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Finding Genetic Differences in *Salmonella enterica* Isolated from Poultry

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Abstract | Deciding the hereditary variations of *S. enterica* disconnected from broiler chicken faeces can provide critical insights to control this infection in poultry. A 286-length genomic part encoding locale of the symptomatic quality (*invA*) was used to assess the diversity of *S. enterica* in 15 bacterial samples. A coordinate sequencing methodology was connected to the PCR amplicons recognized within the upgraded genomic locus. A phylogenetic tree was applied within the recognized varieties to overview the particular phylogenetic separations in differentiate with other comparable bacterial progression. The current results suggest that the investigated samples and the *S. enterica* sequences have roughly 99% homology. A total of 19 genetic variants of A34T, A35del, C36G, C36A, T42del, A69del, A69G, A70ins, G71ins, G91ins, A119ins, A124G, G223A, C253T, C278G, C278T, T279G, T279C, and G280C were identified among the *InvA* genes. In most of the samples, some variances were distributed differently. Several nucleotides insertion mutations were found at positions 70, 71, 91, and 119 in the *InvA* gene sequencing. However, deletion mutations were found in most samples at A35, T42, and A69 sites. These finding highlight the genetic diversity of *S. enterica* and may attribute to the prevalence of infection in the poultry.

Keywords | Genetic, *Salmonella enterica*, Poultry, Thi-Qar, chicken meat, diseases

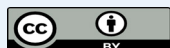
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INTRODUCTION

Since chicken meat is one of the most basic food sources for humans, livestock is among the most crucial economic sectors in underdeveloped countries (Abdulkareem, 2020; Al-Jebory *et al.*, 2024; Salman *et al.*, 2024a, b). Poultry are susceptible to a wide range of diseases, including viral and bacterial ailments such as salmonellosis (Naji and Hanna, 1999; Al-Saeedi *et al.*, 2024; Ajafar *et al.*, 2024). If dangerous microorganisms are present, the carcass's traits and attributes may suffer (Jaffar and Abdulkareem, 2022). Enteric infections are

a major source of illness and death worldwide. Gram-negative enteric microbes are thought to be mindful for approximately 3 million fatalities around the world each year from loose bowels and gastrointestinal illness (Donnenberg, 2000). *Salmonellae* is a gram⁻ bacterium that causes serious illnesses in humans and domestic animals (Makela and Hormaeche, 1997; Eisenstein, 1999). There are around 2668 distinct *Salmonella enterica* serotypes. A major cause of food-borne disease outbreaks and a worldwide public health issue, the condition can harm both people and animals (Akbarmehr, 2011).

Salmonella enterica is the pathogen that causes salmonellosis. *Salmonellosis*, which occurs when feed or water sources are taken after being contaminated by the urine or feces of animals that may act as *Salmonella* reservoirs, causes significant morbidity and mortality worldwide (Raffatellu *et al.*, 2008; Akbermehr, 2012). Depending on the host's susceptibility, *Salmonella enterica* infections presented a range of clinical signs (Crump *et al.*, 2015; Majowicz *et al.*, 2010). These include bacteremia, E.F, gastroenteritis, and ASC (Burns *et al.*, 1997; Andrews-Polymenis *et al.*, 2010). Although human salmonellosis ordinarily up as a self-limiting nourishment harming, it can moreover sometimes result in a major systemic ailment or E. fever. Contaminated food is the essential implies of transmission for non-typhoidal sicknesses, developed *Salmonella*, a zoonotic ailment that influences a large number of animals reservoir. The foremost common of these poultry animal (Akbarmehr, 2011).

Non-typhoidal *Salmonella* is responsible for an estimated 1 million infections, 20,000 hospitalizations, and 378 deaths per year. Three million children are killed by it every year in both wealthy and developing countries (Cardinale *et al.*, 2005; Scallan *et al.*, 2011), *Salmonella*-specific PCR using *invA* primers was found by Lampel *et al.* (2000) to be sensitive, quick, and specific for detecting *Salmonella* in a variety of clinical samples. Furthermore, Kunduk *et al.* (2006) reported that PCR is a quick, accurate, and precise way to diagnose typhoid (Feyer and Karmi, 2013). The invasion of host epithelial cells is dependent on *Salmonella's invA* gene, which contains sequences unique to the genus *Salmonella*. It has also been confirmed to be a basic PCR target with significant diagnostic applications. The molecular approaches are the most straightforward and economical. This gene is consequently a promising target for the detection of *Salmonella* from different forms of biological material, as was previously mentioned, the aimed of study to pinpoint the *invA* gene responsible for cell invasion offer atomic clarification of it. This was accomplished by utilizing the Sanger arrangements procedure to decide the developmental tree of the *Salmonella* segregates beneath consider and analyze the sequencing of the intensified quality portion.

