MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

NGUYEN THANH TRUNG

IDENTIFICATION AND ANALYSIS OF PARTICULAR FUNCTIONAL GENES IN *Salmonella* spp. ISOLATED FROM POULTRY MEAT USING NEXT-GENERATION SEQUENCING TECHNIQUE

SUMMARY OF DOCTORAL THESIS IN MICROBIOLOGY

Code: 942 01 07

Hanoi - 2023

This project was completed at: Graduate University of Science and Technology, Vietnam Academy of Science and Technology

Supervisors:
1. Supervisor 1: Assoc.Prof. Ph.D. Pham Xuan Da,Vietnam Nationanal University, Hanoi - School of Medicine and Pharmacy
2. Supervisor 2: Ph.D. Tran Thi Thanh Huyen, Vinmec Research Institute of Stemcell and Gene Technology

Reviewer 1: …….
Reviewer 2: …….
Reviewer 3: …….

The dissertation will be examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at…….. (time, date……)

The dissertation can be found at:
1. Library of the Academy of Science and Technology.
2. National Library of Vietnam.

PREAMBLE

1. The urgency of the thesis

Salmonella is classified among the hazardous foodborne pathogens by the World Health Organization (WHO). *Salmonella* infection not only diminishes productivity and causes avian mortality, but also contaminates the human food chain, thereby resulting in profound economic repercussions within the poultry farming industry, as well as posing a communal health threat. Annually, a significant number of fatalities and a substantial caseload of *Salmonella*-induced illnesses occur globally and in Vietnam. However, as of the current juncture, our nation lacks a comprehensive national-scale surveillance program for *Salmonella* in poultry. The existing studies are localized in nature, conducted in specific regions, and lack representative inclusiveness.

According to previous records, the incidence rate of *Salmonella* infection through oral ingestion was reported to be as high as 95% (Gut et al., 2018). This can be attributed, in part, to the wide range of food products susceptible to *Salmonella* contamination, encompassing fresh produce, processed foods, ready-to-eat meals, and packaged goods, among others. The Centers for Disease Control and Prevention (CDC) in the United States has identified poultry meat and eggs as the primary sources of *Salmonella* contamination, accounting for over 50% of *Salmonella*-positive food samples in the country (FDA, 2018). As of the present time, our country lacks a national-scale surveillance program for *Salmonella* in poultry. Existing studies are limited in scope, conducted in specific regions, and lack representative coverage. Notably, small-scale food safety monitoring initiatives have revealed that 48.7% of collected chicken samples from various cities across the country (Ta et al., 2014) and 49.62% of chicken samples in Ho Chi Minh City (Truong et al., 2021) were found to be infected with *Salmonella*.

Scientists still have not fully comprehended the intricate transmission mechanisms of antibiotic-resistant *Salmonella* among humans, animals, and environmental factors. The spread of antibiotic-resistant *Salmonella*

among humans, animals, and the environment has been investigated in numerous studies utilizing molecular biology techniques such as pulsed-field gel electrophoresis (PFGE) (Scaltriti et al., 2015) and multi-locus sequence typing (MLST) (Achtman et al., 2012). However, these methods still possess limitations in differentiating between closely related strains or *Salmonella* variants, particularly in cases involving outbreak investigations or distinguishing closely related serovars. To overcome these limitations, a more advanced and superior research method known as whole genome sequencing (WGS) has been employed in molecular epidemiology studies of antibiotic-resistant pathogenic microorganisms (Gilchrist et al., 2015). A WGS study conducted in Denmark reported highly effective results using approaches such as single nucleotide polymorphisms (SNP), pangenome, k-mer, and phylogenetic tree analysis in strain classification and assessment of correlations among isolates involved in foodborne outbreaks caused by *S. Typhimurium* (Leekitcharoenphon et al., 2014). In Vietnam, the application of WGS in in-depth studies for serotyping, analysis of antibiotic resistance-related genes, virulence genes, plasmid existence, and pathogenicity island (SPI) profiles of disease-causing microbial strains, especially in *Salmonella* research, remains limited (Gonzalez-Santamarina et al., 2020). Based on these considerations, the dissertation entitled "*Identification and analysis of functional genes in Salmonella* spp. *isolated from poultry using next-generation genome sequencing techniques*" was conducted with the following specific research objectives and content:

2. The purpose and tasks of the research:

Studying the prevalence of *Salmonella* spp. infection in duck and goose meat as well as investigated the relationship between antibiotic resistance genes and detergent resistance genes in *Salmonella* strains isolated from poultry (chicken, duck, and goose) in Hanoi in 2019;

Performing a comprehensive analysis and evaluation of genes associated with antibiotic resistance, genetic mobility, and virulence factors in various strains of *Salmonella* spp.

3. The object and scope of the research

1. To determine the prevalence of *Salmonella* spp. contamination in poultry meat (chicken, duck, and goose) collected from markets in Hanoi city in 2019.
2. To evaluate the level of antibiotic resistance and resistance to sanitizers in *Salmonella* spp. strains isolated from poultry meat (chicken, duck, and goose).
3. To identify antibiotic resistance genes, virulence factors, and the presence of plasmids in selected multidrug-resistant *Salmonella* spp. strains using genomic analysis techniques.

