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CHARACTERISTIC OF MULTIPLE-ANTIBIOTIC-RESISTANT *Salmonella enteritica* FROM MUSCOVY DUCK IN HANOI

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ABSTRACT

Nowadays, as the global population grows, the demand for food is also becoming higher each day. Together with the rise in food demand, Muscovy duck has been gradually bred industrially as a poultry food supply along with the chicken. The change from traditional to industrial breeding poses a potential risk of pathogenic bacteria infection and antimicrobial resistance bacteria. Especially *Salmonella*, one of the leading pathogens worldwide, is also notable for its antimicrobial resistance. In this study, by using Muscovy duck carcasses collected from wet markets in 05 districts in Ha Noi, we assessed the rate of *Salmonella* infection at first, then conducted an antibiotic susceptibility test utilizing 15 types of antibiotics, from then whole genome sequencing was applied for 8 multidrug resistant isolates. Next, the genomic data after successfully sequenced was used for analyzing antibiotic resistance genes, genotypes, multi-locus sequence-based typing (MLST), virulence factors, and plasmids. 65% of Muscovy duck samples were positive for *Salmonella*, in which 95% (19/20 strains) of *Salmonella* isolated was multidrug resistant. The result of the antibiotics susceptibility test indicated that phenotypic resistance to ampicillin was the most observed (92.3%, 19/20), followed by tetracycline (90%, 18/20), cefuroxime (85%, 17/20), cefazolin (85%, 17/20), ceftriaxone (85%, 17/20), Cefotaxime (85%, 17/20), trimethoprim (70%, 14/20), gentamicin (60%, 12/20), chloramphenicol (55%, 11/20), nalidixic acid (55%, 11/20), ceftazidime (50%, 10/20), ciprofloxacin (2/20). However, all isolates were susceptible to cefoxitin and meropenem. Sixty-five antibiotic resistance genes were identified, including genes that are resistant to aminoglycoside, 3rd generation antibiotics (cefotaxime, cefoperazone, ceftizoxime, ceftazidime, ceftriaxone, etc.). Col, IncA plasmids and some mobile genetic elements were identified. Simultaneously *Salmonella* pathogenic islands were found in all sequenced strains, exclusively SPI 1, SPI 3, and SPI 9 were carried in every isolate.

Keywords: Whole-genome sequencing, *Salmonella*, antimicrobial resistance, virulence factor, serovar, Muscovy duck.

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INTRODUCTION

The increasing human population around the world has placed a huge demand on food in order to feed every individual. This exerts pressure on a number of food industries such as poultry production systems, where growth promotion agents are used in an effort to satisfy the rising food demand. Together with the rising demand for poultry production, consumers, poultry industry professionals, and scientists are also directing their attention toward safety requirements due to the presence of bacterial hazards. Muscovy duck is one of the less often consumed meats in the European Union and the US. However, Muscovy duck is more popular in Asia culture, where its consumption has been increasing in recent years (Gilbert et al., 2018). The prevalent presence of *Salmonella* in poultry meat and its related products has often been addressed as a risk for human consumption (CDC, 2014). *Salmonella* is classified as one of the most common zoonotic foodborne pathogens that cause outbreaks in humans all over the world (CDC, 2022; EFSA & ECDC, 2018; FAO & WHO, 2002, 2009). *Salmonella* spp. may cause systemic infections, particularly in children and immuno-compromised persons, including symptoms such as fever, diarrhea, nausea, abdominal pain, vomiting, and sometimes septicemia, which are characteristic of healthy adult individuals (FAO & WHO, 2002). More than 2600 pathogenic *Salmonella* serotypes have been identified (Lamas et al., 2018), nevertheless, only a minor portion of them is found in poultry regularly. In 2021, a total of 90,105 cases of human salmonellosis were reported by the EU EFSA (EFSA & ECDC, 2021). A survey conducted in the United Kingdom showed that the rate of contaminated duck meat with *Salmonella* spp. is 29.0% and much higher than chicken (5.0%) or meat of other poultry species (8.0%) (Little et al., 2008). In particular, a study in Trinidad and Tobago revealed the rate of *Salmonella* infection in the collected samples from farmed Muscovy duck was 40% (44/110) (Rampersad et al., 2008). Despite the major serovars identified in Rampersad's study (Rampersad et al., 2008) were Kiambu, Orion, Uganda, it still posed an issue of controlling the food safety of this particular product, especially in Vietnam where Muscovy duck has not been a common object for *Salmonella* monitoring.

In Vietnam, most slaughter points (98% at home and 100% at markets) are in the category of low veterinary hygiene according to the Government's regulations on veterinary hygiene for poultry slaughter points. *Salmonella* infection rates were 29.2% in viscous nest samples, 40.6% in carcasses; 2.9% in feed water, 80.6% in wastewater; 30.6% in the floor and 63.9% in slaughter tools. Among the subjects of particular concern is *Salmonella* contaminating carcass at a high rate, which detected 2 serotypes (*Salmonella enteritidis* and *Salmonella typhimurium*) belonging to the group of microorganisms at risk of food poisoning, derived from poultry and contaminated during slaughter (Nguyen et al., 2012). Such an increase in the consumption of Muscovy duck meat suggests that epidemics of salmonellosis will occur in humans. This highlights that Muscovy duck carcasses and related products are becoming more popular among consumers and are often associated with outbreaks of salmonellosis in humans (Noble et al., 2012). The foodborne transmission of *Salmonella* spp. from contaminated Muscovy duck meat has been recognized as an important hazard to human health over the past few decades, and strains of *Salmonella* spp. bacteria that caused disease have long been considered a serious animal-to-human hazard.

Along with the rise of Muscovy duck demand publicly, commercial Muscovy ducks are now being raised as chickens in industrial conditions, thus the risk of *Salmonella* is the same as for chickens. As a consequence of industrial raising, the widespread use of antibiotics may be an important cause in the development and transmission of resistance

determinants from Muscovy ducks to humans across the food chain (Ljubojević Pelić et al., 2021). The emergence of drug-resistant strains of bacteria is another health concern worldwide. Surveillance results of prescription and multidrug-resistant *Salmonella* strains can be used to establish a selection guideline for antimicrobial therapy (Nguyen et al., 2021). Nowadays, Whole genome sequencing (WGS) has had a great influence on the molecular epidemiology of antibiotic-resistant bacteria from human, animal, and environmental sources (Leekitcharoenphon et al., 2014). In addition, WGS can accurately predict antibiotic susceptibility patterns, and virulence genes, determine serotypes, and provide multiple databases for a single *Salmonella* strain that can also be used to predict antibiotic resistance genes in *Salmonella* spp. WGS has begun to streamline the identification of *Salmonella* spp. in the laboratory into a microbiological procedure that can replace traditional standards such as phenotypic, serotype, and genotypic determination (Anhalt & Fenselau, 1975). Therefore, we conducted this study in order to assess the level of *Salmonella* contamination in Muscovy duck, which is most relevant to *S. enteritica*. The main objectives of this research aim to characterize the sequenced genomes and identification of antibiotic resistance genes of *Salmonella* isolate from Muscovy duck carcasses in wet markets in Ha Noi utilizing the power of WGS (Gonzalez-Santamarina et al., 2020).

