UDC 614:31
DOI: 10.21668/health.risk/2023.1.11.eng

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Research article

STRAINS AND VIRULENCE GENES OF SALMONELLA WITH MULTIDRUG RESISTANCE ISOLATED FROM CHICKEN CARCASSES (HANOI, VIETNAM)

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Salmonella enterica is one of dangerous food-borne pathogens listed by the World Health Organization (WHO). In Vietnam, poultry is one of the most widely eaten meats and is reported as a common source of S. enterica contamination.

The aim of this study was to examine multi-resistant Salmonella strains, to identify susceptibility to antibiotics by using 15 different types of medications and to perform sequencing to analyze antibiotic resistance genes, genotypes, multi-locus sequence-based typing (MLST), and plasmids.

The result of the antibiotic susceptibility test indicated that phenotypic resistance to 9–11 types of antimicrobials was confirmed in all strains. Among 06 sequenced strains, we identified 43 genes associated with antibiotic resistance: strains carrying a range of genes that are associated with aminoglycoside resistance (aac(3), aac(6), ant(3), aph(3), aph(6), aadA); all strains carried blaCTX-M-55 or blaCTX-M-65 gene, which were resistant to the 3rd generation antibiotics; there were also frequently observed sul1, sul2, sul3, tet (A), qnrS1, floR, dfrA14 or dfrA27 genes in sequenced isolates. Besides, the genome sequencing also indicated that all strains carried pathogenicity islands SPI 1, SPI 2, and SPI 3 thereby creating many potential triggers of the disease. Additionally, some carried C63PI, SPI 9, SPI 13, SPI 14, and plus some plasmids such as Col156, IncHI2, IncHI2A, IncFIB, Col (MGD2).

Keywords*: antimicrobials, Salmonella, multidrug resistance, virulence factor, plasmid, chicken, antibiotic resistance gen, Salmonella pathogenicity island (SPI), beta-lactam.*

Salmonella enterica is the common factor that causes foodborne outbreaks worldwide (Center for Emerging and Infectious Diseases 2016). *Salmonella enterica* is further subdivided into six subspecies, which compose more than 2600 serovars in total. Among these six subspecies, *S. enterica* subsp. *enterica* is the main cause of most human salmonellosis cases [1]. The common source of animal-originated food products where *Salmonella* is generally found in poultry, in particular chicken and egg (FAO and WHO 2002) [2].

In low- and middle-incomes countries like Vietnam, to control the contamination of bac-

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teria in livestock, antibiotics have been used widely as an effective solution. Inappropriate usage of antibiotics in agricultural and veterinary practice has led to the rise of new multidrug resistant (MDR) bacteria and transferable genetic loci with this property.

MDR *Salmonella* infection in humans has become a concern to public health agencies. Previous studies reported that the persistence and dissemination of multiple resistant *Salmonella* serovars in the environment are due to the excessive application of antibiotics on land [3]. A recent study of the endemic *Salmonella* distribution in raw meat obtained from traditional markets in Ho Chi Minh city revealed that *Salmonella* isolated from 37.89 % were resistant to at least one antibiotic; 22.98 % were resistant to two to five antibiotics; and 8.70 % were resistant to more than 6 antibiotics [4]. In addition to a high prevalence of *Salmonella* noticed in broiler farms environment, 66.85 % of isolated *Salmonella* exhibited resistance to 2–9 antibiotics. Sixty-two multiple resistance patterns were observed in Mekong Delta, Vietnam [5].

Therefore, it is necessary to study the rate of antibiotic resistance bacterial with all the drug classes, for a better understanding of the link between their phenotype and their genotypes, addressing the mutation that might be responsible for their resistance. This can be done by utilizing different classical molecular typing methods to study the subsequence transmission of antibiotic resistant *Salmonella* in humans, animals and environments. Among other available methods, eligible ones are pulsed-field gel electrophoresis (PFGE) [6] and multi-locus sequence-based typing (MLST) [7].

The impact of antibiotic resistance on human health is a great concern in clinical treatment and agriculture since antibiotics have been used frequently for infection control. However, the limitation of these methods lies in insufficient discriminatory power to separate closely related *Salmonella* isolations in outbreak investigations and to differentiate between the intraserovar isolations from different hosts. The use of whole genome sequencing (WGS) has shown a major impact on the study of molecular epidemiology of AR pathogens [8]. A WGS study in Denmark reported that single nucleotide polymorphisms (SNP), pangenome, k-mer, and nucleotide difference trees were superior to the classical typing method and evaluated the association of the isolates to specific outbreaks of *S. Typhimurium* [9].

