Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Microbiology Research

Journal homepage: www.ijmronline.org

Original Research Article

Sequence-based detection of *Salmonella* spp. from symptomatic chicken and characterizing virulence

Anh Thi Quynh Nguyen[1], Chuong Van Huynh[2]*, Hai Thi Hong Truong[2], Han Ngoc Ho[2], Long Tien Nguyen[2], Thuy Thi Thu Nguyen[1], Y Vi Dang Ta[2], Tuan Minh Nguyen[2], Phuong Thi Lan Le[1], Hoa Xuan Nguyen[1]

[1]University of Agriculture and Forestry, Hue University, Hue, Vietnam

[2]Institute of Biotechnology, Hue University, Hue, Vietnam

Abstract

Background: *Salmonella* spp. causes food poisoning in humans and is frequently transmitted from animals to humans. Food poisoning by *Salmonella* spp. is characterized by acute gastritis, diarrhea and can be fatal. *Salmonella* spp. are important foodborne pathogens, causing food poisoning in humans and are often transmitted from animals to humans. Chickens are considered one of the main reservoirs of *Salmonella* and play an important role in the transmission of salmonellosis through contaminated chicken products. However, information on the prevalence and virulence characteristics of circulating *Salmonella* strains in chickens in Hue city, Vietnam, is still limited. Therefore, this study aimed to isolate *Salmonella* spp. from chicken fecal samples and to investigate the presence of selected virulence genes.

Materials and Methods: A total of 270 fecal samples were collected from chickens of different ages (1–13 weeks) showing clinical signs of salmonellosis, including diarrhea, lethargy, reduced feed intake, ruffled feathers, and pale combs. The samples were cultured on HiCrome *Salmonella* Agar for *Salmonella* isolation. Species identification was carried out, using 16S rRNA gene sequences and phylogenetic analysis. PCR was performed to determine virulence genes in the identified strains.

Results: A total of 108 *Salmonella* isolates were obtained from 270 samples (40%). Using 16S rRNA sequencing and phylogenetic analysis, 15 out of 20 *Salmonella* spp. isolates were identified, of which six strains were identified as *Salmonella pullorum*, six strains were identified as *Salmonella gallinarum*, three strains were identified as *Salmonella typhimurium* and other strains of *Salmonella* spp. PCR was performed to identify the virulence genes *Stn, fimA, invA, spvR, ivaB, spvC* of the 15 strains, with the presence being 15/15 (100%), 9/15 (60%), 15/15 (100%), 10/15 (66.7%), 11/15 (73.3%) and 4/15 (26.6%), respectively.

Conclusion: This study showed a relatively high occurrence of *Salmonella* spp. in chickens suspected of fowl typhoid (salmonellosis) in Hue City, Vietnam. The high detection rates of virulence genes such as *stn* and *invA,* together with the presence of *ivaB*, *spvR*, and *fimA*, indicate that the isolated strains possess considerable pathogenic potential, whereas *spvC* was detected at a lower frequency. These findings highlight the need for enhanced surveillance and control of *Salmonella* infections in poultry production to reduce risks to animal health and food safety.

Keywords: *Salmonella* spp., Chicken, Virulence genes, 16s rRNA gene, Hue city.

Received: 15-09-2025; **Accepted:** 12-03-2026; **Available Online**: 23-03-2026

This is an Open Access (OA) journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprint@ipinnovative.com

1. Introduction

Salmonella is a common foodborne pathogen in humans and other animal species, including livestock and poultry.[1-3] Some strains such as *S. enteritidis* and *S. typhimurium* can infect poultry and humans. Other *Salmonella* species such as *S. pullorum* and *S. gallinarum* only cause typhoid in chickens. This disease has caused significant economic losses for the poultry industry.[4-6] The disease is characterized by a number of clinical features such as anorexia, abdominal pain, diarrhea, high mortality rate in chickens, reduced egg production in laying hens, reduced fertility and hatchability. Toxin genes such as *stn, fimA, InvA,* and *spvR, spvC* have

*Corresponding author: Chuong Van Huynh
Email: hvanchuong@hueuni.edu.vn

https://doi.org/10.18231/j.ijmr.13388.1773809904

© 2026 The Author(s), Published by Innovative Publications.

been widely published by many authors to detect virulence genes in *Salmonella* spp.[7-9] There are many methods to identify bacterial species in the gut microbiota. Among them, many reports have shown that molecular methods are useful to address this challenge. Among all the sequencing platforms, 16S rRNA sequencing technology provides sufficient length (1500 bp) for informatics purposes and has been used for phylogenetic studies and bacterial taxonomy.[3,10] Therefore, the objective of this study was to identify *Salmonella* spp. The causative agent of typhoid in chickens is based on *16S rRNA* sequences, building a phylogenetic tree and detecting some toxin genes of isolated strains. This will serve as a foundation for further research on the development of recombinant antigens to immunize chickens, creating egg yolk antibodies against several toxins of *Salmonella* spp. for use in the prevention and treatment of avian typhoid.

2. Materials and methods

2.1. Bacterial strains

A total of 270 fecal samples were collected from chickens of different ages (1–13 weeks) showing clinical signs of salmonellosis, including diarrhea, lethargy, reduced feed intake, ruffled feathers, and pale combs, from three wards in Hue City.

A total of 270 fecal samples (10–50 g) were aseptically collected from chickens suspected of salmonellosis and transported to the Laboratory of Immunology and Vaccines, Institute of Biotechnology, Hue University under refrigerated conditions (2–8 °C) within 24 h. Sample collection and handling followed the Vietnamese National Standard TCVN 8400-12:2011, and bacterial isolation was performed according to TCVN 10780-1:2017. Briefly, samples were homogenized by vortexing and pre-enriched in Luria Bertani (LB) broth, followed by selective enrichment in Rappaport Vassiliadis (RV) broth at 37 °C for 24 h. The cultures were then streaked onto HiCrome™ Improved *Salmonella* Agar and incubated at 37°C for 24 h. Presumptive *Salmonella* colonies were identified based on their characteristic morphology, typically appearing red to pink.

