Presence of metal-resistance and antibiotic-resistance genes in *Salmonella* spp. isolated from broiler chicken farms in Vinh Long province, Vietnam

Luan M. Huynh[1,2], Thuan K. Nguyen[2*], Duy D. Nguyen[2], & Khai T. L. Ly[2]

[1]Faculty of Applied Biological Sciences, Vinh Long University of Technology Education, Vinh Long, Vietnam

[2]Faculty of Veterinary Medicine, College of Agriculture, Can Tho University, Can Tho, Vietnam

ARTICLE INFO

Research Paper

Received: August 17, 2024
Revised: October 01, 2024
Accepted: October 04, 2024

Keywords

Antibiotic resistance
Chicken farm
Metal resistance
Salmonella
Vinh Long

***Corresponding author**

Nguyen Khanh Thuan
Email:
nkthuan@ctu.edu.vn

ABSTRACT

Salmonella can carry multiple antibiotic-resistant and metal-resistant genes and transmit these genes among strains worldwide. This study examined seventy-five *Salmonella* isolates from small-scale chicken farms (chicken feces, bedding, feed, wild animals) in Vinh Long province for the presence and relation of antibiotic and metal-resistance genes in these strains. The single PCR method was applied to detect seven antibiotic-resistance genes (*blaampC, blaTEM, dfrA1, tetA, strA, sul2, mcr1*) and four metal-resistance genes (*pcoR, czcD, cnrA, silE*). The results indicated that those *Salmonella* isolates harbored several patterns of antibiotic-resistance genes. Genes *blaampC* and *tetA* were the most prevalent (48.00%), while genes *mcr1* and *dfrA* were the most minor (1.33%). Of those *Salmonella* isolates, 92.00% harbored one to five antibiotic-resistance genes, and the *blaampC* + *strA* pattern was frequently obtained (12.00%). Moreover, 30.67% of *Salmonella* isolates showed multidrug resistance to three or four antibiotic categories. Among metal-resistance genes, gene *pcoR* encoding for copper resistance was the most predominant (53.33%), and gene *cnrA* encoding for cobalt-nickel resistance was the lowest (5.33%). There were diverse patterns of metal-resistance genes, and one *Salmonella* isolate carried four examined genes (1.33%). Furthermore, these *Salmonella* isolates had several combined patterns of metal-resistance and antibiotic-resistance genes. Among them, *pcoR, czcD,* and *silE* genes had a significant coefficient relation to the examined antibiotic-resistance genes. It indicated the correlation between metal resistance and antibiotic resistance genes and revealed the potential risk of increasing antibiotic resistance in *Salmonella* isolates in chicken farms in Vinh Long province.

Cited as: Huynh, L. M., Nguyen, T. K., Nguyen, D. D., & Ly, K. T. L. (2024). Presence of metal-resistance and antibiotic-resistance genes in *Salmonella* spp. isolated from broiler chicken farms in Vinh Long province, Vietnam. *The Journal of Agriculture and Development* 23 (Special issue 1), 74-87.

1. Introduction

Salmonella is one of the major foodborne pathogens that can pose a significant threat to public health, mainly through the consumption of contaminated poultry products (Chuanchuen et al., 2008; Dantas et al., 2020). *Salmonella* also causes infection in poultry, and contamination in the poultry environment facilitates the transmission of *Salmonella* through both vertical and horizontal pathways (Singh et al., 2010). In a previous report, *Salmonella* was isolated from chicken feces (7.67%), pest animals (5.98%), such as geckos, ants, cockroaches, and environmental samples (4.33%) in the chicken farms in the Mekong Delta, Vietnam (Nguyen et al., 2021). It indicated that *Salmonella* is a potential risk-causing disease for chickens and transmission in the husbandry environment.

On the other hand, the emergence of antibiotic-resistant *Salmonella* strains in food animals, including chickens, is a growing concern (Nair et al., 2018). Most *Salmonella* isolates have developed resistance to multiple drugs because of farmers' indiscriminate and repeated misuse of these antibiotics. The extensive use of antimicrobials in food animal production has been a critical driver of this trend, as it can promote the development and dissemination of resistant strains (Kulwichit et al., 2007). Zhu et al. (2017) reported that *Salmonella* isolated from broiler chickens in slaughterhouses in China exhibited multidrug resistance and harbored several antibiotic-resistance genes, such as *blaTEM, blaCTX-M, tetA, sul2, floR, aadA1, drfA1*, etc. Thus, screening for antimicrobial resistance in *Salmonella* is crucial for managing and treating *Salmonella* infections in poultry.

The extensive application of heavy metals as feed additives in livestock production has resulted in insufficient focus on pathogenic bacteria's resistance to these metals. The occurrence of heavy metal resistance genes in *Salmonella* showed the relationship between these genes and antibiotic-resistance genes (Yang et al., 2020). It has been demonstrated that the co-selection of antibiotic-resistance genes resulting from the presence of heavy metals significantly contributed to the observed rise in antibiotic-resistance genes abundance (Stepanauskas et al., 2006; Mazhar et al., 2021) and acted as a selective factor in their proliferation (Allen et al., 2010). Yang et al. (2020) reported that the presence of metal-resistance genes (*zntA, arsB, merA, pcoR, pcoA, pcoC*, and *chrA*) was found to be significantly associated with one or more antibiotic-resistance genes (*sul1, sul2, sul3, tetA, tetB, tetC, blaTEM, blaSHV*, and *blaCTX*). The interaction of these genes has increased the antibiotic resistance in bacteria, including *Salmonella*, in chicken farms. Moreover, disinfectants are essential in controlling the growth and transmission of pathogens. Nonetheless, the selective pressure imposed by disinfectants and heavy metals on microbial pathogens is increasingly recognized as a significant factor that drives the selection and dissemination of antimicrobial resistance within the food chain of humans and animals (Capita & Alonso-Calleja, 2013; Tezel & Pavlostathis, 2015).

