Detection of virulence genes in multidrug-resistant *Salmonella* serovars isolated from different meat sources

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Abstract

The aim of this study was to detect virulence gene expression associated with multidrug-resistant (MDR) *Salmonella* serovars isolated from retail meats in Ha Noi, Vietnam. Six out of nine MDR *Salmonella* was selected for mRNA sequencing to detect virulence genes. All MDR *Salmonella* serovars produced fimbrial adherence determinants (*bcfC*, *fimD*, *safC*, *sthC*, *sthB*, and *mgtB*), nonfimbrial adherence determinants (*ratB*, *shdA*, and *phoQ*), SPI-1 (*hilA*, *invA*, *invC*, *invE*, *prgH*, and *spa*), SPI-2 (*ssaC*, *ssaD*, *ssaN*, *ssaU*, *ssaV*, *sseC*, and *ssrA*), SPI-3 (*misL*), SPI-4 (*siiE*), SPI-9 (*bapA* and *slrP*), TTSS-1 translocated effectors (*sptP*, *sipA*, *sipC*, *sipB*, *sopA*, and *sopB/sigD*), TTSS-2 translocated effectors (*sseJ*), and effectors outside of SPIs and other virulence factors (*cheA*, *flgI*, *flgK*, *flhA*, *flhB*, *fliF*, *fliI*, *iroB*, *iroC*, *iroD*, *iroN*, and *STM0570*). The findings in this study indicate that retail meats tested were widely contaminated with drug-resistant *Salmonella* and various virulence genes are expression among the MDR *Salmonella* serovars isolated from retail meats in Vietnam, suggested high potential for pathogenicity. Further surveillance programs and research are a necessity to understand their epidemiology and to limit the spread of MDR *Salmonella* spp.

Keywords: Retail meats; *Salmonella*; Virulence genes; Multidrug-resistant

1. Introduction

Over the past years, typhoid is a gastrointestinal disease caused by *Salmonella enterica* serovar Typhi (S. typhi). This dangerous disease spreads quickly when germs in the stool of an infected person infect food or drink and spread to others. When food enters the intestines, the bacteria penetrate the intestinal wall and are pooled by macrophages. According to the World Health Organization (WHO), approximately 16-33 million people infected with typhoid, 5-600,000 people die each year, and typhoid is classified as an essential public communicable disease. *Salmonella enterica* belongs to family Enterobacteriaceae (intestinal bacteria) which is a species of gram-negative bacteria with rod-shaped form, anaerobic, movable by flagella, 0.7 µm to 1.5 µm diameter size, and 2 µm to 5 µm lengths. *Salmonella enterica* is divided into six subspecies and more than 2,500 serovars with high sequence similarity. The subspecies I serovar adapted to mammals and poultry hosts, is responsible for more than 99 % of human *Salmonella* infections [1, 2], and the primary source of transmission is from animals to humans and vice versa. Also, infections are more likely to occur via food routes, particularly from eggs and meat from cattle and poultry [3, 4]. Therefore, the presence of *Salmonella* in retail meats in the market is a significant food safety risk [5-7]. In Vietnam, at present, the network of food safety testing has been widely established throughout the country. However, the reality of the capacity to control many food safety indicators in localities is still limited. Out of 330 fruit samples, up to 2.7 % of the samples had a high rate of pesticide residue refers. Notably, among 1,416 meat and meat products, 40.9 % of *Salmonella* infections caused digestive diseases. Moreover, in the major metropolitan areas such as Hanoi and Ho Chi Minh City are the localities with the highest rate of *Salmonella* contaminated food samples.
All of *Salmonella* serotypes carry virulence genes on chromosomes, plasmids, and pre-phages. The majority of these gene clusters are in the SPI genome (*Salmonella Pathogenicity Island*). They are present in all *Salmonella* strains, from the lowest evolutionary group *Salmonella bongori* to the highest evolutionary group *Salmonella enterica*. They help the process of infection and pathogenesis in the intestinal wall of humans and animals, such as adhesion, invasion, intracellular survival, systemic infection, antibiotic resistance, toxin production and absorption of Mg and iron uptake [8]. In addition, all genes except pefA, iroN, cdT, sipB and spaN (invJ) have been shown to be indispensable for the full virulence of *Salmonella* in rat models [9]. However, information about the virulence gene of MDR *Salmonella* isolated from raw chicken, pork, and beef is very limited [7]. Therefore, the objective of this study is to identify the virulent gene profile in multi-drug resistant *Salmonella* spp isolated from retail meat in Hanoi, Vietnam.

