, respectively. RNA integrity was confirmed by gel electrophoresis, stained with ethidium bromide, and visualized under UV light using a gel documentation system (Herolab, Wiesloch, Germany). The total RNA samples were reverse-transcribed with oligo-dT primers of SuperScript III (Invitrogen) as the manufacturer's instructions. The prepared cDNA samples were stored at −20 °C until use.

Reverse transcription polymerase chain reaction (RT-PCR) methods. The target genes were detected in the MDR *Salmonella* spp. isolates including Beta-lactams (*blaTEM*), Cephalosporin (*CTX-M3-like*), Quinolones (*gyrB*), tetracyclines (*tetA*, *tetB*, *tetC*), sulfonamides (*sul I*, *sul II*, *sul III*, *sodC1*), chloramphenicol (*cmlA*, *cat2*, *floR*, *avrA*, *ssaQ*, *mgtC*) and

Table 2. Primers used for the detection of antibiotic resistance genes in *Salmonella* spp. isolates

Gene	Forward/ Reverse primer (5′-3′)	Annealing temp (°C)	Size (bp)
tetA	TTGGCATTCTGCATTCACTC / GTATAGCTTGCCGGAAGTCG	55	494
tetB	CAGTGCTGTTGTTGTCATTAA / GCTTGGAATACTGAGTGTAA	55	570
tetC	AGGTAAACGCCATTGTCAGC / AAGCCGCGGTAAATAGCA	55	594
sul I	TGGTGACGGTGTTCGGCATTC / GCGAGGGTTTCCGAGAAGGTG	57	789
sul II	CCTGTTTCGTCCGACACAGA / GAAGCGCAGCCGCAATTCAT	57	434
sul III	ATGAGCAAGATTTTTGGAATCGTAA / CTAACCTAGGGCTTTGGATATTT	57	791
sodC1	AACGGATACGTGGCTGTACC / CGGTCTGCTTTTCACTCCTC	55	243
cmlA	GGCCTCGCTCTTACGTCATC / GCGACACCAATACCCACTAGC	55	682
cat2	AACGGCATGATGAACCTGAA / ATCCCAATGGCATCGTAAAG	55	546
floR	ATGACCACCACACGCCCCG / AGACGACTGGCGACTTCTCG	57	1212
avrA	GTTGAGGACCAAAGCAGCTC / TCACCACACAGACGTTCACA	55	192
ssaQ	AATGAGCTGGGTAGGGTGTG / ATGCAACGCTAGCTGATGTG	55	216
mgtC	TGTCTCTGGGATTGGCTTTC / TTCTCCCTCAGCGGATATTG	55	232
aph3-IIa	TCTGAAACATGGCAAAGGTAG / AGCCGTTTCTGTAATGAAGGA	55	581
aph A1	ATGGGCTCGCGATAATGTC / CTCACCGAGGCAGTTCCAT	55	600
aadA	GTGTCACAGGCGATACGTTG / GAACCAGCTGCGAATAAAGC	55	228
aac3-IIa	CGGCCTGCTGAATCAGTTTC / AAAGCCCACGACACCTTCTC	55	438
strA	CTTGGTGATAACGGCAATTC / CCAATCGCAGATAGAAGGC	55	548
blaTEM	GCACGAGTGGGTTACATCGA / GGTCCTCCGATCGTTGTCAG	57	310
gyrB	CTGCGCTATCACAGCATCAT / CGCGATGGAAATCTGGTACT	55	219
CTX-M3like	GGAATCTGACGCTGGGTAAA / GGTTGAGGCTGGGTGAAGTA	55	232
16SrRNA	AGAGTTTGATCMTGGCTCAG / CCGTCAATTCMTTTRAGTTT	55	907

aminoglycosides (*aph3-IIa*, *aphA*, *aadA*, *aac3-IIa*, *strA*) by RT-PCR method. The sequence primers and annealing temperature conditions were described in Table 2. Briefly, the RT- PCR was performed in 20 µl lumes containing 2 µl of 10X buffer (100 mmol/l Tris-HCl [pH 9], 1.5 mmol/l $MgCl_2$, 500 mmol/l KCl, 0.1% gelatin), 2 µl of 100 µmol/l concentrations each of dATP, dTTP, dGTP and dCTP, 0.5 µl of 5 pmol of each primer, and 0.2 µl of 5U of Taq DNA polymerase (Bangalore Genei, Bangalore, India), with 1.0 µl of cDNA templates. The reactions were carried out by using an Eppendorf's Mastercycler pro (Eppendorf, German). A lume of 8 µl of PCR product was loaded on 2% agarose gel and stained with ethidium bromide. The gel photograph was scanned and analyzed using Quantity One program (Gel Doc EQ; Bio-Rad, Hercules, CA).