MATERIALS AND METHODS

DNA EXTRACTION AND PCR

Follow the directions of the manufacturer (Geneaid Biotech, Taiwan), DNA extraction was performed The PCR specifically targeting *InvA* gene was performed using

the master mix (Table 1) and the thermoprofile outlined in Table 2. Afterwards, PCR products were run on a 1.5% agarose gel, they were stained for 45 minutes at 95 volts in 1X TBE buffer.

Table 1: Master mix for the preparation of PCR solution

Reagents	Volume
Primer forward	1.5 µl
Primer reverse	1.5 µl
DNA template (<250ng)	1 µl
Free water	16 µl
Total	20 µl

DNA SEQUENCING OF PCR AMPLICONS

The PCR amplicon sequences were acquired from the terminal in accordance with the instructions provided by the sequencing provider (Macrogen Inc. Geumchen, Seoul, South Korea). By further analyzing only clear chromatographs obtained from ABI sequence data, the annotation and variations were confirmed to be free of PCR or sequencing artifacts. By comparing the observed nucleic corrosive arrangements of the bacterial tests with the reference arrangements that were retrieved from the bacterial database, the virtual areas and other points of interest of the obtained PCR fragments were determined.

INTERPRETATION OF SEQUENCING DATA

BioEdit Assemble Edit Suite v. 7.1 (D.M. WI, USA) was used to modify the PCR products' sequencing findings, modified, and evaluated alongside the sequences within the corresponding reference database. The nucleic acids sequences detected were counted within both the PCR amplicons and the corresponding regions within the reference genome. Each revealed variant within *S. enterica* genes was described by means of SnapGene Viewer 4.0.4 (<https://www.snapgene.com>).

PHYLOGENETIC TREE

This analyse constructed a particular phylogenetic tree according to (Zhang *et al.*, 2000) and comprehensive tree was constructed as outlined by the Hanan *et al.* (2021); Gharkan *et al.* (2023).

RESULTS

The *invA* gene variant was found in 15 samples with amplicons that were roughly 286 bp long. All of the

Table 2: The condition of PCR for *invA* gene amplification.

Monoplex gene	°C/Time					Cycle number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
inv A	95/5min	94/40 s	58/60s	72/90s	72/10 min	30

Table 3: The 286 bp PCR amplicons used to access a subset of the InvA quality within the genomic DNA groups of *S. enterica* (GenBank accession number NC_003197).

Amplicon	Reference locus sequences (5' - 3')	Length
InvA gene	*TCATCGCACCGTCAAAGGAACCGTAAAGCTGGCTTTCCCTTTCCAGTACGCTTTCGC-CGTTTCGCGCGCGGCATCCGCATCAATAATACCGGCCTTCAAATCGGCATCAATACT-CATCTGTTTACCGGGCATACCATCCAGAGAAAATCGGGCCGCGACTTCCGCGA-CACGTTCTGAACCTTTGGTAATAACGATAAACTGGACCACGGTGACAATAGAGAA-GACAACAAAACCCACCGCCAGGCTATCGCCAATAACGAATTGCCCGAACGTGGCGA-TAATTTTAC**	286 bp

* Refers to the forward primer sequences (placed in a forward direction).