In Vinh Long province, chickens were raised frequently; however, most farms were small-scale. The hygiene in these small-scale farms was not managed well; the pathogens could survive and spread via chickens or the environment (Alali et al., 2010; Nguyen et al., 2021). The prevalence and antibiotic resistance of *Salmonella* was recorded in several previous reports. However, few studies have been published on the prevalence of antibiotic-resistant genes. In contrast, no studies have been published on metal-resistance genes in

Salmonella isolated from chickens or husbandry environments in the Mekong Delta, including Vinh Long province. Therefore, this study aims to clarify the prevalence of antibiotic-resistance and metal-resistance genes in *Salmonella* originating from chickens and the surrounding environment. This research could provide valuable insights into the potential risk of those antibiotic-resistant *Salmonella* strains and inform strategies for mitigating poultry health risks in those farms.

2. Materials and Methods

2.1. The origin of *Salmonella* isolates

This study used 75 *Salmonella* isolates, which were isolated from broiler chicken feces (n = 15), husbandry environment samples: bedding samples (n = 6) and feed (n = 4), pests: geckos (n = 38), rats (n = 8), and ants (n = 4) in four different small-scale farms in Tam Binh and Mang Thit districts, Vinh Long province. These positive *Salmonella* strains were detected from 1,265 samples (chickens' feces, pests, and husbandry environment) from February 2022 to December 2022. The isolation and identification of *Salmonella* isolates were performed according to the instructions of Barrow & Feltham (2003). These *Salmonella* isolates were kept in Tryptic Soy Broth (TSB, Merck, Germany) supplied with 15% glycerol (Merck, Germany) at -80°C freezer in the Veterinary Food Hygiene Laboratory, Faculty of Veterinary Medicine, College of Agriculture, Can Tho University, Vietnam. One positive *Salmonella* isolate, representative of one positive sample, was selected for use in this study.

2.2. Identification of antibiotic-resistance genes in *Salmonella* isolates

The DNA of 75 *Salmonella* isolates was extracted using the heat-shock method (Ahmed & Dablool, 2017) and stored at -20°C for use in this experiment. The single PCR assay was applied to detect seven antibiotic-resistance genes, including β-lactam (*blaampC*, *blaTEM*), aminoglycoside (*strA*), tetracycline (*tetA*), polypeptide (colistin-*mcr1*), sulfonamide (*sul2*), and diaminopyrimidine (*dfrA1*) (Table 1). The PCR conditions followed the description of references in Table 1, respectively. These genes were often detected in *Salmonella* and *E. coli* isolated from chickens in previous studies in the Mekong Delta (Nguyen et al., 2015; Nguyen et al., 2021) and represented antibiotic types used frequently in our surveys in the small-scale chicken farms.

The MyTaq Mix 2X (BIO25042, Bioline, Meridian Bioscience, USA) was in the PCR reaction as a master mix. One reaction consists of a total of 25.0 µL, including Mastermix 2X (12.5 µL), forward primer (0.5 µL), reverse primer (0.5 µL), distillation water (9.5 µL), and DNA template (2.0 µL). Thermal cycle was modified as follows: 94°C - 5 min; 30 cycles: 94°C - 1 min, 58°C - 45 sec, 72°C - 1 min; 72°C - 10 min. The *Salmonella* isolates harbored those genes, previously isolated from domestic animals in the Mekong Delta, were used as a control. The PCR products were electrophoresed in 1.5% agarose gel at 50V for 60 min. Then, the gels were dyed in ethidium bromide (0.001 mg/L) before capturing the image under UV.

2.3. Identification of metal-resistance genes in *Salmonella* isolates

This study also used the DNA of seventy-five *Salmonella* isolates to detect the presence of metal-resistance genes. The single PCR (25.0 µL/reaction) and electrophoresis procedures were conducted like those used to detect antibiotic-resistance genes.

Four metal-resistance genes were examined for genes encoding resistance to copper (*pcoR*), cobalt-zinc-cadmium (*czcD*), cobalt-nickel (*cnrA*), and silver (*silE*) (Table 1). These metal-resistance genes were reported in several previous studies in *Salmonella* and *E. coli*, and these heavy metals were commonly used in disinfectant products (Woods et al., 2009; Yang et al., 2020; Mustafa et al., 2021).

The PCR conditions and primer sequences (*pcoR, czcD, cnrA*, and *silE*) followed the descriptions of references in Table 1, respectively. The *Salmonella* isolates harboring these metal-resistance genes, previously isolated from domestic animals (pigs, chickens) in our pilot studies in the Mekong Delta, were used as a control. The PCR products were electrophoresed in 1.5% agarose gel at 50V for 60 min. Then, the gels were dyed in ethidium bromide (0,001 mg/L) before capturing the image under UV.