4. The new scientific contributions of the thesis

1- This thesis is the first systematic study on the prevalence of *Salmonella* spp. infection in duck and goose meat as well as investigated the relationship between antibiotic resistance genes and detergent resistance genes in *Salmonella* strains isolated from poultry (chicken, duck, and goose) in Hanoi in 2019.

2- This thesis proved that mobile genetic elements IVSs3, ISEc57, ISEc59, IS102, IS26 carrying several antibiotic resistance genes: *floR*, *qnrS1*, $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M65}}$, *aac (3)*, *aac (4)*

5. The theoretical and practical meaning of the thesis

5.1. Theoretical meaning

- Providing scientific data on the prevalence of *Salmonella* spp. infection in different poultry species, including chicken, duck, and goose, in Hanoi city in 2019;
- Contributing to the understanding of antibiotic resistance profiles and genotypic characteristics of *Salmonella* spp. strains isolated from poultry (chicken, duck, and goose) in Hanoi city;
- Providing data on the resistance ability of these strains to surface-active sanitizers;
- Contributing to the database for further research on risk assessment of *Salmonella* spp. strains in poultry in particular, as well as other foodborne bacteria in general.

5.2. Practical meaning

- The research has assessed the prevalence of *Salmonella* spp. infection in intact chicken, duck, and goose in Hanoi city, as well as the antibiotic resistance profiles of circulating *Salmonella* spp. strains in poultry, both in terms of phenotypic and genotypic characteristics.

- Furthermore, the research findings provide a deeper understanding of the antibiotic resistance of *Salmonella* spp. strains. This contributes to enhancing awareness and promoting appropriate antibiotic and sanitizer usage in poultry farming practices, while actively supporting efforts to ensure food safety in general.

6. The structure and layout of the thesis:

The thesis consists of 184 pages, with a breakdown of the sections as follows: the introduction section spans 4 pages, the literature review section comprises 28 pages, the materials and methods section covers 20 pages, the results and discussion section spans 67 pages, the conclusion and recommendations section is 1 page, the publications section is 1 page, and the reference list spans 25 pages. The dissertation includes 11 tables and 14 figures (excluding appendices).

CHAPTER 1. OVERVIEW

1.1. Biological characteristics of *Salmonella*

1.1.1. general characteristics of Salmonella

1.1.2. Morphological characteristics

1.1.3. Cultural characteristics

1.1.4. Salmonella resistance

1.1.5. Classification of Salmonella

1.1.6. Pathogenicity of Salmonella

1.1.7. Genomic of Salmonella

1.2. *Salmonella* infection, antibiotic resistance, and sanitizer resistance situation

1.2.1. Global situation of infection and antibiotic resistance

1.2.2. Situation of infection and antibiotic resistance in Vietnam

1.2.3. Situation of sanitizer resistance in Salmonella

1.3. Resistance mechanisms in *Salmonella*

1.3.1 Antibiotic resistance mechanisms in Salmonella and the Beta-lactam antibiotic family

1.3.2. Virulence genes, SPI inversion in Salmonella

CHAPTER 2: OBJECTIVE, MATERIALS AND METHODS

2.1. Study subjects, locations, and timeline

2.1.1. Study subjects

- A total of 182 samples of intact chicken, duck, and goose meat were collected from markets in Hanoi.

2.1.2. Study locations

- Sampling locations: 70 markets located in 5 districts of Hanoi city.

- Research conducted at the Department of Microbiology (renamed as the Department of Microbiology and Genetic Engineering since January 1,

2020) of the National Institute of Food Safety and Hygiene Testing, Ministry of Health.

2.1.3. Timeline

- The study was conducted from April 2019 to June 2022:
- Poultry sampling was conducted from September to October 2019.

2.2. Media, Chemical, equipment

2.2.1. Culture media, chemicals

2.2.2. Reference strains

2.2.3. Salmonella antisera.

2.2.4. Antibiotics

2.2.5. Laboratory equipment and apparatus.

2.3. Research methods

2.3.1. Sampling method

Sampling was performed using a cross-sectional study design, and samples were randomly collected.

2.3.2. Isolation and confirmation method for Salmonella spp.

Samples were tested for the presence (detection) of *Salmonella* spp. following the USDA method (MLG 4.04) (Laboratory Guidebook Notice of Change Title: Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges, n.d.) section 4.5.5 (Whole Bird and Parts Rinses).