Statistical analyses. Data were analyzed by one-way ANOVA, followed by Tukey's test for multiple comparisons of columns. Statistical analysis was performed using GraphPad Prism5 for Windows Edition (GraphPad Software Inc., La Jolla, CA). A P value of < 0.05 was considered statistically significant. The occurrence of differences in ratio of sensitive/ resistant antibiotics among *Salmonella* isolates or antibiotic groups was verified using Fisher's exact probability test. The rejection value for the null hypothesis was $P \leq 0.05$.

Results

Antimicrobial susceptibility of the Salmonella isolates. All the fourteen isolates of *Salmonella* were tested to a panel of eight antibiotics. Total 13/14 (93%) of these *Salmonella* isolates were resistant to at least one antimicrobial agent and 9/14 (64.29%) of the strains demonstrated the multi antimicrobial resistance (e.g., Salm 1, 4, 5.1, 6, 7, 7.1, 8, 11, and 12). The *Salmonella* strains (n = 13) exhibited antimicrobial resistance towards S (84.6%, 11⁄13), TE (84.6%, 11⁄13), C (61.54%, 8⁄13), AM (53.84%, 7⁄13), SXT (53.84%, 7⁄13),

Table 3. Susceptibility results of *Salmonella* isolates

Isolate	Antibiotics								Ratio R/S	(*P*)
	AM	CAZ	GN	S	CIP	C	TE	SXT		
Salm 1	S	S	S	S	S	R	R	R	3/5	(0.62)
Salm 2	S	S	S	R	S	S	R	S	2/6	(0.13)
Salm 3	S	S	S	R	S	S	S	S	1/7	(0.01)
Salm 4	R	S	R	R	R	R	R	R	7/1	(0.01)
Salm 5	S	S	S	R	S	S	R	S	2/6	(0.13)
Salm 5.1	R	S	S	R	S	R	R	R	5/3	(0.61)
Salm 6	R	S	S	R	S	S	R	S	3/5	(0.62)
Salm 7	R	S	S	R	S	R	R	R	5/3	(0.61)
Salm 7.1	I	S	S	R	S	R	R	R	4/3	(1.00)
Salm 8	R	S	S	R	S	R	R	R	5/3	(0.62)
Salm 9	S	S	S	S	S	S	S	S	0/8	(0.02)
Salm 10	S	S	S	S	S	R	S	S	1/7	(0.01)
Salm 11	R	S	R	R	S	R	R	R	6/2	(0.13)
Salm 12	R	S	S	R	S	S	R	S	3/5	(0.62)
Ratio R/S	7/6	0/14	2/12	11/3	1/13	8/6	11/3	7/7		
(*P*)	(*P* = 1)	(*P* = 0.001)	(*P* = 0.004)	(*P* = .007)	(*P* = 0.007)	(*P* = 0.7)	(*P* = 0.007)	(*P* = 1)		

GN (16.66%, 2⁄13), and CIP (7.69%, 1/13). All strains were susceptible to CAZ (Table 3). The most common multi-drug resistant profiles were observed (C, TE, SXT, S, and AM) and (AM, S, and TE) (Table 4).

Antibiotic resistance gene expression of MDR *Salmonella* isolates. All of the nine MDR *Salmonella* isolates were selected to determine the association between the antimicrobial resistance results and mRNA levels of the antibiotic resistance genes by RT-PCR amplification of the twenty-one genes. A total of 20 different antibiotic resistance genes were identified in all MDR *Salmonella* isolates (Tables 4 and 5).