MATERIALS AND METHODS

Sampling

In 2019, 31 samples of Muscovy duck carcass were collected from wet markets in Hanoi. Samples were placed in a sterilized bag and delivered to the Food microbiology and Genetically modified food Laboratory, National Institute for Food Control, Vietnam.

Detection of *Salmonella* spp.

Salmonella spp. was detected by using the USDA method (MLG 4.10) (USDA, 2019). Isolated strains were further confirmed using the Maldi TOF technique on the Vitek MS system. *Salmonella* strains were stored at -80 ^{o}C for further analyses (Hasman et al., 2019). *S. typhimurium* WDCM 00031 and *S. enteritidis* WDCM 00030 were used as the quality control standard.

Antibiotic susceptibility test

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), ESBL disc kit (acc. to CLSI): cefotaxime (CTX, 30 µg); cefotaxime + clavulanic acid (CTL, 30 + 10 µg); ceftazidime (CAZ, 30 µg), ceftazidime + clavulanic acid (CAL, 30 + 10 µg), AmpC disc kit: 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) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Wayne, PA, USA) [34]. Briefly, prepare *Salmonella* strains suspension ($1{,}0 \times 10^{6}$ 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 ^{o}C for 16–18 h. *Escherichia coli* (ATCC 25922) was used as the quality control standard. *Salmonella* spp. resisted more than three classes and more than one antibiotic in a single class was designated as an MDR strain.

Whole genome sequencing of *Salmonella* strains

Eight multidrug resistance isolates were randomly selected among 20 isolates for whole genome sequencing (WGS). Genomic DNA was extracted from overnight culture BHI broth 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.

Analysis of raw whole genome sequence

The raw sequenced reads were analyzed in the *Salmonella in Silico* Typing Resource for serovar identification (Yoshida et al., 2016). Abricate (Seemann, 2016) was applied for screening antibiotic resistance genes, plasmid replicons, and virulence genes. The antibiotic resistance gene was performed by screening the draft genome against Resfinder (Zankari et al., 2012), CARD (McArthur et al., 2013), and ARG-ANNOT (Gupta et al., 2014) databases. The search for plasmid replicons was performed by screening the draft genome against the PlasmidFinder database (Carattoli et al., 2014). The virulence genes were performed by screening the draft genome against Virulence Factors Base (VFDB) (Chen et al., 2005).

RESULTS

Phenotype of antibiotic resistance

The prevalence of *Salmonella* from Muscovy duck carcass samples was 65% in total. The antibiotic resistance results of all isolates are shown in Figure 2. Among 20 isolated *Salmonella* strains, 95% (19/20 strains) of them were resistant to at least three of the 15 tested antimicrobials. There are some images of antibiotic susceptibility test in Figure 1.

The result of the antibiotic susceptibility test indicated that phenotypic resistance to ampicillin was the most observed (92.3%, 19/20), followed by tetracycline (90%, 18/20), cefuroxime (85%, 17/20), cefazolin (85%, 17/20), Ceftriaxone (85%, 17/20), Cefotaxime (85%, 17/20), Trimethoprim (70%, 14/20), gentamicin (60%, 12/20), chloramphenicol (55%, 11/20), nalidixic acid (55%, 11/20), ceftazidime (50%, 10/20), ciprofloxacin (2/20). However, all isolates were susceptible to cefoxitin and meropenem.

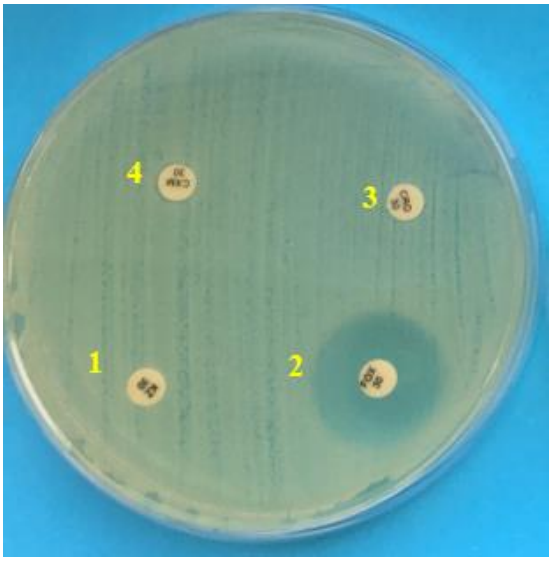

Figure 1. Antibiotic susceptibility test result of a Salmonella isolate resistance to cefazolin, cefuroxime, ceftriaxone, cefotaxime, ceftazidime and susceptible to cefoxitin: (1) CZ, diameter 6 mm (≤ 14 mm, Resistance); (2) FOX, diameter 28 mm (≥ 18 mm, Susceptible); (3) CRO, diameter 6 mm (≤ 14 mm, Resistance); (4) CXM, diameter 6 mm (≤ 14 mm, Resistance)

Fifteen isolates (75%) have the ability to synthesize AmpC β-lactamase enzyme, and seventeen isolates (85%) were identified as ESBL strains. In total, 95% of strains (19/20) were considered multidrug resistance strains.

Whole-genome sequence of multidrug resistance *Salmonella* strains was submitted to the SRA database, and after assembly, genomic data statistics showed in Table 1.

The genome length of 8 isolates ranges from 4,707,459 bp to 5,068,882 bp. *De novo* assembly with velvet algorithm yielded from 173 contigs to 443 contigs, and N50 is in the 24,717–79,467 bp range.

In Silico predictions, antibiotic resistance genes were identified by screening the draft genomes using Resfinder (Fig. 2 and Table 2).

In Silico prediction, the sequenced genomes of MRD isolates were predicted to carry 65 different antimicrobial resistance genes in total (Fig. 3), which belong to 16 different drug classes.