2.3.3. MALDI-TOF microbial identification technique

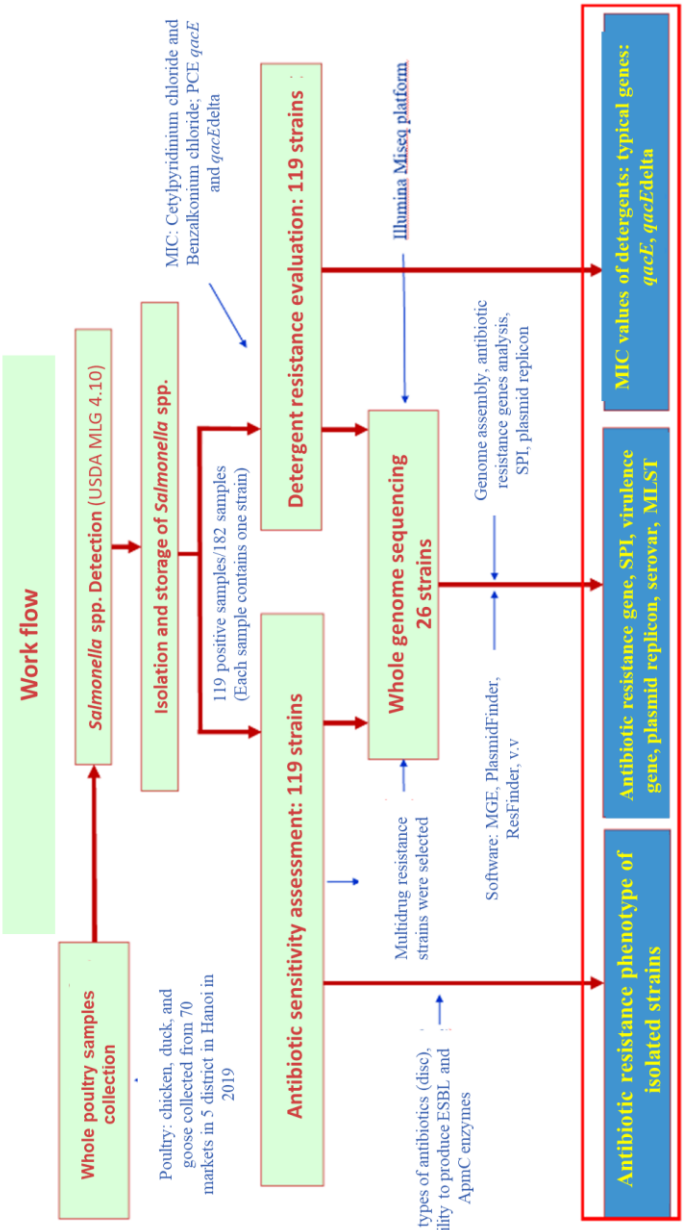
2.3.4. Determination of antibiotic susceptibility

2.3.5. Identification of β-lactamase enzymes in Salmonella

2.3.6. Whole-genome sequencing of isolated Salmonella strains

2.3.7. Analysis of whole-genome sequencing results of Salmonella strains

2.3.8. MIC determination and qac gene analysis of sanitizers


Work flow
Whole poultry samples collection
Poultry: chicken, duck, and goose collected from 70 markets in 5 district in Hanoi in 2019
Salmonella spp. Detection (USDA MLG 4.10)
Isolation and storage of Salmonella spp.
119 positive samples/182 samples (Each sample contains one strain)
MIC: Cetylpyridinium chloride and Benzalkonium chloride; PCE qacE and qacEdelta
Antibiotic sensitivity assessment: 119 strains
Detergent resistance evaluation: 119 strains
Illumina Miseq platform
Whole genome sequencing 26 strains
Multidrug resistance strains were selected
Genome assembly, antibiotic resistance genes analysis, SPI, plasmid replicon
Software: MGE, PlasmidFinder, ResFinder, v.v
15 types of antibiotics (disc), ability to produce ESBL and AmpC enzymes
Antibiotic resistance phenotype of isolated strains
Antibiotic resistance gene, SPI, virulence gene, plasmid replicon, serovar, MLST
MIC values of detergents: typical genes: qacE, qacEdelta

CHƯƠNG 3: RESULTS AND DISCUSSION

3.1. Determination of *Salmonella* spp. contamination rate in poultry meat (chicken, duck, and goose) collected from local stores and supermarkets in Hanoi.

A total of 182 samples, including chicken, duck, and goose meat, were collected from markets in the 5 districts of Hanoi.

All 182 samples were tested using the USDA MLG 4.10 method and simultaneously confirmed positive samples using the Maldi TOF technique (positive confirmed isolates by Maldi TOF). The positive isolates were labelled and stored at -80°C for further studies.

119 *Salmonella* positive samples were tested using the USDA MLG 4.10 method with the typical biochemical characteristics of *Salmonella*: acid production from glucose (+) in Triple Sugar Iron (TSI) test, gas production from glucose (+) in TSI test, acid production from lactose (-) in TSI test, acid production from sucrose (-) in TSI test, hydrogen sulfide production (+) in TSI test, urea hydrolysis (-), lysine decarboxylase (+), β-galactosidase reaction (-), indole production (-), and agglutination inhibition in multi-valent O and H serum testing. Positive *Salmonella* isolates (119 positive samples) were selected for further testing using the Maldi TOF machine to identify the species. The Maldi TOF results showed that 100% of the isolated strains were *Salmonella enterica*. The isolated strains were stored at -80°C for antibiotic resistance, sanitizer resistance, and genomic sequencing experiments. The proportion of positive samples by district is shown in Table 3.1, indicating that most districts had an infection rate higher than 55%. This suggests that over 50% of the consumed poultry is contaminated with *Salmonella* spp. Particularly, Hoang Mai district and Thanh Xuan district had an infection rate of approximately 70%. Despite poultry being typically cooked before consumption, such a high infection rate can easily lead to cross-contamination with other foods and pose a significant risk of food poisoning.