All MDR *Salmonella* isolates possessed antibiotic resistant genes, in which 17 genes were found in Salm 8; 16 genes in Salm 11; 12 genes in each Salm 4, 1, and 5.1; 11 genes in

Table 4. Multi-drug antimicrobial resistance profile of *Salmonella* isolates

Number of antimicrobial resistance	Antimicrobial resistance pattern (number of isolates)	Number of isolates (%)
Three	C, TE, SXT (1); AM, S, TE (2)	3 (33.33)
Four	C, TE, SXT, S (1)	1 (11.13)
Five	C, TE, SXT, S, AM (3)	3 (33.33)
Six	C, TE, SXT, S, AM, GN (1)	1 (11.13)
Seven	C, TE, SXT, S, AM, GN, CIP (1)	1 (11.11)
Total		9

Abbreviations: S: sensitive, R: resistant, I: intermediate; AM (ampicillin), CAZ (ceftazidime), GN (gentamicin), S (streptomycin), CIP (ciprofloxacin), C (chloramphenicol), TE (tetracycline), SXT (trimethoprim-sulfamethoxazole).

Table 5. Antibiotic resistance gene profiles of MDR *Salmonella* isolates

Isolates	Antimicrobial-resistant gene(s)	Number of genes (%)
Salm 8	*tetA*, *tetB*, *tetC*, *sul II*, *sul III*, *sodC1*, *blaTEM*, *gyrB*, *cmlA*, *cat2*, *floR*, *avrA*, *ssaQ*, *mgtC*, *aph3-IIa*, *aadA*, *aac3-IIa*	17/21 (80.95)
Salm 11	*tetA*, *tetB*, *tetC*, *sul II*, *sodC1*, *blaTEM*, *gyrB*, *floR*, *avrA*, *ssaQ*, *mgtC*, *aph3-IIa*, *aphA1*, *aadA*, *aac3-IIa*, *strA*	16/21 (76.19)
Salm 1	*tetA*, *sul I*, *sul II*, *blaTEM*, *gyrB*, *cmlA*, *floR*, *avrA*, *ssaQ*, *mgtC*, *aph3-IIa*, *aadA*	12/21 (57.14)
Salm 4	*tetA*, *sul I*, *sul II*, *blaTEM*, *gyrB*, *cmlA*, *floR*, *ssaQ*, *mgtC*, *aph3-IIa*, *aphA1*, *aadA*	12/21 (57.14)
Salm 5.1	*tetA*, *sul II*, *sul III*, *blaTEM*, *gyrB*, *cmlA*, *cat2*, *floR*, *avrA*, *ssaQ*, *mgtC*, *aadA*	12/21 (57.14)
Salm 7	*tetA*, *sul II*, *sul III*, *blaTEM*, *gyrB*, *cmlA*, *cat2*, *floR*, *avrA*, *ssaQ*, *aadA*	11/21 (52.38)
Salm 12	*tetA*, *sul II*, *sodC1*, *blaTEM*, *gyrB*, *cmlA*, *avrA*, *ssaQ*, *mgtC*, *aadA*, *strA*	11/21 (52.38)
Salm 6	*tetA*, *blaTEM*, *gyrB*, *cmlA*, *floR*, *avrA*, *ssaQ*, *aadA*	8/21 (38.1)
Salm 7.1	*gyrB*, *floR*, *avrA*, *ssaQ*, *mgtC*, *aadA*	6/21 (28.57)

Salm 12, and 7; 8 genes in Salm 6; and 6 genes in Salm 7.1. There were 3 antibiotic resistance genes per total 20 differentially gene expression (e.g., *ssaQ*, *aadA*, and *gyrB*) were expressed in all isolates, whereas Cephalosporin-resistant gene (*CTX-M3-like*) was not detected in any isolates (Table 5). Table 6 has shown the fold change of mRNA levels between the resistant genes and housekeeping gene (*16S rRNA* gene) from all *Salmonella* isolates. The results were shown the unrelationship between the prevalence of an antibiotic resistance gene expression and the resistant phenotype in some *Salmonella* isolates. For example, the *tetA*, *tetB*, *tetC* genes were undetected in TE-resistant as Salm 7.1, and the SXT-resistant Salm 7.1 did not carry *sul I*, *sul II*, *sul III*, and *sodC1* genes, while the *blaTEM* gene was expression (0.5 fold) in AM-susceptible Salm 1, *gyrB* gene expression varied from 0.6 fold to 1.32 fold in CIP-susceptible strains. In other words, these antibiotic resistance genes expressions did not correlate with the number of antibiotics to which a particular strain showed resistance. The cross-resistance to antibiotics is generally a combination of mechanisms, permeability (several antibiotics use the same way to enter or leave the cell), and changes in target and enzymes. Therefore, further studies are needed.