The aim of this study was to assess the prevalence of *Salmonella* contamination in chicken and to analyze the antibiotic-resistant genes, genotypes, MLST, virulence factors, and plasmids in WGS of various *Salmonella* serovars isolated from infected samples.

Materials and methods. Six strains in this study were isolated from whole chicken samples, which were collected in Hanoi in September 2019, by following the United States Department of Agriculture (USDA) isolation method MLG 4.10 (USDA 2019)[1].

Antibiotic susceptibility was determined using:

- the Liofilchem discs (Roseto degli Abruzzi (TE), Italy) with the following antibiotics: cefuroxime (CXM, 30 μg), ceftriaxone (CRO, 30 μg), cefoxitin (FOX, 30 μg), cefazoline (CZ, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg);
- the ESBL disc kit according to the recommendations by the Clinical and Laboratory Standards Institute (CLSI)[2]: cefotaxime (CTX, 30 μg); cefotaxime + clavulanic acid (CTL, 30 + 10 μg); ceftazidime (CAZ, 30 μg), ceftazidime + clavulanic acid (CAL, 30 + 10 μg);
- the AmpC disc kit according to the CLSI recommendations[2]: cefotaxime (CTX, 30 μg); cefotaxime 30 μg + cloxacillin (CTC); ceftazidime (CAZ, 30 μg), ceftazidime 30 μg + cloxacillin (CAC), gentamicin (CN, 10 μg), tetracycline (TE, 30 μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (C, 10 μg), ampicillin (AMP, 10 μg), meropenem (MRP, 10 μg), imipenem (IMI 10 μg), nalidixic acid (NA, 30 μg), trimethoprim (TM, 5 μg).

[1] Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges. Laboratory Guidebook. *USDA*, 2019.

[2] Performance Standards for Antimicrobial Susceptibility Testing, 32nd ed. *Clinical and Laboratory Standards Institute (CLSI)*, 2022.

The procedure in brief: prepare *Salmonella* spp. strains suspension ($1.0 \cdot 10^6$ cfu/mL); dip a sterile cotton swab into the standardized bacterial suspension; inoculate the agar by streaking with the swab containing the inoculum; place the antibiotic disk on the surface of the inoculated and dried plate; incubate plates in an inverted position at 37 °C for 16–18 h.

Escherichia coli (ATCC 25922) were used as the quality control standard. *Salmonella* spp. that resisted more than three classes and more than one antibiotic in a single class were designated as a MDR strain.

Genomic DNA was extracted from 1 mL of overnight culture grown in Brain Heart Infusion broth (BHI; BD, USA) using a PureLink™ Genomic DNA Mini Kit (Invitrogen, Thermofisher scientific) according to the manufacturer's protocol. A library was prepared for sequencing and WGS sequencing was performed using the Illumina MiSeq system (Illumina, San Diego, CA, USA), as described by the respective manufacturers.

The raw sequenced reads were analyzed in the *Salmonella* In Silico Typing Resource for serovar identification [10]. ABRicate was applied for screening of the antibiotic resistance genes, plasmid replicon [11]. The antibiotic resistance gene was performed by screening of the draft genome against Resfinder [12], CARD [13] and ARG-ANNOT [14] databases. The search of plasmid replicons was performed by screening of the draft genome against the PlasmidFinder database [15].

The antibiotic resistance profiles of all *Salmonella* isolates are shown in Table 1. These six isolates were multi-resistant to at least 9 of the 15 tested antimicrobials.

The antibiotic susceptibility test indicated that 7 out of 15 antibiotics were 100 % resistant by 6 *Salmonella* strains, including cefuroxime, ceftriaxone, cefazolin, cefotaxime, tetracycline, and ampicillin. Other antibiotics such as trimethoprim, chloramphenicol, and nalidixic acid were also highly resistant by 5 out of 6 isolates.

Four out of six strains were resistant to gentamicin, while half of the total isolates were resistant to ceftazidime. On the other hand, all six strains were susceptible to cefoxitin and ciprofloxacin. Another similar result was obtained in test with two 4th generation antibiotics, imipenem and meropenem, no resistance detected in all 6 strains.