Table 3.1. Salmonella analysis results in chicken, duck, and goose meat by district in Hanoi city in 2019

Distict	Positive sample	Total sample	Positive rate
Ba Đình	19	33	57,58%
Cầu Giấy	24	37	64,86%
Hoàng Mai	26	37	70,27%
Thanh Xuân	27	39	69,23%
Đống Đa	23	36	63,89%
Total	119	182	65,38%

The results showed that the positive rates for chicken, duck, and goose meat were 62.50%, 72.34%, and 64.52% respectively, and the overall positive rate for all three types of poultry was 65.38%.

3.2. Evaluation of antibiotic resistance levels of *Salmonella* spp. isolated from poultry meat

Assessment of the antibiotic resistance capabilities of 119 *Salmonella* strains isolated from positive samples.

3.2.1. Antibiotic susceptibility of Salmonella strains isolated from chicken

The antibiotic susceptibility of *Salmonella* strains isolated from chicken is presented in Table 3.2. Out of the 65 *Salmonella* strains isolated from chicken, 63/65 strains (96.9%) exhibited resistance to at least one of the 15 tested antibiotics. All strains were susceptible to cefoxitin, meropenem, and imipenem. In total, 93.85% of the strains (61/65) were multidrug-resistant (resistant to at least 3 antibiotic classes).

3.2.2. Antibiotic susceptibility of Salmonella strains isolated from duck

The antibiotic susceptibility of *Salmonella* strains isolated from duck is shown in Table 3.2. Out of the 34 *Salmonella* strains isolated, 32/34

strains (94.12%) exhibited resistance to at least one of the 15 tested antibiotics. All *Salmonella* strains isolated from duck were susceptible to cefoxitin, meropenem, and imipenem. In total, 82.35% of the isolated strains (28/34) were multidrug-resistant (resistant to at least 3 antibiotic classes).

3.2.3. Antibiotic susceptibility of Salmonella strains isolated from goose

The antibiotic susceptibility of *Salmonella* strains isolated from goose is presented in Table 3.2. All *Salmonella* strains isolated from goose were susceptible to cefoxitin, meropenem, and imipenem. In total, 95.00% of the strains (19/20) were multidrug-resistant (resistant to at least 3 antibiotic classes).

3.2.4. The antibiotic resistance patterns of Salmonella strains isolated from chicken, duck, and goose meat

The antibiotic resistance capabilities of all *Salmonella* strains isolated from chicken, duck, and goose meat are presented in Table 3.2. Out of a total of 119 *Salmonella* strains isolated, 118/119 strains (99.16%) exhibited resistance to at least one of the 15 tested antibiotics. The antibiotic susceptibility testing results showed the highest resistance pattern to ampicillin (89.08%; 106/119), cefazolin (88.24%; 105/119), tetracycline (87.39%; 104/119); followed by cefuroxime and cefotaxime (79.83%; 95/119); ceftriaxone (78.99%; 94/119), chloramphenicol (77.31%; 92/119), trimethoprim (68.07%; 81/119), nalidixic acid (66.39%; 79/119), gentamicin (57.98%; 69/119), ceftazidime (47.06%, 56/119), and finally ciprofloxacin (5.88%; 7/119). All *Salmonella* strains isolated from poultry meat were susceptible to cefoxitin, meropenem, and imipenem. In total, 90.76% of the strains (108/119) were multidrug-resistant (resistant to at least 3 antibiotic classes). Regarding the production of β-lactamase

enzymes, 94/119 (78.99%) strains were identified as ESBL producers, and 87/119 (73.11%) strains were AmpC enzyme producers.

The minor differences in antibiotic resistance among *Salmonella* strains isolated from chicken, duck, and goose can be explained by their similar rearing and slaughtering conditions. In Vietnam, chickens, ducks, and geese are often raised together, and therefore, they are exposed to similar natural conditions, feed, shared or closely located living spaces, and similar slaughter conditions. This explains why they exhibit similar resistance or susceptibility patterns to the tested antibiotics.

Table 3.2. Table of antibiotic susceptibility results

Antibiotic		**Poultry samples: chickens, ducks and geese**		**Chicken**		**Duck**		**Geese**	
		Number of strains	**Rate (%)**	**Number of strains**	**Rate (%)**	**Number of strains**	**Rate (%)**	**Number of strains**	**Rate (%)**
cefazolin (CZ)	S	2	1,68	1	1,54	1	2,94	0	0,00
	I	12	10,08	4	6,15	5	14,71	3	15,00
	R	105	88,24	60	92,31	28	82,35	17	85,00
cefoxitin (FOX)	S	117	98,32	64	98,46	33	97,06	20	100,00
	I	2	1,68	1	1,54	1	2,94	0	0,00
	R	0	0,00	0	0,00	0	0,00	0	0,00
cefuroxime (CXM)	S	23	19,33	9	13,85	11	32,35	3	15,00
	I	1	0,84	1	1,54	0	0,00	0	0,00
	R	95	79,83	55	84,62	23	67,65	17	85,00
ceftriaxone (CRO)	S	23	19,33	10	15,38	10	29,41	3	15,00
	I	2	1,68	1	1,54	1	2,94	0	0,00
	R	94	78,99	54	83,08	23	67,65	17	85,00
ceftazidime (CAZ)	S	44	36,97	20	30,77	15	44,12	9	45,00
	I	19	15,97	13	20,00	5	14,71	1	5,00