Table 1. The nucleotide sequence of antibiotic-resistance and metal-resistance primers used in this study

Genes	Sequence 5'-3'	Size (bp)	References
Antibiotic-resistance genes			
blaampC	AATGGGTTTTCTACGGTCTG GGGCAGCAAATGTGGAGCAA	191	Caroff et al. (1999)
blaTEM	ATTCTTGAAGACGAAAGGGC ACGCTCAGTGGAACGAAAAC	1.150	Jouini et al. (2007)
strA	CCTGGTGATAACGGCAATTC CCAATCGCAGATAGAAGGC	546	Carattoli et al. (2002)
tetA	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	577	Randall et al. (2004)
mcr-1	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	309	Elnahriry et al. (2016)
sul2	CGGCATCGTCAACATAACC GTGTGCGGATGAAGTCAG	722	Sáenz et al. (2010)
dfrA1	GGAGTGCCAAAGGTGAACAGC GAGGCGAAGTCTTGGGTAAAAAC	367	Peirano et al. (2006)
Metal-resistance genes			
pcoR	CAGGTCGTTACCTGCAGCAG CTCTGATCTCCAGGACATATC	636	Yang et al. (2020)
czcD	CAGGTCACTGACACGACCAT CATGCTGATGAGATTGATGATC	398	Anton et al. (2004)
cnrA	CCTACGATCTCGCAGGTGAC GCAGTGTCACGGAAACAACC	422	Mustafa et al. (2021)
silE	AGGGGAAACGGTCTGACTTC ATATCCATGAGCGGGTCAAC	432	Woods et al. (2009)

2.4. Statistical analysis

The statistical analysis was used to clarify the difference in the presence of antibiotic-resistant and metal-resistant genes among those *Salmonella* isolates. The Chi-square method was used to define the significant difference in the presence of antibiotic-resistant and metal-resistant genes at a confidence level of 95%. Spearman's correlation coefficient was used to determine the relation between antibiotic-resistant and metal-resistant genes. These analyses were performed in Minitab software version 17.0 (Minitab LLC, USA).

3. Results and Discussions

3.1. The presence of antibiotic-resistance genes in *Salmonella* isolates

The results (Table 2, Figure 1) indicated that *blaampC* and *tetA* genes were detected at the highest rate (48.00%), followed by *strA* (42.67%), and the most minor ones were genes *dfrA1* and *mcr-1* (1.33%). Those genes encode resistance to favored antibiotic groups (β-lactam, cycline, and aminoglycoside) frequently used to treat salmonellosis in poultry and used in small-scale farms in Vinh Long province, according to our previous studies and other reports (Nguyen et al., 2017; Nguyen et al., 2021). This indicated that *Salmonella* isolated from small-scale farms could resist antibiotics currently used, causing challenges in choosing suitable antibiotics for treating diseases there. Yildirim et al. (2011) stated that the variations in resistance were also linked to the specific serovar of *Salmonella*, the type of poultry (broilers or layers), individual farms, and the specific antimicrobial agents used.

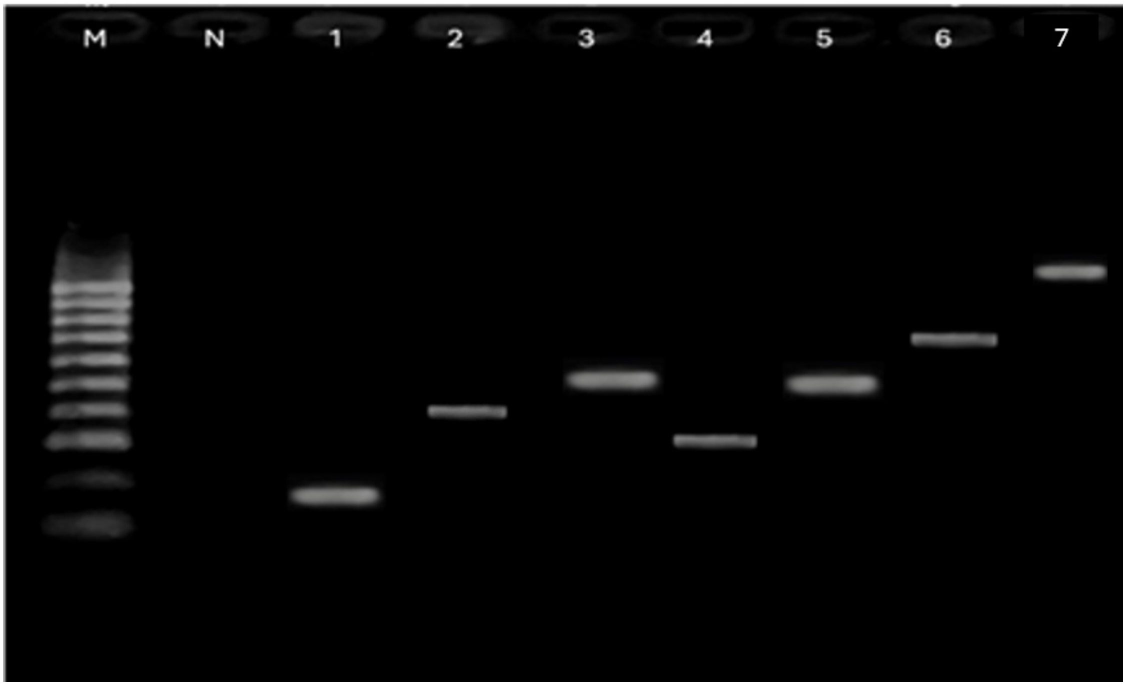

Figure 1. The electrophoresis image of PCR products in detecting antibiotic-resistance genes in *Salmonella* spp. isolates. M: ladder (100 bp), N: negative control (Distilled water), 1: *blaampC* (191 bp), 2: *dfrA* (367 bp), 3: *tetA* (577 bp), 4: *mcr-1* (309 bp), 5: *strA* (546 bp), 6: *sul2* (722 bp), 7: *blaTEM* (1.150bp).