Table 1. Whole genome sequencing characteristics

Sample	Contigs	Genome Length	N50	GC
32_S8	383	4,707,459	29,742	52.38
37_S9	443	4,923,944	24,717	52.39
89_S1	319	5,020,645	33,336	51.63
74_S1	149	4,835,519	79,467	50.47
148_S5	154	4,722,564	66,521	51.55
109_S2	230	4,968,615	49,847	52.00
129_S3	173	4,838,537	59,252	51.67
146_S4	179	5,068,882	73,813	51.24

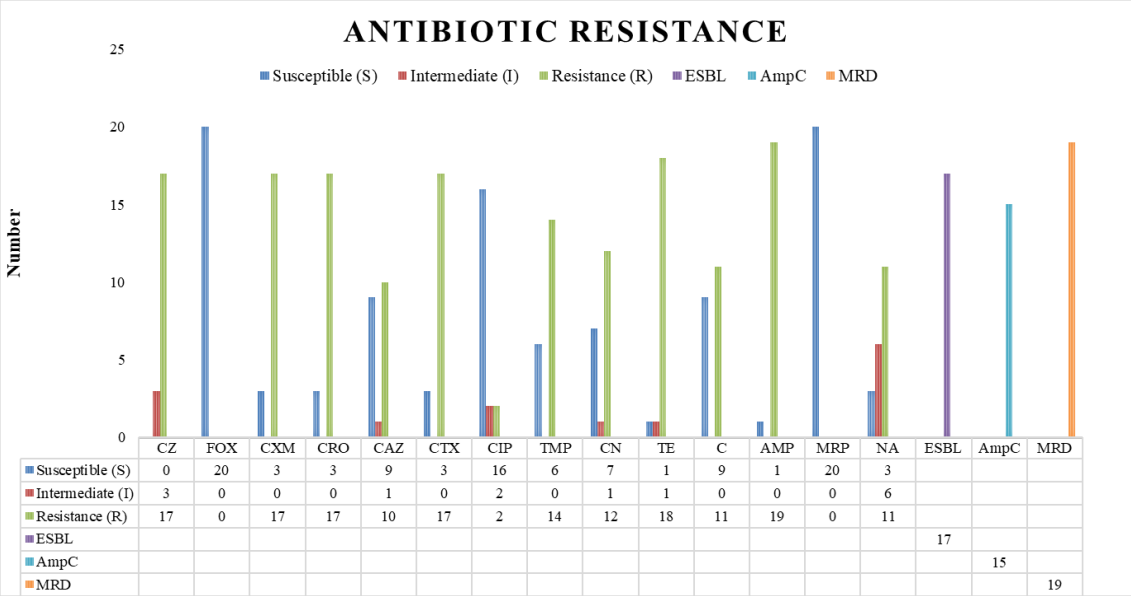

Figure 2. Antibiotic susceptibility of *Salmonella* isolates. Cefazolin (CZ), Cefoxitin (FOX), Cefuroxime (CXM), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Ciprofloxacin (CIP), Trimethoprim (TMP), Gentamicin (CN), Tetracyscline (TE), Chloramphenicol (C), Ampicillin (AMP), Meropenem (MRP), Nalidixic acid (NA), Extended spectrum Beta-lactam (ESBL), AmpC, Multi Drug Resistance (MDR)

Table 2. Antimicrobial resistance gene in *Salmonella* isolates

Antibiotic Resistance	Code strain	Strains	Drug Classes										
			Aminoglycoside	Aminocoumarin	Beta-lactam	Chloramphenicol	Fluoroquinolone + Quinolone	Macrolides + Fosfomycin	Tetracyclin + Nitroimidazole	Diaminopyrimidine + Sulfonamides	Rifampin	Lincosamide + polypeptide+	Multi-drug classes
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-AMP	32_S8	Infantis	*aac(3)-Iia;* *aac(3)-IId_1;* *aac(6)-Iaa_1;* *aac(6)-Iy;* *aadA22;* *ant(3)-Ia_1;* *aph(6)-Id_1*		*bla*$_{CTX-M-55\_1}$; *bla*$_{TEM-1B\_1}$		*qnrS1_1*		*tet(A)_6;* *tetR*	*dfrA14_5;* *sul3_2*	*ARR-3_4;* *ARR-2*	*lnu(F)_1;* *linG*	*golS;* *mdsA;* *mdsB;* *mdsC;* *mdtK;* *sdiA*
CXM-CRO-CZ-CTX-CAZ-TM-TE-AMP	37_S9	Muenster	*aac(3)-IVa_1;* *aac(6)-Iaa_1;* *aac(6)-Iy;* *aadA1-pm;* *ant(3)-Ia_1;* *aph(4)-Ia_1*		*bla*$_{CTX-M-65\_1}$				*tet(A)_6;* *tetR;*	*sul1_5;* *dfrA14_5*			*golS;* *mdsA;* *mdsB;* *mdsC;* *mdtK;* *sdiA*
CXM-CRO-CZ-CTX-CAZ-CN-TE-AMP	74_S1	Kentucky	*aac(3)-Id_1;* *aadA7_1;* *aph(6)-Id_1;* *aph(3)-Ib_5;* *aac(6)-Iaa_1;* *aph(3)-Ia_7;* *acrD*	*mdtB; mdtC;* *baeR;*	*bla*$_{CTX-M-14b\_1}$; *ampH;* *bla*$_{CTX-M-9}$		*emrB;* *emrA;* *emrR*		*tet(A)_6;* *msbA*	*sul1_5*		*yojI; bacA*	*H-NS;* *acrB;* *acrA;* *marA;* *golS;* *mdsA;* *mdsB;* *mdsC;* *CRP;* *cpxA;* *tolC;* *mdtK;* *sdiA;*

Antibiotic Resistance	Code strain	Strains	Drug Classes										
			Aminoglycoside	Aminocoumarin	Beta-lactam	Chloramphenicol	Fluoroquinolone + Quinolone	Macrolides + Fosfomycin	Tetracyclin + Nitroimidazole	Diaminopyrimidine + Sulfonamides	Rifampin	Lincosamide + polypeptide+	Multi-drug classes
CXM-CRO-CZ-CTX-TM-TE-C-AMP	89_S1	Agona	*aph(3)-Ia_7; aph(3)-Ib_5; aph(6)-Id_1; aadA7_1; aac(3)-Id_1; aac(6)-Iaa_1; aph(3)-IIa_2; kdpE; acrD*	*mdtB; mdtC; baeR*	*bla*$_{CTX-M-14b\_1}$; *bla*$_{CTX-M-9}$; *ampH*		*emrB; emrA; emrR*		*tet(A)_6; msbA*	*sul1_5*		*yojI; bacA*	*H-NS; sdiA; marA; CRP; cpxA; mdsA; mdsB; mdsC; golS; acrB; acrA; tolC; mdtK;*
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-AMP	109_S2	Infantis	*aac(6)-Iaa_1; ant(3)-Ia_1; aph(4)-Ia_1; aac(3)-IVa_1; acrD; aac(6)-Iy*	*mdtB; mdtC; baeR*	*bla*$_{CTX-M-65\_1}$	*floR_2*	*emrB; emrA; emrR*		*tet(A)_6; msbA*	*sul1_5; dfrA14_1*		*yojI; bacA*	*H-NS; cpxA; mdsA; mdsB; mdsC; golS; acrB; acrA; tolC; mdtK; CRP; marA; sdiA;*