For further genetic analysis, 6 isolates were then sequenced with the next generation sequencing Illumina platform. Quality check showed that sequencing results yield from 441,192 reads of sample 25_S6 to 811,290 reads out of sample 56_S15, with an average read length of around 235–239 bp. After successful assembly, *Salmonella* genome size range from 4.6 million bp to 4.9 million bp with approximately 52 % of GC content as shown in Table 2.

Table 1

Antibiotic resistance profile of *Salmonella* isolates

Samples	CXM	CRO	FOX	CZ	CTX	CAZ	TMP	TE	C	CN	NA	CIP	AMP	IMI	MRP	Resistance, number of drugs
64_S19	R	R	S	R	R	I	S	R	R	R	R	S	R	S	S	9
13_S3	R	R	S	R	R	S	R	R	R	I	R	S	R	S	S	9
25_S6	R	R	S	R	R	R	R	R	R	S	S	S	R	S	S	9
52_S14	R	R	S	R	R	I	R	R	R	R	R	S	R	S	S	10
56_S15	R	R	S	R	R	R	R	R	S	R	R	S	R	S	S	10
21_S5	R	R	S	R	R	R	R	R	R	R	R	S	R	S	S	11
Resistance, number of drugs	6/6	6/6	0/6	6/6	6/6	3/6	5/6	6/6	5/6	4/6	5/6	0/6	6/6	0/6	0/6	

Note: R is resistance, S is sensitivity, I is intermediate state; cefuroxime (CXM), ceftriaxone (CRO), cefoxitin (FOX), cefazolin (CZ), cefotaxime (CTX), ceftazidime (CAZ), trimethoprim (TMP), tetracycline (TE), chloramphenicol (C), gentamicin (CN), nalidixic acid (NA), ciprofloxacin (CIP), ampicillin (AMP), imipenem (IMI), meropenem (MRP).

Table 2

Assembled genome data characteristics

Sample	Readings	Average length	Contigs	Genome length	Average contig length	N50	GS
13_S3	740,518	236	393	4,788,214	116,030	29,823	52.21
21_S5	763,692	239	428	4,931166	146,003	24,548	52.40
25_S6	441,192	235	530	4,878,881	85,034	18,804	52.51
52_S14	676,386	239	506	4,924,654	102,730	22,592	52.54
56_S15	811,290	237	383	4,678,161	262,392	30,011	52.36
64_S19	771,120	237	508	4,918,718	65,335	22,505	52.48

According to *In silico* prediction, the sequenced genomes of MDR isolates were predicted to carry 43 different antimicrobial resistance genes in total (Table 3), which belong to different drug classes (Table 4).

The presence of antimicrobial resistance (AMR) genes in Table 3 demonstrated a close association between genotype and phenotype of six strains analyzed in this study. All of the analyzed strains carried numerously AMR genes, especially genes related to aminoglycoside resistance. There were 17 aminoglycoside resistance genes in total, divided into three resistant mechanisms. All strains carry at least one gene coding for aminoglycoside acetyltransferases, which are *aac(6)-Iaa_1, aac(6)-Ib-cr_1,* and *aadA16_1*. These genes encode aminoglycoside acetyltransferase in *S.* Enteritidis and *S.* Enterica; this enzyme is resistant to aminoglycoside - broad-spectrum antibiotics. Specifically, genes that encode for resistance to aminoglycoside also include *ant(3)-Ia_1* encoding aminoglycoside nucleotidyltransferase (05/06); aph group: *aph(3)-Ib_5, aph(3)-Ia_3, aph(3)-Ia_7, aph(4)-Ia_1,* and *aph(6)-Id_1*, which encode aminoglycoside phosphotransferases (06/06).

The sequenced genome of all six isolates showed the presence of beta-lactam resistance related genes, especially $bla_{\text{CTX-M-55\_1}}$ and $bla_{\text{CTX-M-65\_1}}$. These two genes are involved in resistance of broad-spectrum beta-lactam antibiotic group. Isolate number 56_S15 was predicted to contain gen $bla_{\text{TEM-1B\_1}}$, another gene in beta-lactam resistance gene group. Two out of six strains contained *qnrS1_1* gene. It is considered to be involved in the mechanism of resistance to Fluoroquinolones antibiotics 1 (QnrS1_1 is a plasmid-mediated quinolone resistance protein). These genes were found to be located on mobile genetic elements in those isolates as well.