Table 2. The prevalence of antibiotic-resistance genes in *Salmonella* isolates in the small-scale farms in Vinh Long province

Genes	Feces (n = 15)		Husbandry environment (n = 10)		Pests (n = 50)		Total* (n = 75)	
	No. of positive isolates	Percentage (%)	No. of positive isolates	Percentage (%)	No. of positive isolates	Percentage (%)	No. of positive isolates	Percentage (%)
blaampC	3	20.00	1	10.00	32	64.00	36	48.00
blaTEM	2	13.33	2	20.00	8	16.00	12	16.00
strA	5	33.33	1	10.00	26	52.00	32	42.67
tetA	11	73.33	6	60.00	19	38.00	36	48.00
mcr-1	0	0.00	1	10.00	0	0.00	1	1.33
sul2	4	26.67	2	20.00	16	32.00	22	29.33
dfrA1	0	0.00	1	10.00	0	0.00	1	1.33

**There was no significant in the prevalence of antibiotic-resistance genes among those samples (P > 0.05).*

In the current study, most genes could be detected from chickens' feces and pests; however, genes *dfrA1* and *mcr-1* were not found. These two genes were found in *Salmonella* isolates originating from the husbandry environment (bedding and feed) (Table 2). The widespread presence of *Salmonella* spp. can be attributed to their ability to adapt to hosts, their resilience to adverse conditions, and their increased capability to form biofilms. These factors lead to persistent contamination of the environment, animal feed, and livestock. Furthermore, the emergence and proliferation of antimicrobial resistance in *Salmonella* spp. presents further challenges (Velhner et al., 2018). In the Mekong Delta, there were a few large-scale chicken farms. Thus, the antibiotic resistance of *Salmonella* isolated from chickens and the environment in these farms was limited compared to this study. In other published research, Ramatla et al. (2019) reported that *Salmonella* spp. isolated from chickens and rats in poultry houses in South Africa, exhibited significant antibiotic resistance, and ultimately highlighted the importance of rats as carriers and transmitters of antibiotic-resistant bacteria to chickens and humans. In India, genes *tetA*, *tetB*, *blaTEM*, and *CTX-M* were found at a relatively high rate in *Salmonella* isolated from chickens and the environment and indicated selective pressure for adopting resistance against the tetracycline antibiotic group in *Salmonella* (Waghamare et al., 2018). In Bangladesh, Das et al. (2021) also reported that 94% of *Salmonella* isolates from broiler chickens were multidrug-resistant, 81.4% of the isolates carrying the *tetA* gene, while genes *blaTEM* and *sul-I* were at 95.4% and 37.2 %, respectively. Thus, the husbandry environment could be a contaminated source from which antibiotic-resistant *Salmonella* isolates survived and spread out.

Table 3. The antibiotic-resistance patterns of *Salmonella* isolates in Vinh Long province (n = 75)

No. of antibiotic-resistance genes	Patterns	No. of positive isolates	Percentage (%)
1	*blaampC*	11	14.67
	strA	7	9.33
	tetA	11	14.67
2	*blaampC + strA*	9	12.00
	blaTEM + dfrA1	1	1.33
	blaTEM + strA	1	1.33
	tetA + mcr-1	1	1.33
	tetA + strA	3	4.00
	tetA + sul2	2	2.67
3	*blaampC + strA + sul2*	1	1.33
	blaampC + tetA + strA	3	4.00
	blaampC + tetA + sul2	6	8.00
	blaTEM + strA + sul2	1	1.33
	blaTEM + tetA +sul2	4	5.33
	tetA + strA + sul2	1	1.33
4	*blaampC + blaTEM + tetA + sul2*	3	4.00
	blaampC + tetA + strA + sul2	2	2.67
	blaTEM + tetA + strA + sul2	1	1.33
5	*blaampC + blaTEM + tetA + strA + sul2*	1	1.33
Total		69	92.00

Moreover, of those *Salmonella* isolates, 92.00% harbored one to five antibiotic-resistance genes (Table 3). The *blaampC + strA* pattern was frequently obtained (12.00%), and one *Salmonella* isolates (1.33%) harbored five antibiotic-resistance genes: *blaampC + blaTEM + tetA + strA + sul2*. This showed that *Salmonella* isolated in those small-scale farms in Vinh Long province could have been diverse antibiotic resistance and caused essential challenges in selecting and combining antibiotics for treating diseases in poultry. It might be due to the indiscriminate use of prescribed antibiotics, horizontal transfer, and clonal spread of resistance genes (Ngoi & Thong, 2013; Fardsanei et al., 2016). Furthermore, 23/75 (20.67%) *Salmonella* isolates showed multidrug resistance to three or four antimicrobial categories in this study, including β-lactam, tetracycline, aminoglycoside, sulfonamide, etc. The multiresistance of *Salmonella* isolates could cause difficulties in selecting antibiotics for treating chickens' diseases in these farms and a potential risk to public health. The findings of Hai et al. (2020) in China in 80% of the *Salmonella* isolated from chicken farms in Nanjing, China, isolates were resistant to three or more antibiotics, suggesting that the percentage of *Salmonella* strains resistant to antimicrobials had also increased over time. These results indicate that antibiotic-resistant *Salmonella* isolates in chicken farms in Vinh Long should be managed and controlled strictly to protect chickens and public health.