Antibiotic Resistance	Code strain	Strains	Drug Classes										
			Aminoglycoside	Aminocoumarin	Beta-lactam	Chloramphenicol	Fluoroquinolone + Quinolone	Macrolides + Fosfomycin	Tetracyclin + Nitroimidazole	Diaminopyrimidine + Sulfonamides	Rifampin	Lincosamide + polypeptide+	Multi-drug classes
CXM-CRO-CZ-CTX-TM-CN-TE-C-AMP	129_S3	Newport	*aac(6)-Iaa_1; aph(3)-Ia_3; aph(6)-Id_1; ant(3)-Ia_1; acrD; aac(6)-Iy; kdpE; ant(3'')-IIa*	*mdtB; mdtC; baeR;*	*$bla_{CTX-M-55\_1}$; bla_{TEM-1B_1}; ampH; bla_{LAP-2}*	*floR_2;*	*emrB; emrA; emrR; qnrS1_1*	*mph(A)_2*	*tet(A)_6; msbA*	*sul3_2; dfrA14_5*	*ARR-3_4; ARR-2*	*lnu(F)_1; linG; yojI; bacA*	*H-NS; marA; acrA; acrB; cpxA; mdsA; mdsB; mdsC; golS; CRP; tolC; mdtK; sdiA; ramA;*
CXM-CRO-CZ-CTX-CAZ-CN-TE-C-AMP	146_S4	Agona	*aph(3)-IIa_2; aph(3)-Ib_5; aph(6)-Id_1; aac(6)-Iaa_1; acrD; aac(6)-Iy; kdpE*	*mdtB; mdtC; baeR*	*$bla_{CTX-M-55\_1}$; bla_{TEM-1B_1}; ampH*	*floR_2;*	*emrB; emrA; emrR; qnrS1_1;*	*fosA7_1;*	*tet(A)_6; msbA;*	*sul2_2; dfrA14_5*		*yojI; bacA*	*H-NS; marA; tolC; acrA; acrB; golS; mdsA; mdsB; mdsC; CRP; mdtK; cpxA; sdiA; ramA*
CXM-CRO-CZ-CTX-CAZ-TM-CN-TE-C-AMP	148_S5	Muenster	*aac(6)-Iaa_1; aph(6)-Id_1; aac(6)-Iy; acrD; kdpE*	*mdtB; mdtC; baeR*	*$bla_{CTX-M-55\_1}$; ampH*	*floR_2*	*emrB; emrA; emrR; qnrS1_1*		*tet(A)_6; msbA*	*sul3_2; dfrA14_5*	*arr-3_4; arr-2;*	*bacA*	*H-NS; acrA; acrB; mdsA; mdsB; mdsC; golS; marA; mdtK; sdiA; tolC; CRP; cpxA*

Drug classes	Sample	32_S8	37_S9	74_S1	89_S1	109_S2	129_S3	146_S4	148_S5
	Genes/Number of genes	24	17	34	36	33	44	38	34
Aminoglycoside	*aac(3)-Iia*	+	−	−	−	−	−	−	−
	aac(3)-IId_1	+	−	−	−	−	−	−	−
	aadA22	+	−	−	−	−	−	−	−
	aadA1-pm	−	+	−	−	−	−	−	−
	aac(3)-Id_1	−	−	+	+	−	−	−	−
	aadA7_1	−	−	+	+	−	−	−	−
	aph(6)-Id_1	+	−	+	+	−	+	+	+
	aph(3)-Ib_5	−	−	+	+	−	−	+	−
	aac(6)-Iaa_1	+	+	+	+	+	+	+	+
	aph(3)-Ia_7	−	−	+	+	−	−	−	−
	acrD	−	−	+	+	+	+	+	+
	aph(3)-IIa_2	−	−	−	+	−	−	+	−
	kdpE;	−	−	−	−	−	+	+	+
	ant(3)-Ia_1	+	+	−	−	+	+	−	−
	aph(4)-Ia_1	−	+	−	−	+	−	−	−
	aac(3)-IVa_1	−	+	−	−	+	−	−	−
	aac(6)-Iy	+	+	−	−	+	+	+	+
	aph(3)-Ia_3	−	−	−	−	−	+	−	−
	ant(3'')-Iia	−	−	−	−	−	+	−	−
Aminocoumarin	*mdtB*	−	−	+	+	+	+	+	+
	mdtC	−	−	+	+	+	+	+	+
	baeR	−	−	+	+	+	+	+	+
Beta-lactam	$bla_{\text{CTX-M-14b\_1}}$	−	−	+	+	−	−	−	−
	bla_{ampH}	−	−	+	+	+	+	+	+
	$bla_{\text{CTX-M-9}}$	−	−	+	+	−	−	−	−
	$bla_{\text{CTX-M-65\_1}}$	−	+	−	−	+	−	−	−
	$bla_{\text{CTX-M-55\_1}}$	+	−	−	−	−	+	+	+
	$bla_{\text{TEM-1B\_1}}$	+	−	−	−	−	+	+	−
	$bla_{\text{LAP-2}}$	−	−	−	−	−	+	−	−
Chloramphenicol	*floR_2*	−	−	−	−	+	+	+	+
Quinolone	*qnrS1_1*	+	−	−	−	−	+	+	+
Fluoroquinolone	*emrB*	−	−	+	+	+	+	+	+
	emrA	−	−	+	+	+	+	+	+
	emrR	−	−	+	+	+	+	+	+
Macrolides	*mph(A)_2*	−	−	−	−	−	+	−	−
Tetracyclin	*tetR*	+	+	−	−	−	−	−	−
	Tet(A)_6	+	+	+	+	+	+	+	+
Sulfonamides	*sul1_5*	−	+	+	+	+	−	−	−
	sul3_2	+	−	−	−	−	+	−	+
	sul2_2	−	−	−	−	−	−	+	−
Diaminopyrimidine	*dfrA14_1*	−	−	−	−	+	−	−	−
	dfrA14_5	+	+	−	−	−	+	+	+
Fosfomycin	*fosA7_1*	−	−	−	−	−	−	+	−
Rifampin	*ARR-3_4*	+	−	−	−	−	+	−	+
	ARR-2	+	−	−	−	−	+	−	+
Polypeptide	*yojI*	−	−	+	+	+	+	+	−
	bacA	−	−	+	+	+	+	+	+
Nitroimidazole	*msbA*	−	−	+	+	+	+	+	+
Lincosamide	*lnu(F)_1*	+	−	−	−	−	+	−	−
	linG	+	−	−	−	−	+	−	−
Multi-drug classes	*H-NS*	−	−	+	+	+	+	+	+
	acrB	−	−	+	+	+	+	+	+
	acrA	−	−	+	+	+	+	+	+
	marA	−	−	+	+	+	+	+	+
	golS	+	+	+	+	+	+	+	+
	mdsA	+	+	+	+	+	+	+	+
	mdsB	+	+	+	+	+	+	+	+
	mdsC	+	+	+	+	+	+	+	+
	CRP	−	−	+	+	+	+	+	+
	cpxA	−	−	+	+	+	+	+	+
	tolC	−	−	+	+	+	+	+	+
	mdtK	+	+	+	+	+	+	+	+
	sdiA	+	+	+	+	+	+	+	+
	ramA	−	−	−	−	−	+	+	−