All six strains carried at least one of two genes (*catA2_1* or *floR-2*) encoding for Chloramphenicol acetyltransferase. Two out of six strains carried *mph(A)_2* gene encoding Macrolide phosphotransferases enzyme. All the sequenced strains carried *tet(A)_6* gene associated with resistance to the tetracycline group. Five out of six strains carried genes (*sul1_5 or sul2_2* or *sul3_2*) related to Sulfonamide resistance by replacing the antibiotic target of Sulfonamide. Two out of six isolates carried the gene *fosA3_1* or *fosA7_1* gene encoding Fosfomycin thiol transferase.

These genes are involved in antibiotic inactivation during resistance to fosfomycin. The genomes of five out of six isolates appeared to carry *dfrA14_5* or *dfrA27_1* gene. These genes are associated with Trimethoprim resistance through the formation of Trimethoprim resistant dihydrofolate reductase Dfr. Three out of six strains were established to have *arr-3_4* or *arr2* gene encoding Rifampin ADP-ribosyltransferase. Two out of six strains turned out to have *lnu(F)_1* gene (equivalent to *lin(F)*), which encodes an integron-mediated nucleotidyltransferase thereby leading to resistance to Lincomycin, and Lindamycin. All strains carried genes associated with multidrug resistance (*golS; mdsA; mdsB; mdsC; mdtK; sdiA; Mrx*).

In silicon serotyping and Multi-Locus Sequence Typing (MLST). The results of MLST analysis showed that the MDR *Salmonella* strains isolated from different areas were clustered into different sequence types and were phenotypically different depending on a serovar, serogroup, and the presence of H and O antigens as well (Table 5).

Table 3

Distribution of antimicrobial resistance genes in *Salmonella* serovars based on *in silico* predictions

Drug classes	Genes	Samples					
		21 S5	25 S6	64 S19	13 S3	52 S14	56 S15
		Number of AMR genes					
		17	18	20	27	27	27
Rifampin	*arr-3_4*						
	arr2						
	arr3						
Aminoglycoside	*aac(3)-IIa*						
	aac(3)-IId_1						
	aac(3)-IVa_1						
	aac(6)-Iaa_1						
	aac(6)-Ib-cr_1						
	aac(6)-Iy						
	aadA1-pm						
	aadA16_1						
	aadA22						
	ant(3)-Ia_1						
	aph(3)-Ib_5						
	aph(3)-Ia_3						
	aph(3)-Ia_7						
	aph(4)-Ia_1						
	aph(6)-Id_1						
Beta-lactam	*bla* $_{\text{CTX-M-55 1}}$						
	bla $_{\text{CTX-M-65 1}}$						
	bla $_{\text{TEM-1B 1}}$						
Diaminopyrimidine	*dfrA14_5*						
	dfrA27_1						
Chloramphenicol	*catA2_1*						
	flor_2						
Fosfomycin	*fosA3_1*						
	fosA7_1						
Lincosamide	*linG*						
	Inu(F)_1						
Multidrug classes	*golS*						
	mdsA						
	mdsB						
	mdsC						
	mdtK						
	Mrx						
	sdiA						
Macrolides	*mph(A)-2*						
Quinolone	*qnrS1_1*						
Sulfonamides	*sul1_5*						
	sul2_2						
	sul3_2						
Tetracyclin	*tet(A)_6*						
	tetR						

Note:

Absence (negative)

Presence (positive)