2.2. Genomic DNA extraction

Twenty suspected *Salmonella* spp. strains were randomly selected and named (SalHue1-SalHue20). All were cultured in Rappaport-Vassiliadis medium (Himedia, India) and incubated at 37°C with shaking at 100 rpm for 14 hours. The cell pellets were harvested by centrifugation at 10,000 rpm for 5 minutes. DNA was extracted using the CTAB method as previously described by Doyle and Doyle (1996) and Sambrook and Russell,[11,12] briefly described as follows: 250 µL of CTAB buffer was added to the bacterial pellets, followed by 5.0 µL of Proteinase-K (20 mg/mL), and then incubated at 70°C for 10 minutes. Next, 2 volumes of phenol/chloroform/isoamyl alcohol (25:24:1) were thoroughly mixed with the suspension and centrifuged for 5 minutes at 13,000 rpm at 4°C. The DNA was precipitated with cold 100% ethanol, then dissolved in TE buffer (50 µL) and stored at -20°C for further analysis.

2.3. Molecular detection of Salmonella spp.

Twenty fecal samples were collected from chickens exhibiting typical clinical signs of salmonellosis, including diarrhea, lethargy, reduced feed intake, ruffled feathers, and pale combs. Bacterial isolates showing characteristic colony morphology and typical biochemical properties of Salmonella were selected for molecular identification. The specific 16S rRNA gene region was amplified by PCR to confirm the identity of the bacteria. The primer sequences used for PCR amplification and the thermal cycling conditions were applied according to previously described methods.[3,13] Successful amplification of the desired sequences was visualized by resolving the PCR products in 2% agarose gel (Bioline, Japan) at 60 V for 35 min and stained with 1 µL of 6x GelGreen® (Biosharp, China) for each DNA sample. DNA bands were observed and photographed using the runVIEW Mini system (Thistle Scientific, UK).

2.4. 16S rRNA gene sequencing and phylogenetic analysis

The amplified DNA was purified with the Wizard PCR SV Gel and PCR Clean-Up System kit (Promega, USA) and sequenced at DNA Sequencing Limited Company, Vietnam. Sequencing of PCR products was performed using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, USA), and capillary electrophoresis was done using 3130xl Genetic Analyzers (Applied Biosystems, USA). The obtained sequences were compared with the NCBI database using the BLASTn tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). In this comparison, sequences of type strains most closely related to the sequences of the isolates were determined. The sequences were aligned using Clustal W. MEGA 11 was used for the comparative analysis of molecular sequence data for reconstructing the evolutionary histories of species and inferring the nature and extent of selective forces shaping the evolution of genes and species.

2.5. Detection of virulence genes in identified Salmonella strains

After identifying the *Salmonella* strains, total DNA was extracted following the procedure mentioned above. Oligonucleotide primers were synthesized by Azenta Life Sciences (**Table 1**). The amplification reactions were performed in a final volume of 25 µL, containing 12.5 µL MyTaq HS Mix, 2X (Bioline, UK), 5 µL DNA template, 5.5 µL Nuclease-Free Water, and 0.5 µL of each primer (20 pmol).

PCR was carried out using gradient temperature for each primer pair (**Table 1**). The gradient temperature was set up

for each primer at 5°C below melting temperature with 35 cycles. The PCR condition included an initial denaturation at 95°C for 2 min, followed by 35 cycles of amplification (denaturation at 95°C for 30 sec, annealing temperature at 46.9°-50.8°C for 30 sec, and extension at 72°C for 45 sec), and final extension at 72°C for 7 min.[15,16] PCR products were resolved on agarose gel, stained and observed as described above.

2.5. Data analysis

Data were processed in Microsoft Excel 2021. The obtained sequences were compared with the NCBI database via BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned using Clustal W. Comparative analysis of sequence data was performed with MEGA 11. Phylogenetic tree using partial sequencing of 16S rRNA gene constructed with the Maximum Likelihood method with the Tamura–Nei model was applied in MEGA 11, and branch robustness was assessed using 1,000 bootstrap replicates.

3. Results

3.1. Characterization of Salmonella spp. isolates

In terms of the morphology, all *Salmonella* spp. isolated were Gram-negative and rod-shaped. The bacterial colonies appeared pink to red on HiCrome *Salmonella* Agar (**Figure 1**).

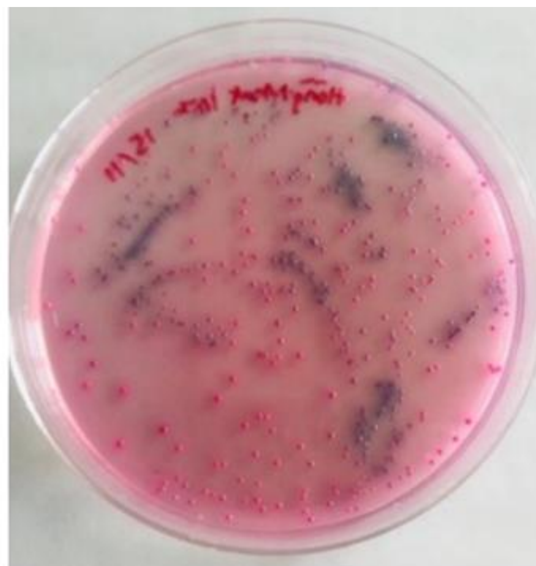

Figure 1: *Salmonella* spp. colonies on HiCrome *Salmonella* Agar.