	R	56	47,06	32	49,23	14	41,18	10	50,00
cefotaxime (CTX)	S	23	19,33	9	13,85	11	32,35	3	15,00
	I	1	0,84	1	1,54	0	0,00	0	0,00
	R	95	79,83	55	84,62	23	67,65	17	85,00
ciprofloxacin (CIP)	S	101	84,87	60	92,31	25	73,53	16	80,00
	I	11	9,24	3	4,62	6	17,65	2	10,00
	R	7	5,88	2	3,08	3	8,82	2	10,00
trimethoprim (TM)	S	38	31,93	24	36,92	8	23,53	6	30,00
	I	0	0,00	0	0,00	0	0,00	0	0,00
	R	81	68,07	41	63,08	26	76,47	14	70,00
gentamycin (CN)	S	33	27,73	15	23,08	11	32,35	7	35,00
	I	17	14,29	16	24,62	0	0,00	1	5,00
	R	69	57,98	34	52,31	23	67,65	12	60,00
tetracycline (TE)	S	5	4,20	2	3,08	2	5,88	1	5,00
	I	10	8,40	5	7,69	4	11,76	1	5,00
	R	104	87,39	58	89,23	28	82,35	18	90,00
chloramphenicol (C)	S	27	22,69	9	13,85	9	26,47	9	45,00
	I	0	0,00	0	0,00	0	0,00	0	0,00
	R	92	77,31	56	86,15	25	73,53	11	55,00
	S	13	10,92	5	7,69	7	20,59	1	5,00

ampicillin (AMP)	I	0	0,00	0	0,00	0	0,00	0	0,00
	R	106	89,08	60	92,31	27	79,41	19	95,00
meropenem (MRP)	S	119	100,00	65	100,00	34	100,00	20	100,00
	I	0	0,00	0	0,00	0	0,00	0	0,00
	R	0	0,00	0	0,00	0	0,00	0	0,00
nalidixic acid (NA)	S	17	14,29	7	10,77	7	20,59	3	15,00
	I	23	19,33	8	12,31	9	26,47	6	30,00
	R	79	66,39	50	76,92	18	52,94	11	55,00
imipenen (IMI)	S	119	100,00	65	100	34	100,00	20	100,00
	I	0	0,00	0	0	0	0,00	0	0,00
	R	0	0,00	0	0	0	0,00	0	0,00
Chủng sinh ESBL		94	78,99	54	83,08	23	67,65	17	85,00
Chủng sinh AmpC		87	73,11	54	83,08	18	52,94	15	75,00
Kháng đa kháng sinh		108	90,76	61	93,85	28	82,35	19	95,00

Note: Cefazolin (CZ), cefoxitin (FOX), cefuroxime (CXM), ceftriaxone (CRO), ceftazidime (CAZ), cefotaxime (CTX), ciprofloxacin (CIP), trimethoprim (TMP), gentamicin (CN), tetracycline (TE), chloramphenicol (C), ampicillin (AMP), meropenem (MRP), imipenen (IMI), nalidixic acid (NA), Extended spectrum Beta-lactam (ESBL), AmpC β-lactamase (AmpC), Multi Drug Resistance (MDR)

3.3. Evaluation of the detergent resistance capabilities of select multidrug-resistant *Salmonella* spp. strains

The assessment involved 119 *Salmonella* strains isolated from positive samples to evaluate their resistance to detergents, aiming to assess the spread and complexity of *Salmonella* contamination in agricultural production, particularly in poultry farming and poultry slaughter: determining the presence of two genes, qacE and qacEdelta, and determining the minimum inhibitory concentration (MIC) values of quaternary ammonium compounds (QACs).

The evaluation included the assessment of resistance to two QACs: Benzalkonium chloride (BKC) and Cetylpyridinium chloride (CPCP) at different concentrations, and the results were summarized in Table 3.3.

Table 3.3. Summary of results for determining surface-active resistance of 119 Salmonella strains

	*qac*E	*qac*Edelta
Average MIC value with BKC for gene-carrying strains	20,5	20,5
Average MIC value with CPC for gene-carrying strains	10,5	10,6
Average MIC value resistant to BKC for non-gene-carrying strains	16,3	16,5
Average MIC value resistant to CPC for non-gene-carrying strains	7,2	7,2
Number of gene-carrying strains	73	72
Percentage of gene-carrying strains	61,34%	60,54%
Number of strains carrying both *qac*E and *qac*Edelta genes	71 (59,66%)	

Results showed that out of 119 strains, 71 strains carried both *qac*E and *qac*Edelta. The average resistance values of *qac*E strains to BKC and CPC were 20.5 and 10.5 ppm, respectively. On the other hand, the MIC

values for BKC and CPC in non-gene-carrying strains were 16.3 and 7.2 ppm, respectively, similar to the *qac*Edelta gene.