Discussion

The present study, about the global antibiotic resistance genes in MDR *Salmonella* serovars isolated from Hanoi, revealed a high antimicrobial resistance in *Salmonella* spp. isolated from retail chicken, beef and pork meats. The high levels of resistance were found to three or more of antimicrobials (64.28% of isolates), which were higher than other studies conducted in Vietnam [30,35,36], China [5] and somewhere in the world [4,27], but lower than figures reported from elsewhere as Romania, Egypt, Japan, and Laos [1,3]. This difference may be due to the increasing rate of inappropriate utilization of antibiotics in the farms and humans, which increased the advantage of maintaining resistance genes in bacteria [29,42].

Some researchers reported that the isolates of *Salmonella* from food items were resistant to the commonly used [5,21,38]. The results of the current research also indicated the resistance of *Salmonella* isolates to commonly used antimicrobials, including streptomycin and tetracycline (78.57%). Followed by resistance to chloramphenicol (57.14%), ampicillin and trimethoprim-sulfamethoxazole (50%), gentamicin (14.28%) and ciprofloxacin (7.14%). The high resistance to tetracycline was not surprising since tetracycline is widely used in Vietnam, both in human and veterinary medicine. This finding is in line with previous reports from Romania (66.6% of isolates) [38], Tokyo (77.8% of isolates) [21], Colombia (60.8% of isolates) [11b] and China (66.3% of isolates) [25]. Here, ceftazidime showed a good antimicrobial activity against in all isolates and no cephalosporin-resistant gene was detected. This result is the same as previously reported by Donado-Godogy [11b], Tiziu [38], Andoh [2] and lower than the reports by Katoh [21] from Tokyo who reported a resistance rate of 0.2%, or by Mihaiu from Romania with a resistance rate of 11.4% [28], and Thong (8%) [37]. Even though ceftazidime has been widely available the reason for its effectiveness until this time need investigations. Besides, the resistance to ampicillin and trimethoprim-sulfamethoxazole were found to be 50%, notably higher than the resistance re-

Table 6. Antibiotic resistance gene expression studies contributing to antimicrobial resistance. Numbers express the level of gene expression (fold change*)

	Isolate								
Gen	Salm 1	Salm 6	Salm 12	Salm 5.1	Salm 7.1	Salm 4	Salm 7	Salm 8	Salm 11
tetA	0.54	0.14	0.20	0.58	–	0.66	0.80	1.01	1.01
tetB	–	–	–	–	–	–	–	0.16	1.09
tetC	–	–	–	–	–	–	–	0.44	1.05
sul I	0.27	–	–	–	–	1.03	–	–	–
sul II	0.30	–	1.13	0.52	–	0.22	0.16	0.31	0.36
sul III	–	–	–	0.64	–	–	0.87	0.98	–
sodC1	–	–	0.97	–	–	–	–	1.42	1.67
blaTEM	0.50	0.78	0.77	0.93	–	0.64	0.86	0.89	1.02
gyrB	1.32	0.97	0.98	0.89	0.60	0.91	0.78	0.95	1.00
cmlA	0.62	0.29	0.28	0.76	–	0.64	0.79	1.00	–
cat2	–	–	–	0.84	–	–	0.95	0.92	–
floR	1.44	0.17	–	1.31	1.52	0.83	0.17	0.95	0.87
avrA	1.15	1.03	1.14	1.40	1.40	–	0.47	0.58	0.68
ssaQ	1.15	1.02	1.12	0.81	1.01	0.87	0.89	0.98	0.83
mgtC	0.35	–	1.11	0.76	0.42–	0.64	–	0.84	0.81
aph3-IIa	0.07	–	–	–	–	0.66	–	0.71	1.11
aph A1	–	–	–	–	–	0.67	–	–	1.10
aadA	0.93	0.96	1.15	1.03	1.40	0.58	0.76	0.70	0.64
aac3-IIa	–	–	–	–	–	–	–	0.31	1.07
strA	–	–	0.97	–	–	–	–	–	1.09

* Means of the relative expression of the antibiotic resistant genes and *16S rRNA* gene from all MDR *Salmonella* isolates analyzed as inidicated in Materials and methods description. – Negative gene expression

ported in other countries [4,37,41], may be due to both ampicillin and SXT are frequently used in animal and humans therapy in Vietnam.