Table 1: PCR primers for the detection of virulence genes in identified *Salmonella* strains

Target Gene	Oligonucleotide sequences (5´- 3´)	Expected size (base pairs)	Reference
fimA	F: CCT TTC TCC ATC GTC CTG AA R: TGG TGT TAT CTG CCT GAC CA	512	(Cohen *et al.*, 1996)[37]
invA	F-ACAGTGCTCGTTTACGACCTGAAT R-AGACGACTGGTACTGATCGATAAT	244	(Lee *et al.*, 2009)[12]
spvR	F: CAG GTT CCT TCA GTA TCG CA R: TTT GGC CGG AAA TGG TCA GT	310	(Chaudhary *et al.*, 2015)[24]
ivaB	F: GTT ATT TCA GCA TAA GGA G R: ACT TGT CCG TGT TTT ACT C	599	
Stn	F: CTT TGG TCG TAA AAT AAG GCG R: TGC CCA AAG CAG AGA GAT TC	260	
spvC	F: ACT CCT TGC ACA ACC AAA TGCGGA R: TGT CTT CTG CAT TTC GCC ACC ATCA	571	(Phumkhachorn & Rattanachaikunsopon, 2017)[19]

3.2. Genomic DNA extraction and molecular detection of Salmonella spp.

In this study, DNA quality was assessed by agarose gel electrophoresis and clear, sharp and high molecular weight bands without degradation were obtained, indicating the high quality of purified DNA. Amplification of the 16S rRNA gene of 20 isolates was carried out using a universal primer pair (27F and 1492R). The PCR products were analyzed by gel electrophoresis (2%), in which single fragments of about 1500 bp were observed (**Figure 2**).

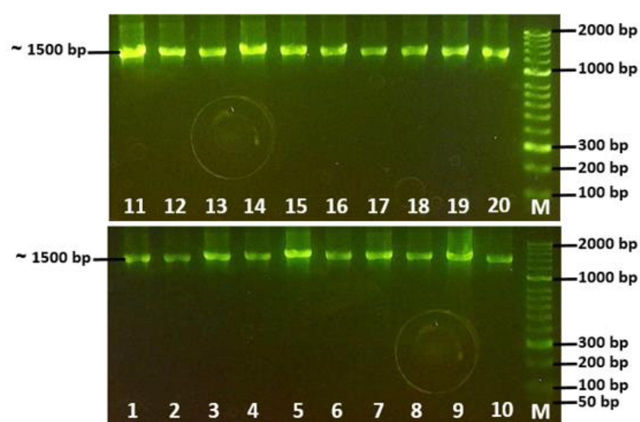

Figure 2: PCR amplification of 16S rRNA gene of 20 different isolates.

Lane M: DNA Marker with size of 50-2000 bp, Bioline; Lane 1-20 (SalHue1-SalHue20): *16S rRNA* gene with size of 1500 bp.

To characterize the 16S rRNA, the PCR products were sequenced by Sanger sequencing. The 16S rRNA sequences obtained in this study showed a similarity of 98.32-99.90% when compared with the 16S rRNA sequences of known strains on the NCBI database. We successfully identified 15 out of 20 (75%) strains as *Salmonella* spp. Of these 15 strains, six strains (SalHue1,8,9,14,15 and 20) were *Salmonella pullorum* (40%); six strains (SalHue 2,3,6,7,10 and 12) were *Salmonella gallinarum* (40%); three strains (SalHue5,16 and 17) were *Salmonella typhimurium* and other strains of *Salmonella* spp. (20%) (**Table 2**).

The phylogenetic tree (**Figure 3**) confirmed the taxonomic position of the isolated strains. The phylogenetic tree demonstrated that the *Salmonella* isolates examined in this study were divided into three major clades corresponding to the serovars *S. gallinarum*, *S. pullorum* and *S. typhimurium*. All major clades were supported by high bootstrap values, indicating strong reliability of the genetic clustering. Clade I consisted of *S. gallinarum* isolates forming a distinct lineage with high genetic similarity. Sequence analysis revealed that all six isolates exhibited 68–100% similarity in the *16S rRNA* gene compared with reference strains of *Salmonella enterica* subsp. enterica serovar *Gallinarum* (GenBank accession numbers: OK035700.1 and MN160602.1). Clade II comprised *S. pullorum* isolates. All six isolates exhibited 89–100% sequence similarity to reference strains of *Salmonella enterica* serovar *Pullorum* (GenBank accession numbers: OM988162.1, MF445124.1, MH352155.1), indicating close genetic relatedness. Clade III comprised *S. typhimurium* isolates and was strongly supported by high bootstrap values (78–100%). The isolates within this clade exhibited 100% sequence similarity to the reference strain *Salmonella enterica* subsp. enterica serovar *Typhimurium* ATCC 13311.

Table 2: Percent identity with Genbank Accession number of *16S rRNA* sequence of 15 isolates compared to those obtained from BLAST search

Isolate No.	Identified strains	Percent identity (%)	Genbank accession
SalHue 1	*Salmonella pullorum*	98.74	MH352155.1
SalHue 2	*Salmonella gallinarum*	99,65	MN160602.1
SalHue 3	*Salmonella gallinarum*	99.53	MN160602.1
SalHue 5	*Salmonella typhimurium*	99.88	MH352194.1
SalHue 6	*Salmonella gallinarum*	98.61	MN160602.1
SalHue 7	*Salmonella gallinarum*	99.12	OK035700.1
SalHue 8	*Salmonella pullorum*	96.76	MH352155.1
SalHue 9	*Salmonella pullorum*	99,88	MH352155.1
SalHue 10	*Salmonella gallinarum*	99.77	PQ577806.1
SalHue 12	*Salmonella gallinarum*	98.68	OK035700.1
SalHue14	*Salmonella pullorum*	99.78	MH352155.1
SalHue 15	*Salmonella pullorum*	99.88	MH352155.1
SalHue 16	*Salmonella typhimurium*	98.28	MH346262.1
SalHue 17	*Salmonella typhimurium*	99.90	PQ288806.1
SalHue 20	*Salmonella pullorum*	98.32	MF445124.1