2. Material and methods

2.1. Collection and preparation of samples

A total of 25 *Salmonella* serovars (including 2 *S. warragul, 1 S. london, 4 S. derby, 2 S. indiana, 1 S. meleagridis, 1 S. give, 2 S. rissen, 11 S. typhimurium and 1 S. assine) was isolated from meat samples (including beef, pork, and chicken) of retail markets in Hanoi provided by the Microbiology Genetics Laboratory, Genome Research Institute. Chemicals used in the study such as RNA extraction (RNeasy Mini kit, Qiagen, Germany), synthesis of cDNA (Promega kit, USA), PCR reaction component (Thermo Scientific, USA).

2.2. Antibiotic susceptibility tests

This testing has been carried out following the Clinical and Laboratory Standards Institute (CLSI-2015) and used the disk diffusion method as described by Kirby-Bauer. Isolated samples were grown overnight in Brain Heart Broth Infusion (Biolife-Italia) and prepared on Mueller-Hinton agar medium. The antibiotic dishes were added and incubated at 37 ºC for 16-18 hours. We used eight tested antimicrobial drugs which are commonly used in animal husbandry and treatment of animal farms as well as human diseases in Vietnam, such as ampicillin (AM) 10 µg, cefazidime (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 (Diagnosis BD).

2.3. RNA sequencing and virulence gene detection

*Total RNA extraction method:* The strains of *Salmonella* were selected and cultured on Nutrient Agar. Enrichment in the Brain Heart Infusion Broth (BHI) medium was used to obtain *Salmonella* bacteria concentration of 10^8. The process of RNA separation is done according to the instructions of the Mini RNeasy kit (Qiagen, Germany). The process includes: 1 ml of the broth was added into an eppendorf tube of 1.5 ml, centrifuge at 14,000 rpm/ 10 minutes/ 4 ºC. Remove the supernatant liquid, add 1 ml of TRIzol solution, sonicate for 30 seconds to completely dissolve. Add 0.2 ml of chloroform/ 1 ml of TRIzol. Vortexing in 15 seconds, centrifuge 14,000 rpm/ 10 minutes/ 4 ºC. Transfer to a new 1.5 ml eppendorf tube, precipitation of RNA with Isopropanol (Isopropyl alcohol, C_3H_7OH). Add 0.6 ml of Isopropanol, then lightly invert 6 - 8 times. Incubate the tube at -20 ºC/ overnight. Centrifugal 14,000 rpm/ 10 minutes/ 4 ºC, washing with 75 % Ethanol. The RNA sample was tested for concentration, purity (OD 260/280) on the visible spectrophotometer (NanoDrop) and the 5S, 18S and 28S deposition coefficients of rRNA by electrophoresis on 1 % agarose gel. Correct the RNA sample (RNAadj) to a concentration of 1 µg/µl. Store the product at -80 ºC until the next steps.

*Synthesis of cDNA:* All of steps to synthesize cDNA are performed according to the instructions of Promega Manufacturer (United States). The process as follows: RNA samples were fully dissolved with room temperature. Denaturation the RNAadj (2 µl) at 70 ºC/ 5 minutes, immediately put into ice for 5 minutes. Prepare a synthetic mixture of cDNA including: 5 X buffers (4 µl); 0.1 MDTT (2 µl); dNTP (2 µl); Random Primer (2 µl); RNase Inhibitor (0.5 µl); M MLV-RT (0.8 µl) and DEPC-DW (6.7 µl). The amount of the above components is calculated for 1 sample. Allow 18 µl of cDNA + 2 µl RNAadj mixture to incubate at 37 ºC/ 60 minutes. Continue to denature the product at a temperature of 95 ºC/ 5 minutes, take out immediately put in the ice box for 5 minutes. The cDNA product is stored at -20 ºC until further steps are taken.