3.2. The presence of metal-resistance genes in *Salmonella* isolates

According to previous studies, this study defined four common metal-resistance genes cooperating in antibiotic resistance (Allen et al., 2010; Yang et al., 2020; Mazhar et al., 2021). The results in Table 4 and Figure 2 exhibited that gene *pcoR* encoding for copper resistance was at the highest rate (53.33%), followed by *silE* (32.00%), *czcD* (18.67%), and *cnrA* (5.33%). Most genes were detected in *Salmonella* isolates from different samples; however, gene *czcD* was not found in *Salmonella* originating from husbandry environments in this study. It is recognized that heavy metals are resistant to degradation, thereby posing a persistent selection pressure that may play a significant role in the development and sustenance of heavy metal-resistant genotypes (Baker-Austin et al., 2006). In addition, Hobman & Crossman (2015) stated that copper is also widely used as a feed additive to promote growth and to treat diarrhea, and the prevalence of *pcoR* encoding for copper resistance could decrease the preventive methods in treatment for animals. Deng et al. (2018) researched *Salmonella* isolated from retail meat (pork, chicken) and stated that the prevalence of metal-resistance genes in the *Salmonella* strains might create conditions that favor the co-selection of strains exhibiting acquired resistance to other antimicrobial agents when the application of disinfectants for decontamination or the use of metals in livestock. The research of Yang et al. (2020) also reported the prevalence of heavy metal resistance genes in *Salmonella* isolates from broiler farms and retail meat harbored several genes, such as *zntA* and *zntB* confer resistance to zinc (Zn), *pcoR, pcoC, and pcoA* confer resistance to copper (Cu), *arsB* confers resistance to mercury (Hg), *merA* confers resistance to arsenic (As), and *chrA* confers resistance to chromium (Cr).

Table 4. The prevalence of antibiotic-resistance genes in *Salmonella* isolates in the small-scale farms in Vinh Long province

Genes	Feces (n = 15)		Husbandry environment (n = 10)		Pests (n = 50)		Total* (n = 75)	
	No. of positive isolates	Percentage (%)	No. of positive isolates	Percentage (%)	No. of positive isolates	Percentage (%)	No. of positive isolates	Percentage (%)
pcoR	8	53.33	7	70.00	25	50.00	40	53.33
czcD	2	13.33	0	0.00	12	24.00	14	18.67
cnrA	1	6.67	1	10.00	2	4.00	4	5.33
silE	4	26.67	2	20.00	18	36.00	24	32.00

**There was no significant in the prevalence of antibiotic-resistance genes among those samples (P > 0.05).*

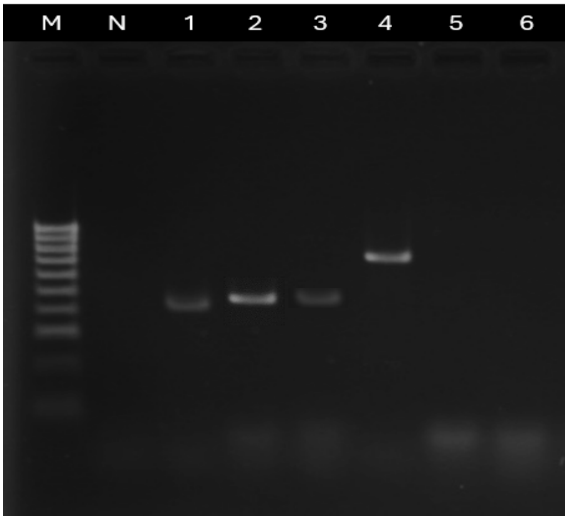

Figure 2. The electrophoresis image of PCR products in detecting metal-resistance genes in *Salmonella* spp. Isolates. M: ladder (100 bp), N: negative control (Distilled water), 1: *pcoR* (636 bp), 2: *czcD* (398 bp), 3: *cnrA* (422 bp), 4: *silE* (432 bp), 5-6: negative samples.

Table 5 presents the results of the patterns of metal-resistance and antibiotic-resistance genes. It shows that several patterns were obtained, and those genes were accompanied. In this study, Spearman's correlation coefficient analysis revealed that *pcoR*, *czcD*, and *silE* were related to all antibiotic-resistance genes ($P < 0.01$). In contrast, gene *cnrA* did not show a relation. It demonstrated that metal-resistance genes could enhance antibiotic resistance or resist disinfectant products in these *Salmonella* isolates isolated from chickens and environments in small-scale farms. Other research has shown that sub-inhibitory levels of heavy metals due to metal-resistance genes can facilitate the horizontal transfer of plasmid-mediated antibiotic resistance among bacterial populations (Zhang et al., 2018; Lu et al., 2020). In China, antibiotic resistance was highly associated with specific heavy metal resistance genes, such as the association among Cu-resistance genes (*pcoC, pcoR*) and tetracycline and sulfonamide resistance genes (*tet, sul*) (Deng et al., 2018). Ji et al. (2012) also reported that various environmental mechanisms have facilitated the co-selection of metal-resistance genes alongside antibiotic-resistance genes.

Besides, Mustafa et al. (2021) stated that the introduction of the Cr-Zn-Cd-resistance gene *czcD*, the Cu-resistance gene *pcoC*, and the Co-Ni-resistance gene *cnrA* into *E. coli* and the enhanced Cu-resistance observed in the transconjugants suggest that these resistance genes are situated on conjugative plasmids. Consequently, the overuse of metals and disinfectants as feed additives and in animal husbandry could potentially encourage the development of antibiotic resistance through co-selection, thereby sustaining and even enhancing antibiotic resistance in environments devoid of antibiotics. Thus, *Salmonella* isolated in the small-scale farms in Vinh Long province showed a severe risk of increased antibiotic resistance due to relationships with antibiotic-resistance genes.