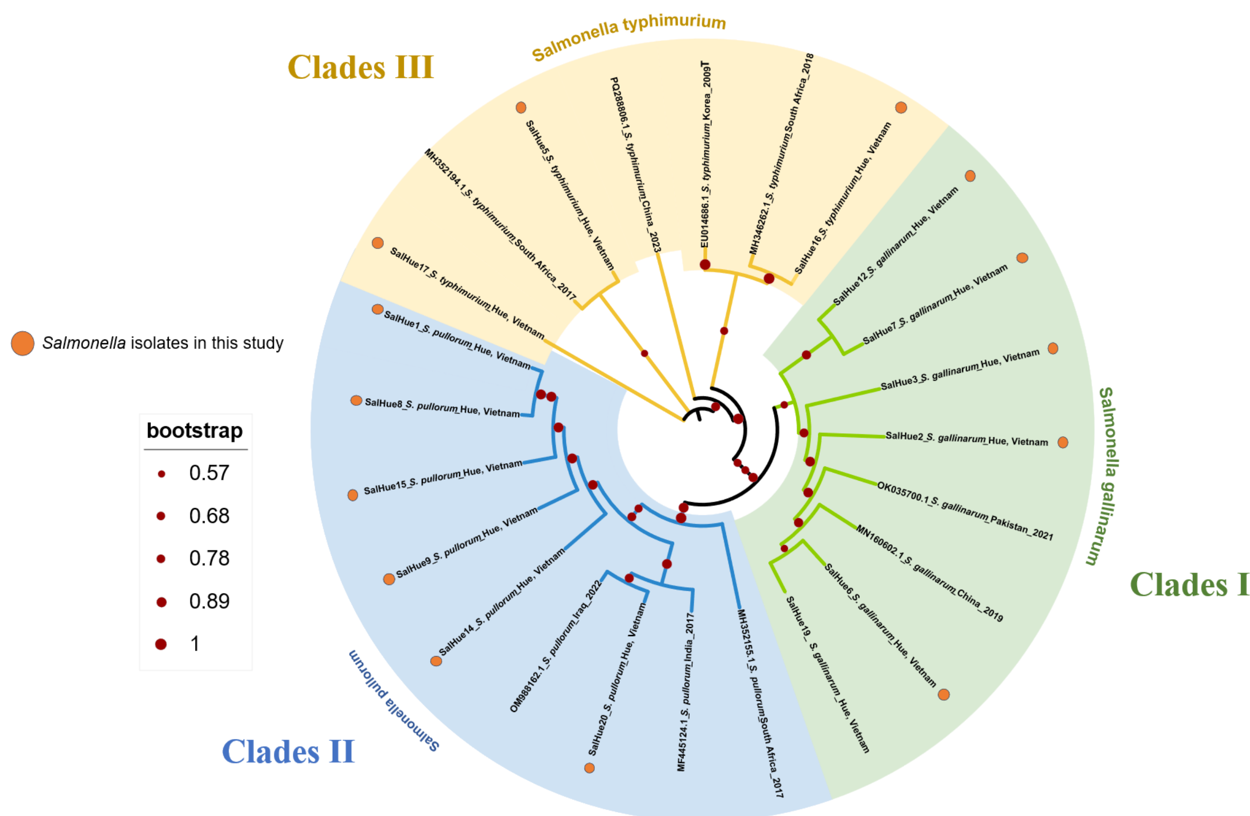

Figure 3: Phylogenetic relationships among 15 *Salmonella* isolates based on 16S rRNA gene sequences. The phylogenetic tree was constructed using the Maximum Likelihood method under the Tamura–Nei model implemented in MEGA 11, with branch robustness assessed by bootstrap analysis using 1,000 replicates. Circular nodes indicate bootstrap support values (%). The scale bar represents an estimated sequence divergence of 1%. Clades I, II, and III are highlighted in green, blue, and yellow, respectively

Table 3: Detection of virulence genes of identified *Salmonella* spp. strains by PCR

Isolate No.	Identified strains	Virulence genes					
		Stn	***fimA***	***invA***	***spvR***	***ivaB***	***spvC***
SalHue 1	*Salmonella pullorum*	+	+	+	-	+	-
SalHue 2	*Salmonella gallinarum*	+	+	+	-	+	-
SalHue 3	*Salmonella gallinarum*	+	-	+	+	+	-
SalHue 5	*Salmonella typhimurium*	+	+	+	-	+	-
SalHue 6	*Salmonella gallinarum*	+	+	+	+	+	+
SalHue 7	*Salmonella gallinarum*	+	-	+	+	-	+
SalHue 8	*Salmonella pullorum*	+	+	+	-	-	-
SalHue 9	*Salmonella pullorum*	+	-	+	+	+	+
SalHue 10	*Salmonella gallinarum*	+	+	+	-	-	-
SalHue 12	*Salmonella gallinarum*	+	-	+	+	-	-
SalHue14	*Salmonella pullorum*	+	+	+	+	+	-
SalHue 15	*Salmonella pullorum*	+	-	+	+	+	-
SalHue 16	*Salmonella typhimurium*	+	+	+	+	+	+
SalHue 17	*Salmonella typhimurium*	+	-	+	+	+	-
SalHue 20	*Salmonella pullorum*	+	+	+	+	+	-
Percentage (%)		15/15 (100)	9/15 (60)	15/15 (100)	10/15 (66.7)	11/15 (73.3)	4/15 (26.6)

+ Postive; *- Negative*

Notably, isolates within each clade exhibited high sequence similarity to *Salmonella* reference strains available in international databases, suggesting that the strains isolated in Hue city are closely related to globally circulating lineages. This observation implies that the local *Salmonella* population may be influenced by widespread international strains and

reflects the genetic conservation of these serovars within poultry populations. Several minor branches displayed lower bootstrap values (0.57–0.68), such as the isolate SalHue17 identified as *Salmonella typhimurium*, indicating reduced stability of phylogenetic relationships among closely related isolates, which may be attributable to limited sequence divergence.

3.3. Detection of virulence genes in identified Salmonella strains

The virulence genes in 15 *Salmonella* spp. strains were probed by PCR. The results showed that *stn* and *invA* toxin genes were present in all strains (15/15). Meanwhile, the *ivaB, spvR, fimA* and *spvC* toxin genes were present in 73.3%, 66.7%, 60% and 26.6% of the 15 strains respectively (**Table 3**).