3.4. Analysis of antibiotic resistance genes in selected *Salmonella* strains isolated

Based on the antibiotic resistance and detergent resistance profiling, 26 strains were selected for whole-genome analysis, including those exhibiting strong and weak resistance patterns.

3.4.1. Salmonella Genomic Characteristics

The sequencing machine produced high-quality results, enabling further downstream analyses.

3.4.2. Serotyping and MLST Results

Two software programs were simultaneously utilized, yielding consistent results. The serovar distribution of the 26 isolated *Salmonella* strains is presented in the following table (Table 3.4)

3.4.3. Antibiotic Resistance Genes

The *Salmonella In Silico* tool was employed to search for antibiotic resistance genes. A total of 82 different antibiotic resistance genes belonging to various gene families were detected in the sequenced strains. The predicted genotypic profiles of antibiotic resistance genes were fully consistent with the observed phenotypic resistance patterns in this study.

Antibiotic resistance genes related to beta-lactam resistance were identified within the genomic repertoire of all 26 *Salmonella* strains in the study. The $bla_{\text{CTX-M-55\_1}}$ gene was present in 15 strains, accounting for 57.69% of the strains. The $bla_{\text{CTX-M-65\_1}}$ gene was found in 5 strains (19.23%). The $bla_{\text{CTX-M-14b\_1}}$ and $bla_{\text{CTX-M-9}}$ and co-occurred in 2 strains (7.69%). However, the $bla_{\text{CTX-M-55\_1}}$ and $bla_{\text{CTX-M-65\_1}}$ as well as the $bla_{\text{CTX-M-14b\_1}}$ and $bla_{\text{CTX-M-9}}$ gene group, did not occur together in any strain. These gene groups were predicted to confer resistance to various antibiotics, including amoxicillin, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, piperacillin, and ticarcillin. In addition, 6 out of 26 strains (23.08%) carried the *ampH* gene, which confers resistance to

beta-lactam antibiotics despite not producing extended-spectrum beta-lactamases (ESBLs).

One notable finding of the study is the presence of genes conferring resistance to the Beta-lactam class of antibiotics on mobile genetic elements. The *bla*$_{\text{CTX-M-65}}$ gene was located on the IS102 genetic element in samples from chickens, ducks, and geese (samples 21, 25, 64, and 109), while the *bla*$_{\text{CTX-M-55}}$ gene was found on the ISKPn19 genetic element along with qnrS1 in sample 55, which also carried the IS6100 mobile genetic element harboring the *mph(A)* gene.

The importance of the research findings is highlighted in the report on antibiotic resistance genes, where the 26 sequenced strains harbored a total of 82 antibiotic resistance genes. All strains carried multiple types of aminoglycoside resistance genes (*aac (3) -Iia, aac (3) -IId_1, aac (6) -Iaa_1* ....). Furthermore, strain 68 carried the rmtB gene encoding the 16S RNA methyltransferase enzyme, which confers resistance to all aminoglycoside antibiotic groups and is of utmost importance in animal husbandry and human disease treatment. Additionally, 24 out of 26 strains with ESBL enzyme phenotypes carried the *bla*$_{\text{CTX-M-55}}$ gene (7 Munster serovars and 1 Kentucky). Surprisingly, strain 68 exhibited five beta-lactam antibiotic resistance genes on a single contig (*bla*$_{\text{TEM-1B}}$; *bla*$_{\text{CTX-M-55}}$; *bla*$_{\text{TEM-206}}$; *bla*$_{\text{TEM-214}}$ và *bla*$_{\text{TEM-141}}$) and sample 51 contained 11 genes related to β-lactam antibiotic resistance, with 10 out of 11 genes located on a single contig (*bla*$_{\text{LAP-2}}$; *bla*$_{\text{TEM-214}}$; *bla*$_{\text{TEM-206}}$; *bla*$_{\text{TEM-33}}$; *bla*$_{\text{TEM-1B}}$; *bla*$_{\text{TEM-216}}$; *bla*$_{\text{TEM-209}}$; *bla*$_{\text{CTX-M-55}}$; *bla*$_{\text{TEM-34}}$; *bla*$_{\text{TEM-210}}$; *bla*$_{\text{TEM-141}}$). This represents the first reported occurrence of this gene cluster in Vietnam.

Table 3.4. Distribution table of serotyping and MLST types of Salmonella spp.

Sample	Poultry	Serovar	Serogroup	H1	H2	O Antigen	MLST
8_S1	Chicken	Agona	B	f,g,s	-	1,4,[5],12	13
12_S2	Chicken	Typhimurium	-	l,v	1,6	3,{10}{15}	155
13_S3	Chicken	Newport	C2-C3	e,h	1,2	6,8,20	4157
19_S4	Chicken	Corvallis	C2-C3	z4,z23	-	8,2	1541
21_S5	Chicken	Infantis	-	r	1,5	6,7,14	32
25_S6	Chicken	Infantis	-	r	1,5	6,7,14	32
52_S14	Chicken	Meleagridis	-	e,h	l,w	3,{10}{15} {15,34}	463
56_S15	Chicken	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
61_S18	Chicken	Typhimurium	-	l,v	1,6	3,{10}{15}	155
64_S19	Chicken	Infantis	-	r	1,5	6,7,14	32
32_S8	Goose	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
37_S9	Goose	Infantis	-	r	1,5	6,7,14	32