*RT-PCR:* The reaction for detecting virulent genes was performed with reaction components according to instructions of use of Thermor Fisher Scientific, USA: 2 µl Dream Taq Buffer 10X; 1 µl of dNTP mixture (2.5 pmol/µl); 0.5 µl downstream (10 pmol/µl); 0.5 µl of primer (10 pmol/µl); 0.15 µl Taq polymerase (5 U/µl); 3 µl of cDNA sample (1 µg/µl); Add deionized water to give a total reaction volume of 20 µl. Thermal cycle: 94 ºC/ 4 minutes. (94 ºC/ 30 seconds; priming temperature/ 45 seconds; 72 ºC/ 1 minute) x 25 cycles. The virulence factor genes were analyzed using virulence factor database (VFDB) (Chen et al., 2005).

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2.4. Statistical analyses

The expression levels of the genes were analyzed by Quantity One analysis program (Gel Doc 1000, version 4.6.3, Bio-Rad, Hercules, CA). The data are statistically calculated by the one-way variance analysis (One-way ANOVA). Significant differences between virulence genes and 16S rRNA genes of Salmonella strains were analyzed by coefficients in Tukey's Multiple Regression Test with P < 0.05 difference (GraphPad Prism version 5.01).

3. Results

3.1. Antibiotic resistance of Salmonella isolates

Our experiment carried out the antibiotic resistance of twenty-five Salmonella serovars obtained. The result showed that all strains were sensitive to ceftazidime (CAZ) with 52.0% of the strain isolates were inhibited at least one antibiotic drug (data not shown). Total 9 Salmonella serovars showed the multiple antimicrobial resistance such as 1 S. meleagridis; 1 S. derby; 1 S. give; 3 S. typhimurium; 1 S. warragal; 1 S. indiana; and 1 S. rissen. Notably, all of 8 antibiotics were resistant by S. indiana strain isolated from chicken (Table 1).

**Table 1** Susceptibility results of multidrug-resistant Salmonella isolates

| Salmonella serovar | Antibiotics | Ratio R/S |
|--------------------|-------------|-----------|
| iniana (Salm 4)    | R           | 7/1       |
| rissen             | S           | 4/4       |
| warragal           | S           | 3/4       |
| typhimurium S384   | R           | 6/2       |
| give (Salm 7)      | R           | 5/3       |
| meleagridis        | R           | 5/3       |
| derby (Salm 6)     | R           | 3/5       |
| typhimurium S181   | R           | 3/5       |
| typhimurium S360   | R           | 5/3       |

Ratio R/S: 7/0 0/0 2/7 8/1 1/0 7/2 9/0 7/2

Abbreviations: R (resistant); S (sensitive)

In addition, S. typhimurium S384 and S. rissen showed resistance to 6 and 4 types of antibiotics (11.11 %), respectively. The multi-antibiotics resistance (AM, S, C, TE, SXT) was found in 3 strains of S. meleagridis; S. give; and S. typhimurium S360; accounting for 33.33 % of the number of isolated strains (Table 2).

**Table 2** Multi-antimicrobial drugs resistance of Salmonella serovars

| Number of antimicrobial resistance | Three | Four | Five | Six | Seven | Total |
|-----------------------------------|-------|------|------|-----|-------|-------|
| Antimicrobial pattern resistance  | C,TE, SXT | C, CE, SXT | AM, S, C, TE, SXT | AM, GN, S, C, C, TE, SXT | AM, GN, S, CIP, C, TE, SXT | AM, GN, S, CIP, C, TE, SXT |
| Number of isolates (%)            | 3 (33.33%) | 1 (11.11%) | 3 (33.33%) | 1 (11.11%) | 1 (11.11%) | 9 (100%) |
3.2. *In silico* virulence gene analysis

The expression (+) and not expression (-) of genes associated with multidrug-resistant *Salmonella* virulence were determined in six *Salmonella* isolates: Salm 4 (*S. indiana*), Salm 6 (*S. derby*), Salm 7 (*S. give*), Salm 8 (*S. typhimurium S360*), Salm 11 (*S. typhimurium S384*), and Salm 12 (*S. typhimurium S181*). A total of 96 virulence genes expression was detected by BLAST search against Virulence Factor Database by Bioedit software [10]. In particular, 73 virulence genes were identified in Salm 4, 73 in Salm 6, 77 in Salm 7, 68 in Salm 8, 77 in Salm 11, and 76 in Salm 12 (Figure 1).