+ Positive − Negative

Figure 3. Distribution of antimicrobial resistance genes in *Salmonella* serovars based on *in Silico* predictions

All strains carry at least one gene coding for Aminoglycoside acetyltransferases, which are *aac(6)-Iaa_1* and *aac(6)-Iy*. These genes are chromosomal-encoded 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 are also included *ant(3)-Ia_1* - encoding for aminoglycoside nucleotidyltransferase (4/8); aph group: *aph(3)-Ib_5*, *aph(3)-Ia_3*, *aph(3)-Ia_7*, *aph(4)-Ia_1*, and *aph(6)-Id_1* - encoding for Aminoglycoside phosphotransferases (8/8). All isolates carried aph group genes, and had at least one *aph* gene. The sequenced genome of all 8 isolates showed the presentation of beta-lactam resistance related genes in 6 out of 8 isolates, especially *blaCTX-M-55_1*, *blaCTX-M-65_1 and two isolates carry blaCTX-M-14b-1*. These three genes are involved in the resistance of the broad-spectrum beta-lactam antibiotic group. Notably, there were 3 isolates that are susceptible to beta-lactam and carry the $bla_{\text{TEM-1B\_1}}$ gene. This gene encodes for the enzyme lactamase class A-lactamase. The resistance to various antibiotics, including amoxicillin, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, piperacillin, and ticarcillin, will be predicted by this group of genes. 04/08 strains contained *qnrS1_1* gene. It is conferred to involve in the mechanism of resistance to fluoroquinolones antibiotics 1 (QnrS1_1 is a plasmid-mediated quinolone resistance protein). Moreover, there were several mutations identified in sequenced isolates. For example isolate numbers 74 (serovar Kentucky) and 89 (serovar Agoda), there were detected to contain *gyrA*:p.D87G, *parC*:p.S80I, *gyrA*:p.S83F, and *parC*:p.T57S mutations. The mutation *parC*:p.T57S was identified in three other isolates, which were number 109, 146, and 148, making it the most prevalent mutation detected in this study. *gyrA*:p.D87Y was also found in isolate number 109. Those mutations caused impacts on phenotypic resistance to nalidixic acid and ciprofloxacin.

In addition, 04/08 strains carried gene *floR-2* encodes for Chloramphenicol acetyltransferase. 01/08 strains carried *mph(A)_2* gene encoding for enzyme Macrolide phosphotransferases. All 8 strains carried *tet(A)_6* genes, involving resistance with the tetracycline group. 08/08 strains carried genes (*sul1_5* or *sul2_2* or *sul3_2*) related to Sulfonamide resistance by replacing the antibiotic target of Sulfonamide.04/08 isolates carried the gene *fosA3_1* or *fosA7_1* gene, encoding for Fosfomycin thiol transferase. These genes are involved in antibiotic inactivation during the resistance to fosfomycin. The genome of 06/08 isolates appeared to carry *dfrA14_5* or *dfrA14_1* gene. These genes are involved in Trimethoprim resistance through the formation of Trimethoprim resistant dihydrofolate reductase Dfr. 03/08 strains showed to have *arr-3_4* gene, encoding Rifampin ADP-ribosyltransferase. 02/08 strain showed to have *lnu(F)_1* gene (equivalent with *lin(F)*), which encodes for an integron-mediated nucleotidyltransferase, resulting in resistance to lincomycin, lindamycin. All strains carried genes associated with multidrug resistance (*golS*; *mdsA*; *mdsB*; *mdsC*; *mdtK*; *sdiA*; *Mrx*).

In Silico serotyping and Multi-Locus sequence typing

The results of Multi-Locus Sequence Typing (MLST) analysis showed that the MDR *Salmonella* strains isolated from different areas were clustered into different sequence types, and phenotypically different in terms of serovar, serogroup and the presence of H and O antigens as well (Table 3).

Within these 8 isolates, 5 MLST were identified. The MLST result was quite evenly distributed with three sequence types (ST) 32, 321, and 13 identified in two isolates for each. The serovars were Infantis, Muenster, and Agona respectively. Other serotypes found in this study are Kentucky (ST 198) and Newport (ST 4157).

The phylogenetic tree was built using Fastree. *Salmonella* strains isolated from Muscovy duck were located together with the strains isolated from duck and chicken in the original study.

Table 3. Serovar and Muti-Locus sequence typing results

Sample	Serovar	Serogroup	H1	H2	O Antigen	MLST
32_S8	Infantis	-	e,h	1,5	3,{10}{15}{15,34}	32
37_S9	Muenster	-	r	1,5	6,7,14	321
74_S1	Kentucky	C2-C3	i	z6	-	198
89_S1	Agona	-	f,g,s	-	-	13
109_S2	Infantis	C1	r	1,5	-	32
129_S3	Newport	C2-C3	e,h	1,2	-	4157
146_S4	Agona	B	f,g,s	-	-	13
148_S5	Muenster	E1	e,h	1,5	-	321