74_S1	Goose	Kentucky	C2-C3	i	z6		198
89_S1	Goose	Agona	-	f,g,s	-		13
109_S2	Goose	Infantis	C1	r	1,5		32
129_S3	Goose	Newport	C2-C3	e,h	1,2		4157
146_S4	Goose	Agona	B	f,g,s	-		13
148_S5	Goose	Muenster	E1	e,h	1,5		321
68_S20	Duck	Kentucky	C2-C3	i	z6	8,2	198
43_S11	Duck	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
45_S12	Duck	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
51_S13	Duck	Muenster	E1	e,h	1,5	3,{10}{15} {15,34}	321
31_S7	Duck	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
42_S10	Duck	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
57_S16	Duck	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321
60_S17	Duck	Muenster	-	e,h	1,5	3,{10}{15} {15,34}	321

3.4.4. Gene Clusters

Multiple antibiotic resistance gene clusters were found in the 26 *Salmonella* strains sequenced in the genomic analysis. Using the Mobile Element Finder tool, 50 gene clusters were identified, with each cluster containing 2 to 10 antibiotic resistance genes. Some clusters even carried genes associated with disinfectant resistance, such as *qacE* or *qac*Edelta. One gene cluster in strain S051, specifically contig00317, contained 10 genes related to antibiotic resistance, including genes associated with cephalosporin resistance ranging from generation 1 to generation 3 and 4.

3.5. Results of Plasmid Replicon, Toxin Genes, and SPI Gene Analysis

The findings regarding toxin factors in the samples were diverse, with numerous pathogenic genes involved in the disease-causing process of *Salmonella* in animals and humans, as shown in Table 3.5.

Table 3.5. Distribution of Plasmid Replicon, SPI, and VFs

Strain	Serovar	Plasmid replicon	Number of VFS	Gene number	SPI
12_S2	Typhimurium	IncA/C2 ColRNAI	23	82	CS54, SPI-1, SPI-2, SPI-3, SPI-9, SPI-13
13_S3	Newport	Col156 IncHI2 IncHI2A	26	90	C63PI, S54, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13
19_S4	Corvallis	IncHI2 Col156 IncHI2A	21	83	SPI-1, SPI-2, SPI-3, SPI-5, SPI-9
21_S5	Infantis	IncF	29	101	SPI-1, SPI-2, SPI-3, SPI-9, SPI-13

25_S6	Infantis		30	93	C63PI, S54, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, SPI-14
52_S14	Meleagridis	IncFIB Col(MGD2)	24	80	C63PI, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9
56_S15	Muenster		22	82	SPI-1, SPI-2, SPI-3, SPI-9, SPI-13, SPI-14
61_S18	Typhimurium	IncA/C2 ColRNAI	21	78	SPI-1, SPI-2, SPI-3, SPI-13, SPI-14
64_S19	Infantis		29	93	C63PI, SPI-1, SPI-2, SPI-3, SPI-9, SPI-13, SPI-14
8_S1	Agona	IncL/M IncX1_1	81	21	C63PI, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9
68_S20	Kentucky		23	83	SPI-1, SPI-2, SPI-3, SPI-9
43_S11	Muenster	IncHI2_1 IncHI2A_1	20	75	SPI-1, SPI-2, SPI-3, SPI-9, SPI-13, SPI-14
45_S12	Muenster		21	79	SPI-1, SPI-2, SPI-3, SPI-5, SPI-13, SPI-14
51_S13	Muenster		23	84	C63PI, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13

31_S7	Muenster		24	82	SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, SPI-14
42_S10	Muenster		23	79	C63PI, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, SPI-14
57_S16	Muenster		23	81	C63PI, SPI-1, SPI-2, SPI-3, SPI-13
60_S17	Muenster	IncL/M(pMU407)_1_pMU407	21	72	SPI-1, SPI-2, SPI-3, SPI-13, SPI-14
32_S8	Infantis		21	80	SPI-1, SPI-2, SPI-3, SPI-9, SPI-13
37_S9	Muenster		29	96	CS54-island, SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13
74_S1	Kentucky	ColRNAI_1	28	95	C63PI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9
89_S1	Agona	ColRNAI_1	28	102	C63PI, SGI1, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9
109_S2	Infantis	IncFIB(K)_1_Kpn3	31	100	C63PI, CS54-island, SGI1, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14

129_S3	Newport	IncHI2A_1 IncHI2_1 RepA_1_pKPC-CAV1321	28	94	C63PI, CS54-island, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14
146_S4	Agona	IncI_Gamma_1 IncFII(pHN7A8) 1_pHN7A8 p0111_1	27	99	C63PI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9
148_S5	Muenster		27	98	C63PI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9

This study revealed a diverse and abundant presence of plasmid replicons and SPIs. Several plasmids carrying antibiotic resistance genes were identified. An interesting finding from the genomic analysis was the presence of toxin genes, including *fyuA, ipr2, traT, astA,* and *terC*. This helps explain the pathogenicity and food poisoning potential of *Salmonella*.