4. Discussion

The 16S rRNA gene is most commonly sequenced to identify *Salmonella* and discover new species.[3,17] They are reliable markers for analysis and construction of phylogenetic trees.[18] The *Salmonella* 16S rRNA gene sequence is highly conserved and takes a long time to change and is therefore widely used for species identification and genetic evolution studies.[19,20] The results of 16S rRNA gene sequencing of 15 isolated strains and the analysis of phylogenetic data showed that six strains (SalHue1,8,9,14,15 and 20) shared high sequence identity (96.78%- 99.88%) with *Salmonella pullorum* strains (Genbank accession numbers MH352155.1; MF445124.1; OM988162.1; MH352155.1); six other strains (SalHue2,3,6,7,10 and 12) shared high sequence identity (99.61%-99.77%) with *Salmonella gallinarum* strains (Genbank accession numbers MN160602.1; OK035700.1; PQ577806.1) and three strains (SalHue5,16 and 17) shared high sequence identity (98.28%-99.9%) with *Salmonella typhimurium* strains (Genbank accession numbers MH346262.1; PQ288806.1). Furthermore, a well-characterized *Salmonella typhimurium* reference sequence (GenBank accession number EU014686.1) was incorporated as a control to confirm the correct branch assignment in the phylogenetic tree.

However, the use of the *16S rRNA* gene region as a taxonomic marker also has certain limitations, among which intragenomic gene redundancy is the most important. The presence of multiple *16S rRNA* gene copies within bacterial genomes can bias abundance estimates based on gene copy numbers, thereby leading to errors in microbial classification. Another limitation is that the variable regions of the 16S rRNA gene exhibit different degrees of sequence heterogeneity, and even conserved regions show a certain level of variability, which affects the design and selection of PCR primers.[21]

As an alternative, the *invA*-specific gene is the internationally recognized standard for detecting the *Salmonella* genus.[22,23] The *InvA* gene carries a unique gene sequence of the genus *Salmonella* and is therefore commonly used to detect *Salmonella* spp.[24] This gene is responsible for encoding a protein that helps *Salmonella* invade host epithelial cells.[22,25,26] Fifteen *Salmonella* spp. were identified and DNA extracted to perform PCR reactions to identify virulence genes. In our study, all 15 strains carried the *invA* gene (PCR product size was approximately 244 bp), a high rate of *invA* gene carriage has also been reported by other authors.[27-30] Nguyen Thi Ngan *et al.* demonstrated that 20/20 (100%) *Salmonella gallinarum pullorum* isolates obtained from eggs and chicken meat were positive for the virulence genes *stn* and *invA* by PCR.[31] Likewise, Indu Sharma and Kashmiri Das (2020) identified the *invA* gene in 40/40 (100%) *Salmonella* isolates from chicken meat using PCR amplification.[32]

The combination of chromosomal and plasmid elements is related to bacterial virulence factors. *Salmonella* strains contain many different virulence genes, among which the *stn* virulence gene encodes the important *stn* protein responsible for causing gastroenteritis leading to fever, abdominal pain, nausea, vomiting and diarrhea.[30] Virulence genes present in *Salmonella* spp. strains are responsible for salmonellosis and food poisoning in humans.[27] The biological activity of *stn* toxin plays an important role in the virulence of *Salmonella* spp., causing acute gastroenteritis.[33] All *Salmonella* serotypes carry the *stn* gene and this gene contains a unique sequence considered suitable for detecting *Salmonella* strains in field samples.[34-36] In this study, all 15 isolated *Salmonella* spp. carried the *stn* gene with a predicted size of about 260 bp. The *stn* gene was also present in all strains of *Salmonella* spp. in previous works by others.[7,30,37] A study published in 2017 by Zowail *et al.* reported that 100% (20/20) of *Salmonella* isolates obtained from chicken internal organs carried the *stn* gene.[38]

FimA is the gene encoding fimbriae in *Salmonella* spp. Fimbria-mediated association with the intestinal epithelium is a key step in the pathogenesis of enteric bacteria.[39] In this study, a 513 bp representing the *fimA* gene was detected in 9/15 (60%) *Salmonella* isolates. These findings are consistent with previous reports.[40] Many *Salmonella* isolates carried the *fimA* gene containing a unique sequence and demonstrated that this gene is suitable for PCR targeting to detect *Salmonella* strains[41]. Several studies have reported that the prevalence of the *fimA* gene in *Salmonella* isolates recovered from chicken meat and internal organs reaches 100%.[42] However, Cohen *et al.* (1996) mentioned that *Salmonella gallinarum* and *Salmonella pullorum* represent particular cases with respect to fimbrial formation; while most *Salmonella* serotypes produce type 1 fimbriae, which confer diverse adhesive properties, *S. gallinarum* and *S. pullorum* predominantly form type 2 fimbriae that lack adhesive capacity.[40] This observation is consistent with our findings, in which the amplification rate of the *fimA* gene was 60%, indicating that the gene is present at a relatively low

frequency. This discrepancy may be attributed to the source of isolation (chicken fecal samples) as well as methodological limitations that may have resulted in failure to detect the *fimA* gene in some isolates. Bacterial adhesion is considered to be an important requirement for infection. Evidence suggests that bacterial surface appendages such as fimbriae or pili enable them to bind to specific receptors on the epithelial or mucous cell surfaces of the intestine.[43] Although the virulence of several *Escherichia coli* fimbriae is known, only type 1 fimbriae (*fimA*) are thought to play an important role in *Salmonella* pathogenicity.[44,45]

Amplifying the *ivaB* gene in fifteen isolates, electrophoresis obtained a band of about 599 bp appearing in 11/15 (73.3%) strains. This result is similar to the previous report.[22] The primers were designed based on the study by Parichat Phumkhachorn and Pongsak Rattanachaikunsopon. The *invB* primers were specifically designed to target the invB gene region of *Salmonella typhimurium*, while showing no specificity toward *S. pullorum* and *S. gallinarum*. Consequently, the detected prevalence of the invB gene at 73.3% is considered appropriate.[22] The function of this gene is to act in the *ivaB* region, where the chromosome locus contains specific structural genes for the expression of the Vi (Virulence) antigen.[46]