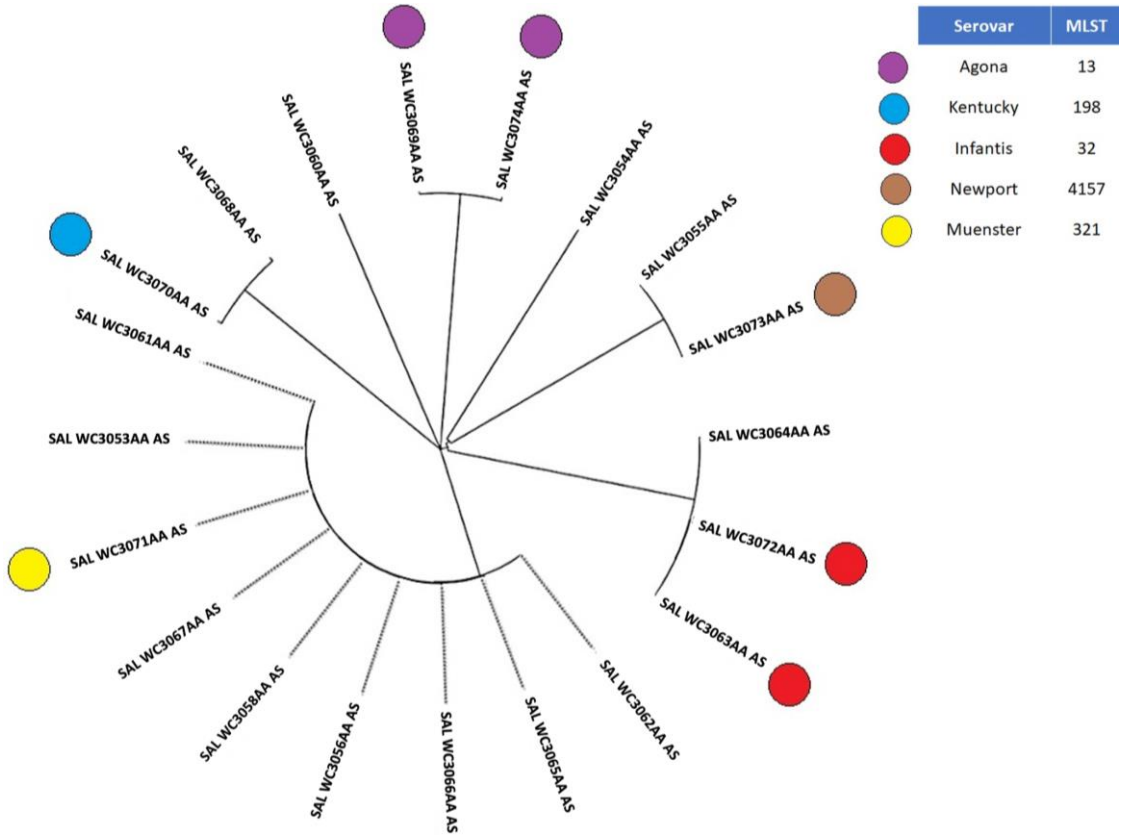

Figure 4. Phylogenetic tree based on core genome alignment representation

Plasmid replicons and virulence gene

In Silico Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing showed results in Table 4.

In addition, using VFDB with Abricate resulted in isolates carrying between 80 and 102 virulence genes and containing 21-29 virulent factors. The SPIFinder-2.0 prediction findings demonstrate the widespread presence of SPI-1, SPI-2, SPI-3, SPI-5, SPI-9, SPI-13, and SPI-14, of which all strains have SPI-1, SPI-3, and SPI-9. There were two isolates belonging to each serovar Infantis, Muenster, and Agona, however, isolates within the same serovar contain distinct pathogenic islands, virulent factors, and virulence genes due to the difference in collecting places.

Table 4. Plasmid, virulence factors, and SPIs results

Strains	Serotype	Plasmid	Numbers of virulence factors	Number of virulence genes	SPIs
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

The mobile genetic element finder (version v.1.0.3, database v.1.0.2, https://cge.food.dtu.dk/services/MobileElementFinder/) revealed results with a wide range of plasmids, transposons, and insertion sequences. ColRNAI_1, IncFIB(K)_1_Kpn3, IncHI2A_1, IncHI2_1, RepA_1_pKPC-CAV1321, IncI_Gamma_1, IncFII(pHN7A8)_1_pHN7A8, and p0111_1 were predicted plasmids that 5 out of 8 strains carried. The *CTX-M 55* and *CTX-M 65* genes, which were conferred to resistance to cefotaxime and ceftriaxone, were frequently found in IncHI2. These plasmids were the most significant plasmid lineages, which were implicated in the transmission of antibiotic resistance in *Salmonella*, particularly in *S. typhimurium* strains. β-lactam ($bla_{\text{OXA-1}}$ and $bla_{\text{TEM-1}}$) and quinolone resistance genes (*qnrA* and *acc(6')-ib-cr*) were horizontally transferred by IncHI2 plasmid.

DISCUSSION

Antimicrobial resistance (AMR) in foodborne pathogens is considered a serious threat to public health (WHO, 2015). Particularly in non-typhoidal *Salmonella* (NTS), one of the most common causes of foodborne disease and an important cause of mortality worldwide (WHO, 2015). *Salmonella* spp. frequently carries virulence factors and mobile elements, allowing them to accumulate and spread antibiotic resistance genes from other *Salmonella* strains and other species developed in the human digestive system. In this study, we investigated the phenotype and genotype of antibiotic resistance *Salmonella* strains that were isolated from whole Muscovy duck samples obtained from wet markets in Ha Noi. Coinciding, genetic data can reveal the potential of pathogens to cause illness in humans and animals.

Statistic data on *Salmonella* infection in Muscovy ducks are rarely public in Vietnam. Our finding showed that 65% of Muscovy duck samples were contaminated with *Salmonella*. Although the difference in *Salmonella* infection rate from district to district in this study may not be reliable enough since the total sample number was not enough to be a representative sample size. The proportion of *Salmonella* positive samples detected in Muscovy duck is higher than in several studies on chicken conducted in Ha Noi. The infection rate in the study of Nghiem et al. (2016) was 36% (n = 30, in 2016). The difference in the prevalence of *Salmonella* might be attributed to differences in sample location, sample collection time, sampling method as well as *Salmonella* detection method. However, the ratio of infection rate in our study is similar to the results of previously published studies and in another province of Vietnam such as Ho Chi Minh city. A study by Khan et al. (2018) resulted in 66.7% of chicken meat samples being positive for *Salmonella*. Remarkably, surveillance of *Salmonella* infection in Muscovy ducks around the world shows dissimilar results. In Emanuella's research in 2014 in Brazil, there was no detection of *Salmonella* in Muscovy duck (Emanuella et al., 2014). The dataset from the study in Trinidad and Tobaga in 2008 indicated approximately 40% of *Salmonella* positive in 110 Muscovy duck fecal samples (Rampersad et al., 2008). However Rampersad's study detected *Salmonella* serovar Uganda, Kiambu, and Orion, which are not commonly found in food-borne outbreaks cases. Until now, it could be explained that Muscovy duck was not raised industrially, thus resulting in not having the same microbial profile as chicken. However, as the food demand of humans grows eventually, Muscovy duck started to be raised the same way as other poultry, which lead to the spread of antimicrobial resistance in *Salmonella* strains.

Our study revealed a critical predominance of anti-microbial resistance in *Salmonella* strains isolated from Muscovy duck for the first time in Ha Noi. The proportion of *Salmonella* that was either mono- or multidrug resistant (resistant to more than three antibiotic families) in our investigation was also significantly higher than what was discovered in chicken, base on one study by the Nguyen Thanh Viet (2016) in which 27.3% (3/11) of the chicken samples (also collected from wet markets in Ha Noi) were monodrug resistant and 36.4% (4/11) were multidrug resistant (Nguyen et al., 2018). It is likely possible that the excessive usage of antibiotics in livestock has encouraged the development of bacteria with progressively higher levels of antibiotic resistance from time to time. *Salmonella* resistance to antibiotics is rising at a comparable rate in Thailand and Cambodia, in Trongjit's research, 90% (n = 345) of *Salmonella* isolates resisted to at least one antibiotic, and multidrug resistance was taken up to 45% of total strains (Trongjit et al., 2017). While in research done in China, 97.7% of *Salmonella* were resistant to at least 1 antimicrobial and 81.1% were multidrug resistant strains (Zhang et al., 2018). Our study and past studies in other countries also found a high proportion of antimicrobial resistant *Salmonella* isolated from poultry.