Table 5. The patterns of metal-resistance and antibiotic-resistance genes of *Salmonella* isolated in the small-scale farms in Vinh Long province (n = 75)

Patterns	No. of positive isolates	Percentage (%)
blaampC + czcD	1	1.33
blaampC + pcoR	3	4.00
blaampC + strA	3	4.00
strA + czcD	2	2.67
strA + pcoR	2	2.67
tetA + pcoR	6	8.00
blaampC + pcoR + silE	2	2.67
blaampC + strA + czcD	2	2.67
blaampC + strA + pcoR	3	4.00
blaTEM + strA + silE	1	1.33
strA + pcoR + czcD	1	1.33
tetA + mcr1 + pcoR	1	1.33
tetA + pcoR + czcD	1	1.33
tetA + strA + pcoR	1	1.33
blaampC + strA + pcoR + silE	1	1.33
blaampC + strA + sul2 + silE	1	1.33
blaampC + tetA + strA + czcD	1	1.33
blaampC + tetA + strA + silE	1	1.33
blaampC + tetA + sul2 + silE	2	2.67
blaTEM + tetA + sul2 + pcoR	1	1.33
tetA + sul2 + pcoR + silE	1	1.33
blaampC + tetA + strA + sul2 + silE	1	1.33
blaampC + tetA + sul2 + pcoR + silE	2	2.67
tetA + strA + sul2 + pcoR + silE	1	1.33
blaampC + blaTEM + tetA + strA + sul2 + silE	1	1.33
blaampC + blaTEM + tetA + sul2 + pcoR + silE	2	2.67
blaampC + tetA + strA + pcoR + czcD + silE	1	1.33
blaampC + tetA + strA + sul2 + pcoR + silE	1	1.33
blaTEM + tetA + sul2 + pcoR + cnrA + silE	2	2.67
blaTEM + tetA + sul2 + pcoR + czcD + silE	1	1.33
blaTEM + strA + sul2 + pcoR + czcD + cnrA + silE	1	1.33
blaTEM + tetA + strA + sul2 + pcoR + cnrA + silE	1	1.33
Total	51	68.00

4. Conclusions

Salmonella isolated from broiler chickens, husbandry environments, and pests in small-scale farms in Vinh Long province harbored several antibiotic-resistance and metal-resistance genes, especially genes *blaampC* and *pcoR*. Moreover, the genes accompanied in those *Salmonella* strains exhibited a potential increase in antibiotic resistance, especially the presence of *pcoR*, *czcD*, and *silE* genes along with antibiotic-resistance genes. Thus, managing the prevalence of antibiotic-resistant *Salmonella* in poultry farms is an essential issue to protect poultry health and ensure the effectiveness of treatment.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

The authors thank the Department of Animal Husbandry, Veterinary, and Fisheries in Vinh Long province for the excellent cooperation with the Faculty of Veterinary Medicine, College of Agriculture, Can Tho University.

References

Ahmed, O. B., & Dablool, S. A. (2017). Quality improvement of the DNA extracted by boiling method in Gram-negative bacteria. *International Journal of Bioassays* 6(4), 5347-5349. http://dx.doi.org/10.21746/ijbio.2017.04.004.

Alali, W. Q., Thakur, S., Berghaus, R. D., Martin, M. P., & Gebreyes, W. A. (2010). Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Foodborne Pathogens Disease* 7(11), 1363-1371. https://doi.org/10.1089/fpd.2010.0566.

Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J., & Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology* 8, 251–259. https://doi.org/10.1038/nrmicro2312.

Anton, A., Weltrowski, A., Haney, C. J., Franke, S., Grass, G., Rensing, C., & Nies, D. H. (2004). Characteristics of zinc transport by two bacterial cation diffusion facilitators from *Ralstonia metallidurans* CH34 and *Escherichia coli*. *Journal of Bacteriology* 186(22), 7499-7507. https://doi.org/10.1128/JB.186.22.7499-7507.2004.

Baker-Austin, C., Wright, M. S., Stepanauskas, R., & McArthur, J. V. (2006). Co-selection of antibiotic and metal resistance. *Trends in Microbiology* 14(4), 176-182. https://doi.org/10.1016/j.tim.2006.02.006.

Barrow, G. I., & Faltham, R. K. A. (2003). *Cowan and Steel's manual for the identification of medical bacteria* (3rd ed.). Cambridge, UK: Cambridge University Press.

Capita, R., & Alonso-Calleja, C. (2013). Antibiotic-resistant bacteria: a challenge for the food industry. *Critical Reviews in Food Science and Nutrition* 53(1), 11-48. https://doi.org/10.1080/10408398.2010.519837.

Carattoli, A., Filetici, E., Villa, L., Dionisi, A. M., Ricci, A., & Luzzi, I. (2002). Antibiotic resistance genes and *Salmonella* genomic island 1 in *Salmonella enterica* serovar Typhimurium isolted in Italy. *Antimicrobial Agents and Chemotherapy* 46(9), 2821-2828. https://doi.org/10.1128/AAC.46.9.2821-2828.2002.

Caroff, N., Espaze, E., Berard, I., Richet, H., & Reynaud, A. (1999). Mutations in the ampC promoter of *Escherichia coli* isolates resistant to oxyiminocephalosporins without extended spectrum β-lactamase production. *FEMS Microbiology Letters* 173(2), 459-465. https://doi.org/10.1111/j.1574-6968.1999.tb13539.x.

Chuanchuen, R., Pathanasophon, P., Khemtong, S., Wannaprasat, W., & Padungtod, P.

(2008). Susceptibilities to antimicrobials and disinfectants in *Salmonella* isolates obtained from poultry and swine in Thailand. *Journal of Veterinary Medical Science* 70(6), 595-601. https://doi.org/10.1292/jvms.70.595.