Table 4

Antimicrobial resistance genes of *Salmonella* isolates

			Drug Class											
Antibiotic Resistance	Strain code	Strain	Aminoglycoside	Beta-lactam	Chloram-phenicol	Quinolone	Macrolides	Tetra-cycline	Sulfo-namides	Fosfo-mycin	Diamino-pyrimidine	Rifampin	Linco-samide	Multidrug classes
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-C-AMP	13_S3	Newport	*aac(3)-IId_1; aac(3)-IIa; aadA22; ant(3'')-Ia_1; aph(3')-Ia_3; aph(6)-Id_1; aac(6')-Iaa_1; aac(6')-Iy;*	*$bla_{CTX-M-55\_1}$; bla_{TEM-1B_1}*	*floR_2*	*qnrS1_1;*	*mph(A)_2;*	*tet(A)_6; TetR*			*dfrA14_5*	*arr-2; arr-3_4*	*lnu(F)_1; linG;*	*golS; mdsA; mdsB; mdsC; mdtK; Mrx; sdiA;*
CXM-CRO-CZ-CTX-CAZ-TM-C-AMP	21_S5	Infantis	*aac(3)-IVa_1; aac(3)-IV; aac(6')-Iaa_1; ant(3'')-Ia_1; aph(4)-Ia_1; aac(6')-Iy; aadA1-pm*	*$bla_{CTX-M-65\_1}$*	*floR_2*			*tet(A)_6; TetR*	*sul1_5;*					*golS; mdsA; mdsB; mdsC; mdtK; sdiA;*
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-C-AMP	25_S6	Infantis	*aac(3)-IVa_1; aac(6')-Iaa_1; ant(3'')-Ia_1; aph(4)-Ia_1; aac(6')-Iy; aadA1-pm;*	*$bla_{CTX-M-65\_1}$*	*floR_2*			*tet(A)_6; TetR*	*sul1_5;*		*dfrA14_5*			*golS; mdsA; mdsB; mdsC; mdtK; sdiA;*
CXM-CRO-CZ-CTX-CAZ-TM-TE-C-AMP	52_S14	Mele-agridis	*aac(3)-IId_1; aac(3)-IIa; aac(6')-Iaa_1; aac(6')-Ib-cr_1; aadA16_1; aph(3'')-Ib_5; aph(6)-Id_1;*	*$bla_{CTX-M-55\_1}$; bla_{TEM-1B_1}*	*catA2_1; floR_2*		*mph(A)_2*	*tet(A)_6; TetR*	*sul1_5; sul2_2;*	*fosA7_1;*	*dfrA27_1*	*arr-3_4; arr-3;*		*golS; mdsA; mdsB; mdsC; mdtK; sdiA; Mrx*
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-C-AMP	56_S15	Muenster	*aac(3)-IId_1; aac(6')-Iaa_1; ant(3'')-Ia_1; aph(3')-Ia_3; aph(6)-Id_1; aac(3)-IIa; aac(6')-Iy; aadA22;*	*$bla_{CTX-M-55\_1}$; bla_{TEM-1B_1}*	*floR_2*	*qnrS1_1;*		*tet(A)_6; TetR*	*sul3_2*		*dfrA14_5*	*arr-3_4; arr-2*	*lnu(F)_1; linG;*	*golS; mdsA; mdsB; mdsC; mdtK; sdiA;*
CXM-CRO-CZ-CTX-CAZ-TM-CN-C-AMP	64_S19	Infantis	*aac(3)-IVa_1; aac(6')-Iaa_1; ant(3'')-Ia_1; aph(3')-Ia_7; aph(4)-Ia_1; aac(6')-Iy; aadA1-pm;*	*$bla_{CTX-M-65\_1}$*	*floR_2*			*tet(A)_6; TetR*	*sul1_5*	*fosA3_1*	*dfrA14_5*			*golS; mdsA; mdsB; mdsC; mdtK; sdiA;*

Table 5

Serotyping and MLST of *Salmonella* isolates

Sample code	Serovar	Serogroup	H1	H2	antigen O	MLST
13_S3	Newport	C2–C3	e,h	1,2	6,8,20	4157
21_S5	Infantis	-	r	1,5	6,7,14	32
25_S6	Infantis	-	r	1,5	6,7,14	32
52_S14	Meleagridis	-	e,h	l,w	3,{10}{15} {15,34}	463
56_S15	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
64_S19	Infantis	-	r	1,5	6,7,14	32

Within these six isolates, 4 MLST were identified. Three out of 6 strains were classified as sequence type (ST) 32. These 3 isolates were also identified as serovar Infantis, which is the most prevalent serovar in this study. Other serotypes found in this study are Newport, which also classified as serogroup C2–C3 (n = 1); Meleagridis (n = 1); and Muenster (n = 1).

Plasmid replicons and *Salmonella* pathogenicity islands (SPIs). We performed In Silico Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. The results are shown Table 6.

Table 6

Plasmid, and *Salmonella* pathogenicity islands (SPIs) of isolates

Strains	Serotype	Plasmid	Number of virulence genes	SPI
13_S3	Newport	Col156 IncHI2 IncHI2A	90	C63PI, S54, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13
21_S5	Infantis	IncF	101	SPI-1, SPI-2, SPI-3, SPI-9, SPI-13
25_S6	Infantis		93	C63PI, S54, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, SPI-14
52_S14	Meleagridis	IncFIB Col(MGD2)	80	C63PI, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9
56_S15	Muenster		82	SPI-1, SPI-2, SPI-3, SPI-9, SPI-13, SPI-14
64_S19	Infantis		93	C63PI, SPI-1, SPI-2, SPI-3, SPI-9, SPI-13, SPI-14

The SPIFinder-2.0 prediction findings demonstrated the widespread presence of SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, and SPI-14; all strains turned out to have SPI-1, SPI-2, SPI-3, and SPI-9. Strains 21 S5, 25 S6, and 64_18 are all Infantis serovars; however, they contain distinct pathogenic islands, and virulence genes due to the difference in collecting places.