Phenotype and genotype prediction of antibiotic-resistant organisms showed all 06 strains resistant to cefotaxime (3rd generation cephalosporin) contain genes $bla_{\text{CTX-M-65}}$ or $bla_{\text{CTX-M-55}}$. The fact that all of the strains analyzed in the research harbored the gene $bla_{\text{CTX-M-65}}$ or $bla_{\text{CTX-M-55}}$ *or* $bla_{\text{CTX-M-14b}}$ demonstrated a prominent and widespread level of AmpC and/or ESBL-related gene carrier number in Muscovy duck. The $bla_{\text{CTX-M-65}}$ or $bla_{\text{CTX-M-55}}$ *or* $bla_{\text{CTX-M-14b}}$ gene is associated with antibiotic resistance to a variety of essential drugs, including cefotaxime, ceftriaxone, aztreonam, ceftazidime, amoxicillin, ampicillin, ticarcillin, piperacillin, and cefepime. These genes including $bla_{\text{CTX-M}}$, *floR, qnrS1* and *fosA7* were predicted to be located on mobile genetic elements, this prediction addressed a great concern since it represents a risk of

horizontal transmission between strains of the same species as well as between various species via synaptic plasmids or transposons. Eventually, the threat of horizontal transmission (connected to genetic mobile elements) and *qnrS1* and $bla_{\text{CTX-M}}$ accumulation in *Salmonella* puts humans' therapeutic strategy using antibiotics at risk (Monte et al., 2021). In addition, antibiotics susceptibility tests indicated phenotypes resistant to ceftriaxone and cefotaxime, these two antibiotics are commonly used to treat *Salmonella* infection, especially in children, which pose a serious risk to public health (Iwamoto et al., 2017). Mutations related to fluoroquinolone resistance, an important antibiotic used in clinical treatment, were also a risk that needs public attention.

Together with the presence of variety and severity of ARM genes, the presence of plasmids, VFs, and SPIs combined, make the treatment process against pathogens, especially *Salmonella* become more challenging and more difficult for us in the way we are using antibiotics in clinical treatment and agricultural infection control.

CONCLUSION

Muscovy duck is usually breed along with chicken and duck. Muscovy duck meat is an enjoyable dish in Vietnam. Our study a identified high positive rate of *Salmonella* (65%), as well as multi-drug resistance of *Salmonella* isolates (n = 19/20) from Muscovy duck meat which is sold in wet markets in Hanoi. Isolates showed resistant characteristics to significant antibiotics such as the third-generation cephalosporin, aminoglycoside, and ciprofloxacin. Moreover, the result of genomic data analysis illustrated the existence of antibiotic-resistant genes including *blaCTX-M-9; blaCTX-M-14*, *blaCTX-55*, and *blaCTX-M-65*. Especially, our study proposed the first report on whole genome analysis of *Salmonella* strains from Muscovy duck meat in Vietnam.

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REFERENCES

Anhalt J. P. & Fenselau C., 1975. Identification of Bacteria using Mass Spectrometry. *Analytical Chemistry*, 47(2): 219–225. https://doi.org/10.1021/AC60352A007/ASSET/AC60352A007.FP.PNG_V03

Carattoli A., Zankari E., Garciá-Fernández A., Larsen M. V., Lund O., Villa L., Aarestrup F. M. & Hasman H., 2014. In Silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. *Antimicrobial Agents and Chemotherapy*, 58(7): 3895–3903. https://doi.org/10.1128/AAC.02412-14

CDC, 2022. FoodNet Fast. https://wwwn.cdc.gov/foodnetfast/

Centers for Disease Control and Prevention (CDC), 2014. Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food-Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2006–2013. Morbidity and Mortality Weekly Report (MMWR). https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6315a3.htm

Chen L., Yang J., Yu J., Yao Z., Sun L., Shen Y. & Jin Q., 2005. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Research*: 33(Database issue). https://doi.org/10.1093/NAR/GKI008

EFSA & ECDC, 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal*, 16(12):

e05500. https://doi.org/10.2903/J.EFSA.2018.5500

EFSA & ECDC, 2021. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. *EFSA Journal*, 19(4). https://doi.org/10.2903/J.EFSA.2021.6490

Emanuella E. da S., Elisângela de S. L., Régis S. de C. T., Átilla H. de A., Roberta C. da R. e S., Valdez J. R. G. F., Ruben H. V. & William C. M., 2014. Survey of Enterobacteriaceae in domestic ducks (*Cairina moschata*) from properties located in four cities of the state of Ceará, Brazil. *Arquivos Do Instituto Biológico*.

FAO & WHO, 2002. Risk assessments of Salmonella in eggs and broiler chickens.

FAO & WHO, 2009. M I C R O B I O Salmonella and Campylobacter in chicken meat. www.who.int/foodsafety

Gilbert M., Nicolas G., Cinardi G., van Boeckel T. P., Vanwambeke S. O., Wint G. R. W. & Robinson T. P., 2018. Global distribution data for cattle, buffaloes, horses, sheep, goats, pigs, chickens and ducks in 2010. *Scientific Data*, 5(1): 1–11. https://doi.org/10.1038/sdata.2018.227

Gonzalez-Santamarina B., Busch A., Garcia-Soto S., Abdel-Glil M. Y., Linde J., Fries R., Meemken D., Hotzel H. & Tomaso H., 2020. Draft genome sequence of multi-resistant *Salmonella enterica* subsp. *enterica* serovar Rissen strain 19CS0416 isolated from Vietnam reveals *mcr-1* plasmid mediated resistance to colistin already in 2013. *Journal of Genomics*, 8(2020): 76–79. https://doi.org/10.7150/jgen.42790

Gupta S. K., Padmanabhan B. R., Diene S. M., Lopez-Rojas R., Kempf M., Landraud L. & Rolain J. M., 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrobial Agents and Chemotherapy*, 58(1): 212–220. https://doi.org/10.1128/AAC.01310-13

Hasman H., Agersø Y., Hendriksen R., Cavaco L. M., Guerra-Roman B., Bortolaia V. & Pedersen S. K., 2014. Laboratory Protocol: Isolation of ESBL, AmpC and carbapenemase producing E. coli from caecal samples. Final protocol, November: 1–11.