The *spvR* gene was amplified in the isolate. The size of the amplified DNA was 310 bp, appearing in 10/15 (66.7%). The *spvR* gene encodes a protein that plays a role in the virulence of the *Salmonella* plasmid, which is a 7.8 kb locus consisting of 5 genes. The *spvR* gene is associated with some *Salmonella* serotypes. At the same time, they have the potential to increase the severity of enteritis and infection.[3,7,47-49]. Zavari *et al.* reported a 60% prevalence of the *spvR* gene among *S. typhimurium* isolates from chickens, whereas a lower prevalence (55%) was observed in 22 *Salmonella* isolates recovered from broiler chickens by Lozano-Villegas *et al.*[50,51]

From the result of the amplification, PCR products representing the *spvC* gene (571 bp) were found in 4/15 (26.6%) *Salmonella* isolates. The prevalence of this gene among *Salmonella* isolates obtained from chickens has been reported to be relatively low. Thuan Khanh Nguyen *et al.* reported that the virulence gene *spvC* was detected in 54.70% of the 75 *Salmonella*-positive samples.[52] However, a lower prevalence of the *spvC* gene was reported by Hamza M. Edid *et al.*, who did not detect this gene in any of the 26 *Salmonella* isolates recovered from chickens (0%).[42] Therefore, the 26.6% prevalence observed in the present study is consistent with international reports.Gene *spv* (*Salmonella* plasmid virulence) is a 7.8 kb locus consisting of 5 genes namely *spvRAB*.[48,53]

5. Conclusion

In this study, *Salmonella* spp. was detected in 108 out of 270 chicken samples (40%) collected from chickens suspected of having salmonellosis in Hue city, Vietnam. Molecular analysis of the 15 *Salmonella* strains showed that all strains carried major virulence genes such as *stn* and *invA,* while most strains also carried *fimA*, *ivaB*, and *spvR*, whereas *spvC* was detected at a lower frequency. The presence of multiple virulence genes indicates that the circulating *Salmonella* strains have the potential to cause significant disease in poultry. These findings highlight the importance of continuous monitoring and effective control measures to reduce Salmonella infection in chickens and minimize potential risks to animal health and food safety.

6. Source of Funding

None.

7. Conflict of Interest

Authors declare that they have no conflict of interest in current work.

8. Acknowledgements

This work was supported by the Ministry of Science and Technology of Thua Thien Hue Province (Grant No. TTH.2022-KC.07). The authors also acknowledge partial support of Hue University under the Core Research Program, Grant No. NCTB.DHH.2024.03.

References

1. Swamy SC, Barnhart HM, Lee MD, Dreesen DW. Virulence determinants invA and spvC in salmonellae isolated from poultry products, wastewater, and human sources. *Appl Environ Microbiol*. 1996;62(10):3768–71. https://doi.org/10.1128/aem.62.10.3768-3771.1996
2. Alikhan NF, Moreno LZ, Castellanos LR, Chattaway MA, McLauchlin J, Lodge M, *et al.* Dynamics of Salmonella enterica and antimicrobial resistance in the Brazilian poultry industry and global impacts on public health. *PLoS Genet*. 2022;18(6):e1010174. https://doi.org/10.1371/journal.pgen.1010174
3. Abdhalla F, Al-Gburi NM. Molecular Identification and Phylogenetic Analysis of Salmonella species Isolated from Diarrheal Children and Dogs in Baghdad Governorate, Iraq. *Iraqi J Vet Med*. 2023;47(2):50–8. https://doi.org/10.30539/ijvm.v47i2.1541
4. Soria MC, Soria MA, Bueno DJ. Comparison of 2 culture methods and PCR assays for *Salmonella* detection in poultry feces. *Poult Sci*. 2012;91(3):616–26. https://doi.org/10.3382/ps.2011-01831
5. Anejo-Okopi JA, Isa SE, Audu O, Fagbamila IO, Iornenge JC, Smith IS. Isolation and polymerase chain reaction detection of virulence invA gene in Salmonella spp. from poultry farms in Jos, Nigeria. *J Med Trop*. 2016;18(2):98. https://doi.org/10.4103/2276-7096.192237
6. Guo R, Li Z, Zhou X, Huang C, Hu Y, Geng S, et al. Induction of arthritis in chickens by infection with novel virulent Salmonella Pullorum strains. *Vet Microbiol*. 2019;228:165–72. https://doi.org/10.1016/j.vetmic.2018.11.032
7. Bekoz Yarar M, Turkyilmaz MK, Turkyilmaz S. Investigation of Integron, Antimicrobial Resistance and Virulence Gene Profiles of Salmonella enterica Subspecies enterica Serovar Infantis Isolates Obtained from Broiler Chickens. *Isr J Vet Med*. 2023;78(4):12–27.
8. Matsui H, Bacot CM, Garlington WA, Doyle TJ, Roberts S, Gulig PA. Virulence plasmid-borne spvB and spvC genes can replace the 90-kilobase plasmid in conferring virulence to Salmonella enterica

serovar Typhimurium in subcutaneously inoculated mice. *J Bacteriol*. 2001;183(15):4652–8. https://doi.org/10.1128/JB.183.15.4652-4658.2001