![Figure 1 Heatmap of virulence genes expression in six *Salmonella* isolates. In this figure, each row represents a sample ID and each column represents a virulence gene. Legend description: Blue color (expression), grey color (not expression).](image)

Homologs of 48 virulence genes were identified in multidrug-resistant *Salmonella* spp., including: fimbrial adherence determinants (*bcfC, fimD, safC, stbC, sthE, sthC, sthB, and mgtB*), nonfimbrial adherence determinants (*ratB, shdA*, and *phoQ*), SPI-1 (*hilA, invA, invC, invE, invG, prgH*, and *spaS*), SPI-2 (*ssaC, ssaD, ssaN, ssaU, ssaV, sseC*, and *ssrA*), SPI-3 (*misL*), SPI-4 (*siiE*), SPI-9 (*bapA*), slrP, TTSS-1 translocated effectors (*sptP, sipA, sipC, sipB, sopA*, and *sopB/sigD*), TTSS-2 translocated effectors (*sseJ*), and effectors outside of SPIs and other virulence factors (*cheA, flgI, flgK, flhA, flhB, flfF, filI, iroB, iroC, iroD, iroN*, and *STM0570*). A list of the virulence genes is shown in Table 3.

The results indicated that virulence determinants located in SPIs 1–3, SPI-9 were highly conserved. In most isolates all SPI-1 genes were expression. Three strains of *S. typhimurium* (Salm 8, Salm 11, and Salm 12) were deficient *invJ* and *sipD* genes. Four isolates (Salm 4, Salm 8, Salm 11, and Salm 12) lacked *saal* (Secretion system apparatus) from SPI-2. Virulence determinants of SPI-3, SPI-4 and SPI-9 were conserved in all isolates. Gifsy-1-associated gene *gogB* were present in only two isolates (Salm 8 and Salm 12).