The mobile element finder revealed a wide range of plasmid and transposons. The plasmids Col156, IncHI2, IncHI2A, IncFIB, Col(MGD2), IncF are among the expected ones (3/6 strains). The CTX-M 55 or CTX-M 65 genes, which are thought to bear responsibility for resistance to cefotaxime and ceftriaxone, were frequently found in Col156 and IncHI2. These plamids were the most significant plasmid lineage involved in the transmission of antibiotic resistance in *Salmonella*, particularly in *S.* Typhimurium strains. β-lactam (blaOXA-1 and blaTEM-1) and quinolons resistant genes (qnrS1_1 and acc(6′)-ib-cr) were horizontally transferred by IncHI2 plasmid.

Results and discussion. The results of our study focus on the situation of MDR Salmonella strains in Hanoi, Vietnam. The growing number of drug classes that Salmonella is capable to resist has become a threat in Vietnam and all over the world. Report for the current multi-resistance was found in 45/46 studies of Salmonella in poultry; Salmonella strains found in the food chain had high rates of resistance to antibiotics such as nalidixic acid (26.8–86.6 %), ampicillin (14.9–68 %), trimethoprim / sulfamethoxazole (16–54.2 %) and were not inducible to carbapenems such as imipenem and meropenem [16].

The fact that all six analyzed strains harbored the gene $bla_{\text{CTX-M-65}}$ or $bla_{\text{CTX-M-55}}$, demonstrates a very high and widespread level of AmpC and/or ESBL-related gene carrier. The $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-65}}$ genes are associated with antibiotic resistance to a variety of essential drugs, including cefotaxime, ceftriaxone, aztreonam, ceftazidime, amoxicillin, ampicillin, ticarcillin, piperacillin, and cefepime. Interestingly, the phenotypic analysis revealed that all of the tested strains were resistant to cefotaxime, ceftriaxone, ceftazidime, and ampicillin. The prevalence of $bla_{\text{CTX-M}}$ represents a risk of drug resistance when all of these strains are frequently associated with horizontal transmission between strains of the same species as well as between various species via synaptic plasmids or transposons [17]. Although many studies demonstrated the prevalence of *Salmonella* harboring $bla_{\text{CTX-M-55}}$ or $bla_{\text{CTX-M-65}}$ worldwide, however, no similar information is obtained from Vietnam. Our study reported identifying *Salmonella* harboring $bla_{\text{CTX-M-55}}$ or $bla_{\text{CTX-M-65}}$ and co-harboring $bla_{\text{CTX-M-55}}$ or $bla_{\text{CTX-M-65}}$ with bla_{TEM}. On the other hand, Nakayama and others reported extended-spectrum β-lactamase-producing *E. coli* co-harboring $bla_{\text{CTX-M-55}}$ or $bla_{\text{CTX-M-65}}$ with bla_{TEM} isolates in chicken meat in Vietnam [18].

Thus, the presence of SPI enhanced the survivability of *Salmonella* cells and this be-

came the challenge in the MDR *Salmonella* treatment with antibiotics.

Conclusion. *Salmonella* has been a serious threat to public health for a long time, especially with the spread of its multidrug resistance and virulence genes. Results in this study indicated that *Salmonella* strains were able to resist several important antibiotics, which were commonly used in clinical treatment and agriculture, notably the third generation of cephalosporins (ceftriaxone, cefotaxime, and ceftazidime). Additionally, genomic sequencing of six isolates revealed the identification of 43 genes associated with antibiotic resistance. The presence of genes $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-65}}$ (resistant to 3rd generation antibiotics) on *Salmonella* isolated from chickens were confirmed in this study. In addition, the sequenced genomes also demonstrated the variety of SPIs and plasmids in isolated strains.

Funding. The authors acknowledge the National Institute for Food Control, Ministry of Health in Vietnam for funding this project within the Specific Task Program 2019 (Vietnam Ministry of Health, Reg. No. 149/QD-BYT).

Competing interests. The authors declare no competing interests.

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Received: 12.10.2022
Approved: 21.03.2023
Accepted for publication: 24.03.2023