Iwamoto M., Reynolds J., Karp B. E., Tate H., Fedorka-Cray P. J., Plumblee J. R., Hoekstra R. M., Whichard J. M. & Mahon B. E., 2017. Ceftriaxone-Resistant Nontyphoidal Salmonella from Humans, Retail Meats, and Food Animals in the United States, 1996–2013. *Foodborne Pathogens and Disease*, 14(2): 74–83. https://doi.org/10.1089/FPD.2016.2180

Khan A. S., Georges K., Rahaman S., Abdela W. & Adesiyun A. A., 2018. Prevalence and serotypes of *Salmonella* spp. on chickens sold at retail outlets in Trinidad. *PLoS One*, 13(8). https://doi.org/10.1371/journal.pone.0202108

Lamas A., Miranda J. M., Regal P., Vázquez B., Franco C. M. & Cepeda A., 2018. A comprehensive review of non-enterica subspecies of *Salmonella enterica*. *Microbiological Research*, 206: 60–73. https://doi.org/10.1016/J.MICRES.2017.09.010

Leekitcharoenphon P., Nielsen E. M., Kaas R. S., Lund O. & Aarestrup F. M., 2014. Evaluation of whole genome sequencing for outbreak detection of *Salmonella enterica*. *PloS One*, 9(2). https://doi.org/10.1371/JOURNAL.PONE.0087991

Little C. L., Richardson J. F., Owen R. J., de Pinna E. & Threlfall E. J., 2008. Prevalence, characterisation and antimicrobial resistance of *Campylobacter* and *Salmonella* in raw poultrymeat in the UK, 2003–2005. *International Journal of Environmental Health Research*, 18(6): 403–414. https://doi.org/10.1080/09603120802100220

Ljubojević Pelić D., Vidaković Knežević S., Pelić M., Živkov Baloš M. & Milanov D., 2021. The epidemiological significance of duck meat as a source of *Salmonella* spp.

a review. *World's Poultry Science Journal*, 77(1): 105–114. https://doi.org/10.1080/00439339.2020.1866960

McArthur A. G., Waglechner N., Nizam F., Yan A., Azad M. A., Baylay A. J., Bhullar K., Canova M. J., de Pascale G., Ejim L., Kalan L., King A. M., Koteva K., Morar M., Mulvey M. R., O'Brien J. S., Pawlowski A. C., Piddock L. J. V., Spanogiannopoulos P., … Wright G. D., 2013. The comprehensive antibiotic resistance database. *Antimicrobial Agents and Chemotherapy*, 57(7): 3348. https://doi.org/10.1128/AAC.00419-13

Monte D. F. M., Nethery M. A., Barrangou R., Landgraf M. & Fedorka-Cray P. J., 2021. Whole-genome sequencing analysis and CRISPR genotyping of rare antibiotic-resistant *Salmonella enterica* serovars isolated from food and related sources. *Food Microbiology*: 93. https://doi.org/10.1016/j.fm.2020.103601

Nghiem M. N., Nguyen V. T., Jeung E. B. & Vo T. T. B., 2019. Alternate antimicrobial resistance genes in multidrug resistant *Salmonella* spp. isolated from retail meats in Vietnam using RNA-sequencing analysis. *Journal of Food Safety*, 39(6). https://doi.org/10.1111/jfs.12707

Nguyen T. K., Nguyen L. T., Chau T. T. H., Nguyen T. T., Tran B. N., Taniguchi T., Hayashidani H. & Ly K. T. L., 2021. Prevalence and antibiotic resistance of *Salmonella* isolated from poultry and its environment in the Mekong Delta, Vietnam. *Veterinary World*, 14(12): 3216. https://doi.org/10.14202/VETWORLD.2021.3216-3223

Nguyen T. V., Nghiem N. M. & Vo T. B. T., 2018. Determination of antibiotic resistance of *Salmonella* isolated from pork, beef, and chicken meat at the retail markets in Hanoi. *Vietnam Journal of Biotechnology*, 16(3): 553–564.

Nguyen V. K., Pham T. N., Dinh X. T., Ma Lucila L., Unger F., Nguyen V. H., Pham D. P., Pham T. N. & Gilbert J. G., 2012. Hygienic practices and microbial contamination of small - scale poultry slaughter houses at peri - urban areas of Hanoi, Vietnam. *Agriculture and Rural Development*, 12(2): 60–67.

Noble D. J., Lane C., Little C. L., Davies R., de Pinna E., Larkin L. & Morgan D., 2012. Revival of an old problem: an increase in *Salmonella enterica* serovar Typhimurium definitive phage type 8 infections in 2010 in England and Northern Ireland linked to duck eggs. *Epidemiology and Infection*, 140(1): 146–149. https://doi.org/10.1017/S0950268811000586

Rampersad J., Johnson J., Brown G., Samlal M. & Ammons D., 2008. Comparison of polymerase chain reaction and bacterial culture for *Salmonella* detection in the Muscovy duck in Trinidad and Tobago. *Revista Panamericana de Salud Pública*, 23(4): 264–267.

Seemann T., 2016. ABRicate: mass screening of contigs for antiobiotic resistance genes. https://github.com/tseemann/abricate

Trongjit S., Angkititrakul S., Tuttle R. E., Poungseree J., Padungtod P. & Chuanchuen R., 2017. Prevalence and antimicrobial resistance in *Salmonella enterica* isolated from broiler chickens, pigs and meat products in Thailand–Cambodia border provinces. *Microbiology and Immunology*, 61(1): 23–33. https://doi.org/10.1111/1348-0421.12462

USDA, 2019. Isolation and identification of *Salmonella* from meat, poultry, pasteurized egg, and siluriformes (fish) products and carcass and environmental sponges. Laboratory Guidebook.

WHO., 2015. Who estimates of the global burden of foodborne diseases. www.who.int

Yoshida C. E., Kruczkiewicz P., Laing C. R., Lingohr E. J., Gannon V. P. J., Nash J. H. E. & Taboada E. N., 2016. The *Salmonella In Silico* typing resource (SISTR): An open web-accessible tool for rapidly typing and subtyping draft

Salmonella genome assemblies. *PLoS One*, 11(1). https://doi.org/10.1371/journal.pone.0147101

Zankari E., Hasman H., Cosentino S., Vestergaard M., Rasmussen S., Lund O., Aarestrup F. M. & Larsen M. V., 2012. Identification of acquired antimicrobial resistance genes. *The Journal of Antimicrobial Chemotherapy*, 67(11): 2640–2644. https://doi.org/10.1093/JAC/DKS261

Zhang L., Fu Y., Xiong Z., Ma Y., Wei Y., Qu X., Zhang H., Zhang J. & Liao M., 2018. Highly prevalent multidrug-resistant *Salmonella* from chicken and pork meat at retail markets in Guangdong, China. *Frontiers in Microbiology*, pp. 9. https://doi.org/10.3389/FMICB.2018.02104