**Table 3** Distribution of virulence genes across multidrug-resistant isolate

| Virulence factors | Related genes | Salm 4 | Salm 6 | Salm 7 | Salm 8 | Salm 11 | Salm 12 |
|-------------------|---------------|--------|--------|--------|--------|---------|---------|
| **Fimbrial adherence determinants** | *bcfC* | + | + | + | + | + | + |
| | *bcfD* | - | - | + | - | - | - |
| | *fimD* | + | + | + | + | + | + |
| | *lpfD* | - | - | - | + | + | + |
| | *lpfC* | - | - | - | + | + | + |
| | *pegD* | - | - | + | - | - | - |
| | *pegC* | - | + | + | - | - | - |
| | *safC* | + | + | + | + | + | + |
| | *staG* | - | + | + | - | - | - |
| | *staC* | - | + | + | - | - | - |
| | *stbD* | + | + | - | + | + | + |
| | *stbC* | + | + | + | + | + | + |
| | *stcC* | - | - | - | + | + | + |
| | *stdB* | - | - | - | + | + | + |
|                      | Number of genes (%) | Number of genes (%) | Number of genes (%) | Number of genes (%) | Number of genes (%) | Number of genes (%) |
|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                      |          13/25        |          17/25        |          16/25        |          17/25        |          17/25        |          16/25        |
| Magnesium uptake     |                      |                      |                      |                      |                      |                      |
| mgtB                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         |
| Nonfimbrial          |                      |                      |                      |                      |                      |                      |
| adherence            |                      |                      |                      |                      |                      |                      |
| determinants         |                      |                      |                      |                      |                      |                      |
| ratB                 | +         (100)        | +         (100)        | +         (100)        | +         (90)        | +         (100)        | +         (100)        |
| shdA                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| shsC                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 3/3         | 3/3         | 3/3         | 2/3         | 3/3         | 3/3         |
| phoQ                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| hilA                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| invA                 | +         (90)         | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| invC                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| invE                 | +         (87.5)       | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| invG                 | +         (90)         | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| invF                 | +         (64)         | +         (87.5)       | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| prgH                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| sipD                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| spaS                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 10/10       | 9/10        | 10/10       | 8/10        | 8/10        | 8/10        |
| Regulation           |                      |                      |                      |                      |                      |                      |
| ssaC                 | +         (87.5)       | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| ssaD                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| ssaL                 | -         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| ssaN                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| ssaU                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| ssaV                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| ssaC                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| ssaA                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 7/8         | 8/8         | 8/8         | 7/8         | 7/8         | 7/8         |
| SPI-2                |                      |                      |                      |                      |                      |                      |
| misL                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         |
| SPI-3                |                      |                      |                      |                      |                      |                      |
| siiE                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         |
| SPI-4                |                      |                      |                      |                      |                      |                      |
| bapA                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         |
| SPI-9                |                      |                      |                      |                      |                      |                      |
| sspH2                | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         |
| SPI-12               |                      |                      |                      |                      |                      |                      |
| sfrP                 | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        | +         (100)        |
| Number of genes (%)  | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         |
| TTSS effectors       |                      |                      |                      |                      |                      |                      |
| translocated via     |                      |                      |                      |                      |                      |                      |
| both systems         |                      |                      |                      |                      |                      |                      |
| Number of genes (%)  | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         | 1/1         |
| Virulence factors          | Gifsy-1 | TTSS-1 translocated effectors | TTSS-2 translocated effectors | Effectors outside of SPIS and other virulence factors |
|---------------------------|---------|-------------------------------|-------------------------------|-------------------------------------------------------|
|                          | gogB    | Number of genes (%)           | Number of genes (%)           | Number of genes (%)                                    |
|                          |         | 0/1 (0)                        | 0/1 (0)                        | 0/1 (0)                                               |
|                          |         | 0/1 (0)                        | 0/1 (0)                        | 0/1 (100)                                            |
|                          |         | 1/1 (100)                      | 1/1 (100)                      | 1/1 (100)                                            |
|                          |         |                                |                                |                                                       |
| spsP                      | +       | +                              | +                              | +                                                      |
| sipA                      | +       | +                              | +                              | +                                                      |
| sipC                      | +       | +                              | +                              | +                                                      |
| sipB                      | +       | +                              | +                              | +                                                      |
| sopA                      | +       | +                              | +                              | +                                                      |
| sopB/sigD                 | +       | +                              | +                              | +                                                      |
| sopD                      | +       | +                              | +                              | -                                                      |
|                          | 7/7 (100)| 7/7 (100)                      | 7/7 (100)                      | 6/7 (85.71)                                           |
|                          | 2/4 (25)| 2/4 (25)                       | 2/4 (75)                       |                                                       |
|                          | 2/4 (50)| 2/4 (50)                       |                                |                                                       |
| pipB2                     | -       | -                              | +                              | +                                                      |
| sifA                      | -       | -                              | +                              | -                                                      |
| sseJ                      | +       | +                              | +                              | +                                                      |
| sseK1                     | -       | -                              | -                              | -                                                      |
|                          | 1/4 (25)| 1/4 (25)                       | 3/4 (75)                       | 2/4 (50)                                              |
|                          | 2/4 (50)| 2/4 (50)                       |                                |                                                       |
| clfV                      | +       | +                              | -                              | +                                                      |
| cnf                       | -       | -                              | -                              | +                                                      |
| cheA                      | +       | +                              | +                              | +                                                      |
| cheB                      | +       | +                              | -                              | -                                                      |
| flgE                      | +       | +                              | -                              | +                                                      |
| flgI                      | +       | +                              | +                              | +                                                      |
| flgK                      | +       | +                              | +                              | +                                                      |
| flsA                      | +       | +                              | +                              | +                                                      |
| flsB                      | +       | +                              | +                              | +                                                      |
| flsC                      | -       | -                              | -                              | +                                                      |
| flsD                      | +       | -                              | +                              | +                                                      |
| flsF                      | +       | +                              | +                              | +                                                      |
| flsI                      | +       | +                              | +                              | +                                                      |
| flsK                      | -       | -                              | +                              | +                                                      |
| flsM                      | +       | -                              | +                              | -                                                      |
|                          | 27/32 (84.38)| 24/32 (75)                      | 25/32 (78.13)                    | 19/32 (59.38)                                         |
|                          | 27/32 (84.38)| 27/32 (84.38)                      |                                |                                                       |
4. Discussion