9. Kumar Naik V, Shakya S, Patyal A, Gade NE. Detection of Virulence Genes in Salmonella Species Isolated from Chevon and Chicken Meat. *J Anim Res*. 2015;5(1):115–8. https://doi.org/10.5958/2277-940X.2015.00019.4
10. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J Clin Microbiol*. 2007;45(9):2761–4. https://doi.org/10.1128/JCM.01228-07
11. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull*. 1987(19):11–5.
12. Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual*. Vols 1-3. 3rd ed. Cold Spring Harbor Laboratory Press; 2001.
13. Heuer H, Krsek M, Baker P, Smalla K, Wellington EMH. Analysis of Actinomycete Communities by Specific Amplification of Genes Encoding 16S rRNA and Gel-Electrophoretic Separation in Denaturing Gradients. *Appl Environ Microbiol*. 1997;63(8):3233–41. https://doi.org/10.1128/aem.63.8.3233-3241.
14. Lee K, Iwata T, Shimizu M, aniguchi T, Nakadai A, Hirota Y, et al. A novel multiplex PCR assay for Salmonella subspecies identification. *J Appl Microbiol*. 2009;107(3):805–11. https://doi.org/10.1111/j.1365-2672.2009.04263.x
15. Sunar NM, Stentiford EI, Stewart DI, Fletcher LA. Molecular techniques to characterize the invA genes of Salmonella spp. for pathogen inactivation study in composting. *ArXiv Cornell Univ Libr*. 2014. https://doi.org/10.48550/arXiv.1404.5208
16. Yulian R, Narulita E, Iqbal M, Sari DR. Detection of virulence and specific genes of Salmonella sp. indigenous from Jember, Indonesia. *Biodiversitas J Biol Divers*. 2020;21(7):2889–92. https://doi.org/10.13057/biodiv/d210703
17. Kaabi HKJA, AL-Yassari AKS. 16SrRNA sequencing as tool for identification of Salmonella spp isolated from human diarrhea cases. *J Phys Conf Ser*. 2019;1294(6). https://doi.org/10.1088/1742-6596/1294/6/062041
18. Yang B, Wang Y, Qian PY. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics*. 2016;17:135. https://doi.org/10.1186/s12859-016-0992-y
19. Whyte P, Mc Gill K, Collins JD, Gormley E. The prevalence and PCR detection of Salmonella contamination in raw poultry. *Vet Microbiol*. 2002;89(1):53–60. https://doi.org/10.1016/s0378-1135(02)00160-8
20. Saeed AA, Hasoon MF, Mohammed MH. Isolation and Molecular Identification of Salmonella typhimurium from Chicken Meat in Iraq. *J Worlds Poult Res*. 2013;3(2):63–6.
21. Regueira-Iglesias A, Balsa-Castro C, Blanco-Pintos T, Tomás I. Critical review of 16S rRNA gene sequencing workflow in microbiome studies: From primer selection to advanced data analysis. *Mol Oral Microbiol*. 2023;38(5):347–99. https://doi.org/10.1111/omi.12434
22. Phumkhachorn P, Rattanachaikunsopon P. Detection of viable Salmonella Typhi using three primer pairs spesific to invA, ivaB, and fliC-d genes. *Emir J Food Agric*. 2017;29(4)312–6. https://doi.org/10.9755/ejfa.2016-12-1867
23. Malorny B, Hoorfar J, Bunge C, Helmuth R. Multicenter Validation of the Analytical Accuracy of Salmonella PCR: towards an International Standard. *Appl Environ Microbiol*. 2003;69(1):290–6. https://doi.org/10.1128/AEM.69.1.290-296.2003
24. Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galán JE, Ginocchio C, et al. Amplification of an invA gene sequence of Salmonella typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. *Mol Cell Probes*. 1992;6(4):271–9. https://doi.org/10.1016/0890-8508(92)90002-f
25. Darwin KH, Miller VL. Molecular Basis of the Interaction of Salmonella with the Intestinal Mucosa. *Clin Microbiol Rev*. 1999;12(3):405–28. https://doi.org/10.1128/cmr.12.3.405
26. Alarjani KM, Elkhadragy MF, Al-Masoud AH, Yehia HM. Detection of Campylobacter jejuni and Salmonella typhimurium in chicken using PCR for virulence factor hipO and invA genes (Saudi Arabia). *Biosci Rep*. 2021;41(9):BSR20211790. https://doi.org/10.1042/BSR20211790
27. Chaudhary JH, Nayak JB, Brahmbhatt MN, Makwana PP. Virulence genes detection of Salmonella serovars isolated from pork and slaughterhouse environment in Ahmedabad, Gujarat. *Vet World*. 2015;8(1):121–4. https://doi.org/10.14202/vetworld.2015.121-124
28. Nagappa K, Shantanu T, Mumtesh KS, Singh SP. (PDF) Isolation of Salmonella Typhimurium from poultry eggs and meat of Tarai region of Uttaranchal. *Indian J Biotechnol*. 2007;6:407–9.
29. Karmi M. Detection of Virulence Gene (inva) in Salmonella Isolated from Meat and Poultry Products. *Int J Genet*. 2013;3(2):7–12. https://doi.org/10.5829/idosi.ijg.2013.3.2.82204
30. Fekry E, Eman-abdeen E, Ammar AM, Hussien A edin. Molecular Detection of InvA, OmpA and Stn Genes in Salmonella Serovars from Broilers in Egypt. *Alex J Vet Sci*. 2018;56(1):69–74. https://doi.org/10.5455/ajvs.288089
31. Ngân NT, Phúc PTH, Thùy PD, Duyên DTH, Thắng TN, Yên NX. Study on the virulence of isolated salmonella strains from chickens infected with salmonellosis and use of nanosan probiotics in treating the disease for chicken at tam duong district, vinh phuc province. *TNU J Sci Technol*. 2022;227(01):159–66. https://doi.org/10.34238/tnu-jst.5390
32. Sharma I. Detection of invA Gene in Isolated Salmonella from Marketed Poultry Meat by PCR Assay. *J Food Process Technol*. 2016;7(3). https://doi.org/10.4172/2157-7110.1000564
33. Chopra AK, Peterson JW, Chary P, Prasad R. Molecular characterization of an enterotoxin from Salmonella typhimurium. *Microb Pathog*. 1994;16(2):85–98. https://doi.org/10.1006/mpat.1994.1010
34. Moore MM, Feist MD. Real-time PCR method for Salmonella spp. targeting the stn gene. *J Appl Microbiol*. 2007;102(2):516–30. https://doi.org/10.1111/j.1365-2672.2006.03079.x
35. Singh Y, Tiwari A, Kumar R, Saxena MK. Cloning, Sequencing and Phylogenetic Analysis of Stn Gene of Salmonella Typhimurium. *Biosci Biotechnol Res Asia*. 2017;14(4):1387–93. http://dx.doi.org/10.13005/bbra/2583
36. Ammar AM, Abdeen EE, Abo-Shama UH, Fekry E, Kotb Elmahallawy E. Molecular characterization of virulence and antibiotic resistance genes among Salmonella serovars isolated from broilers in Egypt. *Lett Appl Microbiol*. 2019;68(2):188-195. https://doi.org/10.1111/lam.13106
37. Raji MA, Kazeem HM, Magyigbe KA, Ahmed AO, Lawal DN, Raufu IA. Salmonella Serovars, Antibiotic Resistance, and Virulence Factors Isolated from Intestinal Content of Slaughtered Chickens and Ready-to-Eat Chicken Gizzards in the Ilorin Metropolis, Kwara State, Nigeria. *Int J Food Sci*. 2021;2021:8872137. https://doi.org/10.1155/2021/8872137
38. Zowail MEM, Azab MS, Nassif SA, Elsadek GM, Mohamed SZ. Some Molecular Studies on Salmonella on Chickens. *Int J Sci Res*. 2017;6(11):349–53. https://doi.org/10.21275/ART20177777
39. Zeiner SA, Dwyer BE, Clegg S. FimA, FimF, and FimH Are Necessary for Assembly of Type 1 Fimbriae on Salmonella enterica Serovar Typhimurium. *Infect Immun*. 2012;80(9):3289–96. https://doi.org/10.1128/iai.00331-12
40. Cohen HJ, Mechanda SM, Lin W. PCR amplification of the fimA gene sequence of Salmonella typhimurium, a specific method for detection of Salmonella spp. *Appl Environ Microbiol*. 1996;62(12):4303–8. https://doi.org/10.1128/aem.62.12.4303-4308.1996
41. Jawad AAK, Al-Hamadani AH. Detection of fimA and fimC genes of Salmonella isolates by using Polymerase Chain Reaction. *J Basrah Res Sci*. 2011;37(4).
42. Eid HM, El-Demerdash AS, Hassonna NM. Molecular Analysis of Some Virulence Genes of Salmonellae Isolated from Chicken at Sharkia Governorate. *Suez Canal Vet Med J SCVMJ*.