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

1. This study determined the prevalence of *Salmonella* spp. contamination in poultry meat (chicken, duck, and goose) collected from markets in Hanoi city as follows: 65.38% of samples were found to be contaminated with *Salmonella* spp. (119/182 samples); the prevalence of *Salmonella* spp. in each poultry group was: chicken 62.50% (65/104), duck 72.34% (34/47), and goose 64.52% (20/31).

2. The antimicrobial and disinfectant resistance profiles of the isolated *Salmonella* spp. strains from poultry meat were assessed. The strains exhibited resistance to various antibiotics and disinfectants,

including ampicillin (89.08%), cefazolin (88.24%), tetracycline (87.39%); cefuroxime and cefotaxime (79.83%); ceftriaxone (78.99%), chloramphenicol (77.31%), trimethoprim (68.07%), nalidixic acid (66.39%), gentamicin (57.98%), ceftazidime (47.06%), and ciprofloxacin (5.88%). Additionally, a correlation has been observed between *Salmonella* strains carrying the *qac*E và *qac*Delta genes and their resistance to the disinfectants Benzalkonium chloride and Cetylpyridinium chloride.

3. Functional genes related to antibiotic resistance in certain *Salmonella* spp. strains have been identified through genomic analysis techniques. A total of 82 genes associated with various types of antibiotics were found. Some genes related to third and fourth generation cephalosporin (Beta-lactam) antibiotic resistance, such as $bla_{\text{CTX-M-55\_1}}$ in 15 strains (57.69%), $bla_{\text{CTX-M-65\_1}}$ in 5 strains (19.23%); $bla_{\text{CTX-M-14b\_1}}$ and $bla_{\text{CTX-M-9}}$ (7,69%, 2/26), as well as $bla_{\text{TEM-33}}$, $bla_{\text{TEM-34}}$, *mcr-3* gene associated with colistin resistance; and *mrtB* gene conferring resistance to all aminoglycoside antibiotics. Additionally, several mobile genetic elements (IVSs3, ISEc57, etc.) carrying important antibiotic resistance genes such as *flo*R, *qnrS1*, $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M65}}$, *aac (3)*, *aac (4have been detected.* The presence of plasmid replicons, including IncA/C, IncHI2, IncL/M, ColRNAI, Col156, IncHI2A, IncF, IncFIB, IncFIB, IncI, has also been observed, indicating the circulation of diverse plasmid types. Moreover, a rich presence of SPIs (*Salmonella* Pathogenicity Islands) and virulence genes has been detected among the isolated strains.

RECOMMENDATIONS

The study investigated the impact of mutations on antibiotic resistance in *Salmonella* strains isolated from poultry in Vietnam.

The expression of virulence genes such as *fyuA, ipr2, traT, astA* and *terC* was examined to assess their toxic properties.

LIST OF PUBLICATIONS

1. **Trung Thanh Nguyen**, Hoa Vinh Le, Yen Thi Ta, Da Xuan Pham, Nam Trung Nguyen (2022). Prevalence and Whole-Genome Analysis of Multidrug-Resistant *Salmonella* Isolated from Chicken Carcasses in Hanoi. *Vietnam Journal of Biotechnology* 20 (4):705-15; https://doi.org/10.15625/1811-4989/17495.

2. **Trung Thanh Nguyen**, Hoa Vinh Le, Yen Thi Ta, Da Pham Xuan, Nam Trung Nguyen, Nguyen Huy Hoang (2022). Characteristic of Multiple-Antibiotic-Resistant *Salmonella enteritica* from Muscovy Duck in Hanoi. *Academia Journal of Biology 44 (4):1-17; https://doi.org/10.15625/2615-9023/17499*.

3. **Trung Thanh Nguyen**, Hoa Vinh Le, Da Pham Xuan, Trung Nghia Vu, Minh Hong Nguyen, Huyen Thi Thanh Tran (2022). Whole-Genome Sequencing of Antimicrobial-Resistance *Salmonella enterica* Isolates from Cairina Moschata Carcass in Vietnam. *Data in Brief, Volume 47, 2023, 108932, ISSN 2352-3409; https://doi.org/10.1016/j.dib.2023.108932*

4. **Trung Thanh Nguyen**, Hoa Vinh Le, Ha Vu Thi Hai, Thanh Nguyen Tuan, Huong Minh Nguyen, Da Pham Xuan, Huyen Tran Thi Thanh, Hao Hong Le Thi (2022). Whole Genome Analysis of Antimicrobial Resistant *Salmonella enteritica* Isolated from Duck Carcasses in Hanoi, Vietnam. *Current Issues in Molecular Biology, 2023, 45(3), 2213-2229; https://doi.org/10.3390/cimb45030143***.**

5. Xuan Da Pham, Hao Le Thi Hong, Huyen Tran Thi Thanh, Long Thanh Le, Hoa Vinh Le, Ninh Hanh Thi, Minh Le Tran, **Nguyen Thanh Trung**. Strains and virulence genes of *Salmonella* with multidrug resistance isolated from chicken carcasses (Ha-noi, Vietnam). *Health Risk Analysis*, 2023, no. 1, pp. 115–123. DOI: 10.21668/health.risk/2023.1.11.eng.