CIP-resistant gene (*gyrB*) occurred most frequently in our study, and also detected in both CIP-susceptible and resistant isolates. All of the nine C-resistant and C-susceptible isolates carried *ssaQ* gene. Likewise, all of the nine aminoglycosides resistant and susceptibility isolates carried *aadA* gene. TET-resistant genes were also detected in one TET-susceptible isolate. Therefore, it can be seen that identification of resistance machanisms based on gene expression analysis may be complicated when several mechanisms affect the same class of antibiotics are at work [12]. Thus, the present results and those of Deekshit [11] have agreed that some antimicrobial resistant genes are "silent" in bacteria in vitro; it further provides an indication that these silent genes can spread to other bacteria or turn on in vi, especially under the selection pressure of antibiotic use. Tetracycline resistance genes (*tetA*, *tetB*, and *tedC*) were detected in 88.88%, 22.22% and 22.22% respectively of the isolates. This result is common as *tetA* has been reported to be widely distributed among *Salmonella* isolates of animal origin. Tetracycline is a broad spectrum antibacterial agent commonly used in the treatment of animals and human. Thus the isolates could have acquired these genes from other enteric bacteria via horizontal transfer of plasmids and transposons [8]. The *blaTEM* genes, which code the β-lactamases, have a tendency to mutate and secrete enzymes with the extended spectrum of activity, this could have accounted for the high resistance to ampicillin in the study population [13], and similarly, the *blaTEM* gene was detected in

100% of ampicillin resistant isolates, and one susceptible strain (Salm 1) in the study.

Aminoglycosides, particularly streptomycin and gentamicin are often used for the treatment of animals and humans in Vietnam. This could also explain the moderate to high acquisition and detection of *aadA* gene encoding for aminoglycoside resistance in 100% of the isolates. The *aadA* family of genes encode aminoglycoside-3″-adenylyltransferases, which confer resistance to streptomycin and spectinomycin by adenylation mechnism[38]. In addition, sulfonamide resistance in Gram-negative bacteria arises from the acquisition of either of the two genes, *sul I* or *sul II*, encoding forms of dihydropteroate synthase that are not inhibited by the drug [13]. In the present study, resistance to sulfamethoxazole was highly expressed in *sul II* gene (6 isolates). Genes *sul II* are located on small nonconjugative plasmids [33] or on a large transmissible multi-resistance plasmid. The presence of *sul II* resistance genes may be as a result of successive pressure exerted by sulfonamides and other antimicrobial agents commonly used. These results were similar to previous studies in other European countries [13,22]. *Sul III* is the new sulfonamide resistance gene, has been detected in Gram-negative bacteria such as *Salmonella* [18]. In our study, the *sul III* gene has now been identified in 4 *Salmonella* isolates.

The consumption of sulfonamides for veterinary use is also generally widespread in Vietnam, particularly in the small farmers and directly sells retail meat to the market. Thus, the appearance of a newly described gene and the simultaneous presence of several *sul* genes may reflect antibiotic abuse in local animal husbandry. In addition, the present study found that antimicrobial resistance genes expression did not correlate with the number of antibiotics to which a particular strain showed resistance, e.g., the multidrug-resistant isolates (Salm 5.1, Salm 7 and Salm 8) and (Salm 6, Salm 12) shared identical phenotype profile (AM, S, C, TE, SXT) and (AM, S, TE) respectively, but exhibited different antimicrobial resistance gene profiles. Multiple mechanisms contribute to the development of resistance to antimicrobial agents in *Salmonella*, including enzyme production to inactivate antimicrobial agents, reduction of cell permeability, activation of the antimicrobial efflux pump and drug target modification. Moreover, the resistance genes could be transferred between organisms via transformation, transduction, and conjugation [14] . A large number of genes are inlved in these pathways and in gene regulation. In some cases, gene presence may not relate to the actual resistance phenotype, and a single gene may not solely be responsible for the resistance profiles.

Taken together, the results have shown the various antibiotic resistance genes are widely distributed in the MDR *Salmonella* isolated in retail meat in Hanoi. However, the relationship between the prevalence of an antibiotic resistance gene expression and the antimicrobial resistant phenotype was not clearly defined in some *Salmonella* isolates. Therefore, to control the further emergence of antimicrobial resistance, monitoring the food processing and the prudent use of antibiotics in animal husbandry is essential.

Acknowledgements. This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-NN.04-2015.41. V.T.N. is grateful to the Medical Microbiology Department, Vietnam National Institute of Burns for assistance in Antibiotic susceptibility testing.

Competing interests. None declared.

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