2020;15(2):261–75. https://doi.org/10.21608/scvmj.2020.40819.1015

43. Kukkonen M, Raunio T, Virkola R, Lähteenmäki K, Mäkelä PH, Klemm P, et al. Basement membrane carbohydrate as a target for bacterial adhesion: binding of type I fimbriae of Salmonella enterica and Escherichia coli to laminin. *Mol Microbiol*. 1993;7(2):229–37. https://doi.org/10.1111/j.1365-2958.1993.tb01114.x
44. Clegg S, Pruckler J, Purcell BK. Complementation analyses of recombinant plasmids encoding type 1 fimbriae of members of the family Enterobacteriaceae. *Infect Immun*. 1985;50(1):338–40. https://doi.org/10.1128/iai.50.1.338-340.1985
45. Aslanzadeh J, Paulissen LJ. Role of Type 1 and Type 3 Fimbriae on the Adherence and Pathogenesis of Salmonella enteritidis in Mice. *Microbiol Immunol*. 1992;36(4):351–9. https://doi.org/10.1111/j.1348-0421.1992.tb02034.x
46. Xiong K, Zhu C, Chen Z, Zheng C, Tan Y, Rao X, et al. Vi Capsular Polysaccharide Produced by Recombinant Salmonella enterica Serovar Paratyphi A Confers Immunoprotection against Infection by Salmonella enterica Serovar Typhi. *Front Cell Infect Microbiol*. 2017;7:135. https://doi.org/10.3389/fcimb.2017.00135
47. Oliveira SD de, Rodenbusch CR, Michael GB, Cardoso MIR, Canal CW, Brandelli A. Detection of virulence genes in Salmonella Enteritidis isolated from different sources. *Braz J Microbiol*. 2003;34:123–4. https://doi.org/10.1590/S1517-83822003000500042
48. Card R, Vaughan K, Bagnall M, Spiropoulos J, Cooley W, Strickland T, et al. Virulence Characterisation of Salmonella enterica Isolates of Differing Antimicrobial Resistance Recovered from UK Livestock and Imported Meat Samples. *Front Microbiol*. 2016;7:640. https://doi.org/10.3389/fmicb.2016.00640
49. Dougnon TV, Legba B, Deguenon E, Hounmanou G, Agbankpe J, Amadou A, et al. Pathogenicity, Epidemiology and Virulence Factors of Salmonella species: A Review. *Not Sci Biol*. 2017;9(4):460-466. https://doi.org/10.15835/nsb9410125
50. Zavari Ali, Badouei MahdiA, Tabar Gholamreza H. Molecular Epidemiology and Genetic Profile of Non-Typhoidal Salmonella Serovars Isolated From Poultry Production Chain in Khorasan Province, Iran: A Comprehensive Analysis of Prevalence, Virulence Genes, and Antibiotic Resistance. *Vet Med Sci*. 2026;12(1):e70757. https://doi.org/10.1002/vms3.70757
51. Lozano-Villegas KJ, Rondón-Barragán IS. Virulence and antimicrobial-resistant gene profiles of salmonella spp. isolates from chicken carcasses markets in Ibague City, Colombia. *Int J Microbiol*. 2024;2024(1):4674138. https://doi.org/10.1155/2024/4674138
52. Nguyen TK, Huynh LM, Vo TVD, Tran DD, Kha TT, Ly KTL, et al. Virulent Genes and Genetic Relationship of Salmonella spp. Isolated from Chickens and Husbandry Environments in Small-Scale Farms in the Mekong Delta, Vietnam. *Worlds Vet J*. 2025;15(1):126–33. https://doi.org/10.54203/scil.2025.wvj15
53. van Asten AJAM, van Dijk JE. Distribution of “classic” virulence factors among Salmonella spp. *FEMS Immunol Med Microbiol*. 2005;44(3):251–9. https://doi.org/10.1016/j.femsim.2005.02.002

Cite this article: Nguyen ATQ, Huynh CV, Truong HTH, Ho HN, Nguyen LT, Nguyen TTT, et al. Sequence-based detection of *Salmonella* spp. from symptomatic chicken and characterizing virulence. *Indian J Microbiol Res*. 2026;13(1):158–166.