This study showed that SPI-located genes were highly conserved in all strains, while pro-phages were generally variable among the strains and serotypes, as reported previously [11]. A core set of fimbrial genes (bcfC, fimD, safC, stbC, sthE, sthC, sthB, and mgtB) were detected in all strains which could contribute to the colonization in a broad host range, while presence of particular fimbrial genes in some isolates, probably contribute to host specificity [11, 12]. All multidrug-resistant *Salmonella* strains were showed presence of SPI-3 *misL* gene, contrasting with previous reports [13]. The spv (*Salmonella* plasmid virulence) has been found only in a small fraction of the subspecies I serovars such as *abortusovis, cholerasuis, dublin, gallinarum, paratyphi C, sendai, enteritidis* and *typhimurium* [14]. In this study, no spv gene expression was found, contrasting with previous reports [13]. *Salmonella* has numerous virulence-associated genes found within clusters in its genome, which are known as *Salmonella* Pathogenicity Islands [15]. To date, 23 SPIs have been reported although the functions of virulence genes contained within each island have not yet been fully elucidated [16, 17]. SPIs are playing a underlying role in pathogenesis and host specificity [18]. While SPI-1 and SPI-2 have been studied in depth, other SPIs have been detected recently and their distribution across *Salmonella* serovars and the role they play in disease much less is known about. The existence of SPIs in the genomes of pathogenic bacteria distinguishes them from closely related nonpathogenic strains or species [19]. Therefore, to better understand the virulence genes expression of multidrug-resistant *Salmonella* strains, we examined the virulence genes content in six multi-antimicrobial resistance *Salmonella* isolates. All of the virulence factors analyzed in multidrug-resistant *Salmonella* in this study as listed in Table 3. Although these results confirmed the variable presence among isolates, they did not indicate a clear origin-related distribution for any of the aforementioned genes.

The major virulence factors in *Salmonella* was identified in all our isolates including adherence, SPI-1, SPI-2, SPI-3, SPI-4 and SPI-9. Interestingly, SPI-5 which was considered to be the major pathogenicity islands in *Salmonella* was found to be absent in all isolates. The genetic islands are situated on the chromosome or on plasmids of bacteria, however, not all bacteria possess every known SPI. SPI-1 through SPI-5 are common among all *S. enterica*. The major pathogenicity islands encompass SPI-1, SPI-2, SPI-3, SPI-4, and SPI-5. The SPI-1 and SPI-2 genes code for proteins forming the type III secretion system (T3SS) which enable the transport of *S. enterica* proteins from the bacterial cell directly into the cytosol of eukaryotic cells [20]. In addition, it has been reported that SPI-1 genes, independent of cell invasion, induce macrophage cytotoxicity [21]. SPI-2 encoded T3SS is required for the transport of *S. enterica* proteins across the phagosomal membrane and increases *S. enterica* survival inside phagocytic cells [22, 23]. The function of genes located on the remaining SPIs is less well defined; SPI-3 genes are involved both in gut colonization due to MisL-dependent fibronectin binding and intracellular survival due to high-affinity magnesium transport encoded by *mgtABC* [24, 25]. SPI-4 genes are required for the intestinal phase of disease by coding for non-fimbrial adhesin [26]. We have identified the expression of a homolog of 48 virulence genes. The common expression of homologs virulence factors in all of the isolates suggests that they might be globally-required by multidrug-resistant *Salmonella* serovars for underscoring potential host-pathogen interactions. The high frequency of the presence of the virulence genes that were investigated highlights the pathogenic potential of the studied *Salmonella* strains, which have been causing disease in humans and contaminating food in Vietnam.

5. Conclusion

Our data manifested a global core of virulence gene expression, which might be required for *Salmonella* pathogenesis. This is the first study aimed to characterize multidrug-resistant *Salmonella* virulence factors expression from Vietnam. The result of this research indicated that retail meats play a role in harboring drug-resistant *Salmonella* serovars. Furthermore, the exhibiting multi-antibiotic resistant and virulence genes of *Salmonella* isolates create a possible risk for humans from consumer products. Therefore, the using of antimicrobial agents in the livestock and poultry industry needs to be strictly controlled. This aims to prevent the acquisition and increase resistance to recent molecules in order to fight against the vertical and horizontal transfer of MDR strains. In addition, the government also needs to build and promote the development of effective intervention strategies for the food supply chain for people to reduce the risk of foodborne illness.

Compliance with ethical standards

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Disclosure of conflict of interest
No disclosure of any conflict of interest.

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