Dantas, S. T. A., Camargo, C. H., Tiba-Casas, M. R., Vivian, R. C., Pinto, J. P. A. N., Pantoja, J. C. F., Hernandes, R. T., Fernandes, Júnior A., & Rall V. L. M. (2020). Environmental persistence and virulence of *Salmonella* spp. Isolated from a poultry slaughterhouse. *Food Research International* 129, 108835. https://doi.org/10.1016/j.foodres.2019.108835,

Das, T., Rana, E. A., Dutta, A., Bostami, M. B., Rahman, M., Deb, P., Nath, C., Barua, H., & Biswas, P. K. (2021). Antimicrobial resistance profiling and burden of resistance genes in zoonotic Salmonella isolated from broiler chicken. *Veterinary Medicine and Science* 8(1), 237-244. https://doi.org/10.1002/vms3.648.

Deng, W. W., Quan, Y., Yang, Z. S., Guo, J. L., Zhang, L. X., Liu, L. S., Chen, J. S., Zhou, K., He, L., Li, B., Gu, F. Y., Zhao, H. S., & Zou, K. L. (2018). Antibiotic resistance in *Salmonella* from retail foods of animal origin and its association with disinfectant and heavy metal resistance. *Microbial Drug Resistance* 24(6), 782-791. https://doi.org/10.1089/mdr.2017.0127.

Elnahriry, S. S., Khalifa, H. O., Soliman, A. M., Ahmed, A. M., Hussein, A. M., Shimamoto, T., & Shimamoto, T. (2016). Emergence of plasmid-mediated colistin resistance gene mcr-1 in a clinical *Escherichia coli* isolate from Egypt. *Antimicrobial Agents and Chemotherapy* 60(5), 3249-3250. https://doi.org/10.1128/aac.00269-16.

Fardsanei, F., Nikkhahi, F., Bakhshi, B., Salehi, T. Z., Tamai, I. A., & Dallal, M. S. (2016). Molecular characterization of *Salmonella enterica* serotype Enteritidis isolates from food and human samples by serotyping, antimicrobial resistance, plasmid profiling, (GTG) 5-PCR and ERIC-PCR. *New Microbes and New Infections* 14, 24-30. https://doi.org/10.1016/j.nmni.2016.07.016.

Hobman, J. L., & Crossman, L. C. (2015). Bacterial antimicrobial metal ion resistance. *Journal of Medical Microbiology* 64(5), 471-497. https://doi.org/10.1099/jmm.0.023036-0.

Ji, L. X., Shen, H. Q., Liu, F., Ma, J., Xu, G., Wang, L. Y., & Wu, H. M. (2012). Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai, China. *Journal of Hazardous Materials* 235-236, 178-185. https://doi.org/10.1016/j.jhazmat.2012.07.040.

Jouini, A., Vinué, L., Slama, K. B., Saenz, Y., Klibi, N., Hammami, S., Boudabous, A., & Torres, C. (2007). Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. *Journal of Antimicrobial Chemotherapy* 60(5), 1137-1141. https://doi.org/10.1093/jac/dkm316.

Kulwichit, W., Chatsuwan, T., Unhasuta, C., Pulsrikarn, C., Bangtrakulnonth, A., & Chongthaleong, A. (2007). Drug-resistant nontyphoidal *Salmonella* bacteremia, Thailand. *Emerging Infectious Diseases* 13(3), 501-502. https://doi.org/10.3201/eid1303.061059.

Lu, J., Wang, Y., Jin, M., Yuan, G. Z., Bond, P., & Guo, H. J. (2020). Both silver ions and silver nanoparticles facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes. *Water Research* 169, 115229. https://doi.org/10.1016/j.watres.2019.115229.

Mazhar, S. H., Li, J. X., Rashid, A., Su, M. J., Xu, Q. J., Brejnrod, A. D., Su, Q. J., Wu, J. Y., Zhu, G. Y., Zhou, G. S., Feng, W. R., & Rensing, C. (2021). Co-selection of antibiotic resistance genes, and mobile genetic elements in the presence of heavy metals in poultry farm environments. *Science of The Total Environment* 755(Pt2), 142702. https://doi.org/10.1016/j.scitotenv.2020.142702.

Mustafa, G. R., Zhao, K., He, P. X., Chen, J. S., Liu, L. S., Mustafa, A., He, L., Yang, Y., XiuYu, M., Penttinen, P., Ao, L. X., Liu, P. A., Shabbir, M. Z., Xu, B. X., & Zou, K. L. (2021). Heavy metal resistance in *Salmonella* Typhimurium and its association with disinfectant and antibiotic resistance. *Frontiers in Microbiology* 12, 702725. https://doi.org/10.3389/fmicb.2021.702725.

Nair, D. V. T., Venkitanarayanan, K., & Johny, A. K. (2018). Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. *Foods* 7(10), 1-24. https://doi.org/10.3390/foods7100167.

Ngoi, S. T., & Thong, K. L. (2013). Molecular characterization showed limited genetic diversity among *Salmonella* Enteritidis isolated from humans and animals in Malaysia. *Diagnostic Microbiology and Infectious Disease* 77(4), 304-311. https://doi.org/10.1016/j.diagmicrobio.2013.09.004.

Nguyen, T. K., Nguyen, L. T., Chau, T. H. T., 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), 3216-3223. https://doi.org/10.14202/vetworld.2021.3216-3223.

Nguyen, V. T, Carrique-Mas, J. J., Nghia, N. H., P. Tu, L. T., Mai, H. H., Tuyen, H. T., Campbell, J., Nhung, N. T., Nhung, H. N., Minh, P. V., B. Chieu, T. T., Hieu, T. Q., N. Mai, N. T., Baker, S., Wagenaar, J. A., Hoa, N. T., & Schultsz, C. (2017). Non-typhoidal *Salmonella* colonization in chickens and humans in the Mekong Delta of Vietnam. *Zoonoses and Public Health* 64(2), 94-99. https://doi.org/10.1111/zph.12270.

Nguyen, V. T., Carrique-Mas, J. J., Ngo, T. H., Ho, H. M., Ha, T. T., Campbell, J. I., Nguyen, T. N., Hoang, N. N., Pham, V. M., Wagenaar, J. A., Hardon, A., Thai, Q. H., & Schultsz, C. (2015). Prevalence and risk factors for carriage of antimicrobial-resistant *Escherichia coli* on household and small-scale chicken farms in the Mekong Delta of Vietnam. *Journal of Antimicrobial Chemotherapy* 70(7), 2144-2152. https://doi.org/10.1093/jac/dkv053.

Peirano, G., Agersø, Y., Aarestrup, F. M., Falavina dos Reis, E. M., & Rodrigues, D. P. (2006). Occurrence of integrons and antimicrobial resistance genes among *Salmonella enterica* from Brazil. *Journal of Antimicrobial Chemotherapy* 58(2), 305-309. https://doi.org/10.1093/jac/dkl248.

Ramatla, T., Taioe, M. O., Thekisoe, O. M., & Syakalima, M. (2019). Confirmation of antimicrobial resistance by using resistance genes of isolated *Salmonella* spp. in chicken houses of North West, South Africa. *World's Veterinary Journal* 9(3), 158-165. https://dx.doi.org/10.36380/scil.2019.wvj20.

Randall, L. P., Cooles, S. W., Osborn, M. K., Piddock, L. J. V., & Woodward, M. J. (2004). Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy* 53(2), 208-216. https://doi.org/10.1093/jac/dkh070.

Sáenz, Y., Vinué, L., Ruiz, E., Somalo, S., Martínez, S., Rojo-Bezares, B., Zarazaga, M., & Torres, C. (2010). Class 1 integrons lacking qacEΔ1 and sul1 genes in *Escherichia coli* isolates of food, animal and human origins. *Veterinary Microbiology* 144(3-4), 493-497. https://doi.org/10.1016/j.vetmic.2010.01.026.

Singh, S., Yadav, A. S., Singh, S. M., & Bharti, P. (2010). Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Research International* 43(8), 2027-2030. https://doi.org/10.1016/j.foodres.2010.06.001.

Stepanauskas, R., Glenn, T. C., Jagoe, C. H., Tuckfield, R. C., Lindell, A. H., King, C. J., & McArthur, J. V. (2006). Coselection for microbial resistance to metals and antibiotics in freshwater microcosms. *Environmental Microbiology* 8(9), 1510-1514. https://doi.org/10.1111/j.1462-2920.2006.01091.x.

Tezel, U., & Pavlostathis, S. G. (2015). Quaternary ammonium disinfectants: microbial adaptation, degradation, and ecology. *Current Opinion in Biotechnology* 33, 296-304. https://doi.org/10.1016/j.copbio.2015.03.018.

Velhner, M., Milanov, D., & Kozoderović, G. (2018). *Salmonella* spp. in poultry: a constant challenge and new insights. *Journal of The Hellenic Veterinary Medical Society* 69(2), 899-910. https://doi.org/10.12681/jhvms.18012.

Waghamare, R. N., Paturkar, A. M., Vaidya, V. M., Zende, R. J., Dubal, Z. N., Dwivedi, A., & Gaikwad, R. V. (2018). Phenotypic and genotypic drug resistance profile of *Salmonella* serovars isolated from poultry farm and processing units located in and around Mumbai city, India. *Veterinary World* 11(12), 1682-1688. https://doi.org/10.14202/vetworld.2018.1682-1688.

Woods, E., Cochrane, C., & Percival, S. (2009). Prevalence of silver resistance genes in bacteria isolated from human and horse wounds. *Veterinary Microbiology* 138(3-4), 325-329. https://doi.org/10.1016/j.vetmic.2009.03.023.

Yang, Z. S., Deng, W. W., Liu, L. S., Yu, M. X., Mustafa, G. R., Chen, J. S., He, L., Ao, L. X., Yang, Y., Zhou, K., Li, B., Han, F. X., Xu, B. X., & Zou, K. L. (2020). Presence of heavy metal resistance genes in *Escherichia coli* and *Salmonella* isolates and analysis of resistance gene structure in *E. coli* E308. *Journal of Global Antimicrobial Resistance* 21, 420-426. https://doi.org/10.1016/j.jgar.2020.01.009.

Yildirim, Y., Gonulalan, Z., Pamuk, S., & Ertas, N. (2011). Incidence and antibiotic resistance of *Salmonella* spp. on raw chicken carcasses. *Food Research International* 44(3), 725-728. https://doi.org/10.1016/j.foodres.2010.12.040.

Zhang, Y., Gu, A. Z., Cen, T., Li, Y. X., He, M., Li, D., & Chen, M. J. (2018). Sub-inhibitory concentrations of heavy metals facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes in water environment. *Environmental Pollution* 237, 74-82. https://doi.org/10.1016/j.envpol.2018.01.032.

Zhu, T. Y., Lai, M. H., Zou, K. L., Yin, S., Wang, T. C., Han, F. X., Xia, L. X., Hu, D. K., He, L., Zhou, K., Chen, J. S., Ao, L. X., & Liu, L. S. (2017). Antimicrobial resistance and resistance genes in *Salmonella* strains isolated from broiler chickens along the slaughtering process in China. *International Journal of Food Microbiology* 259, 43-51. https://doi.org/10.1016/j.ijfoodmicro.2017.07.023.