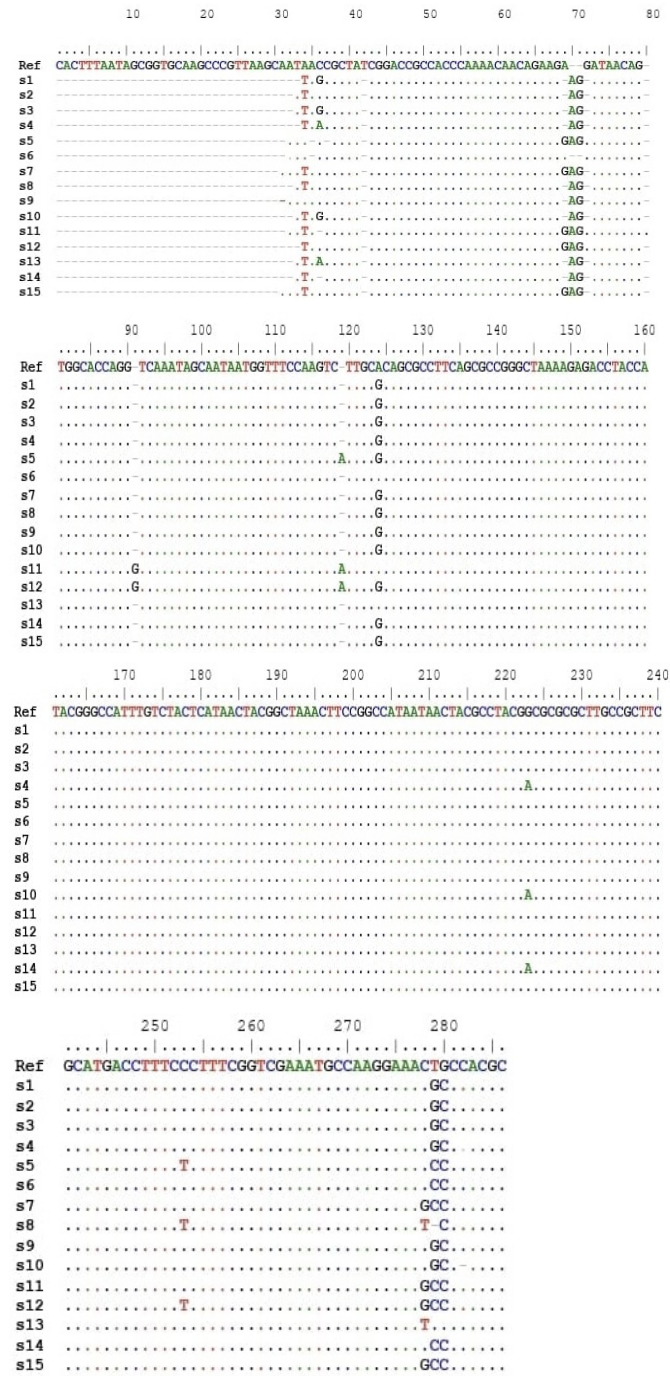


Figure 1: Nucleic destructive course of action course of action of 15 bacterial tests with its comparing reference groupings of the InvA inside the *S. enterica* genomic DNA courses of action. The picture ref: implies to the NCBI reference courses of action, whereas “S: implies to test numbers.

amplified amplicons were checked for clean, distinct, and sharp bands before sending for sequencing. By utilizing NCBI impact, the sequencing responses illustrated the intensified products confirmed manually. The NCBI BLASTn motor uncovered a tall degree of arrangement closeness between the sequenced tests and *S. enterica* arrangements with respect to the 286 bp PCR amplicons of the as of now focused on InvA arrangements. The NCBI BLASTn motor appeared that the InvA quality sequences coding locale was mostly secured by roughly 99% homology with the expecting target. The highlights of these groupings were underscored inside the increased groupings (Table 3) by differentiating the gotten DNA groupings (GenBank acc. NC_003197) with the watched DNA arrangements of the tests beneath examination.

The 286 bp PCR amplicons used to access a subset of the InvA quality within the genomic DNA groups of *S. enterica* (GenBank accession number NC_003197) as are displayed in Table 3 along with their length and location. A total of 19 nucleic acid differences were found in the alignment findings of the 286 bp samples when compared to the matching *S. enterica* referencing sequences (Figure 1). These sequences were created by matching the most related sequences that were deposited in the NCBI database (GenBank accession NC_003197) with the samples that we examined.

According to the analyzed InvA sequences in the bacterial samples under examination, a phylogenetic tree was generated. The three inspected tests (LC762569, LC762570, and LC762571) were included in this phylogenetic tree beside the other stored DNA groupings. These tests were adjusted with their profoundly related groupings in Tamura-Nei mode. There were sixteen adjusted nucleic corrosive groupings within the tree because it was as of now shaped. The inspected InvA groupings were assembled into various neighboring phylogenetic branches based on the hereditary arrangements of *S. enterica*, showing an incredible bargain of inconstancy in this life form with regard to the inspected InvA groupings (Figure 2). *S. enterica* subsp. *enterica* serovar Ouakam, *S. enterica* subsp. *enterica* serovar Newport, *S. enterica* subsp. *enterica* serovar Enteritidis, and *S. enterica* subsp. *enterica* serovar Typhimurium were also included, along with other relevant serovars. All of these subspecies, however, belonged to the enterica serovar.

Table 4: The plan of the observed contrasts inside the 286 bp of the InvA amplicons in comparison with the NCBI reference groupings (GenBank acc. no. NC_003197).

Sample	Variant	Position in the PCR fragment
S1, S2,S3, S4, S7, S8,S10,S11, S12,S13, S14, S15	A34T	34
S5, S6	A35 del	35
S1, S3, S10	C36G	36
S4,S13	C36A	36
S1,S2 S3, S4, S5,S6,S7, S8,S9,S10,S11, S12,S13, S14, S15	T 42del	42
S1, S2,S3,S4, S8,S9,S10, S13, S14	A69del	69
S5, S7, S11, S112	A69G	69
S1,S2 S3, S4, S5, S7, S8,S9,S10,S11, S12,S13, S14, S15	A70ins	70
S1,S2 S3, S4, S5, S7, S8,S9,S10,S11, S12,S13, S14, S15	G71ins	71
S11,S12	G91ins	91
S5,S11,S12	A119ins	119
S4,S10,S14	A124G	124
S5,S8,S12	G223A	223
S7,S11,S12,S15	C253T	253
S8,S13	C278G	278
S1,S2,S3,S4,S9,S10	C278T	278
S6,S7,S8,S11,S12,S13,S14,S15	T279G	279
S1,S2,S3,S4,S5,S6,S7,S8,S9,S10,S11,S12,S13,S14,S15	T279C	279
	G280C	280

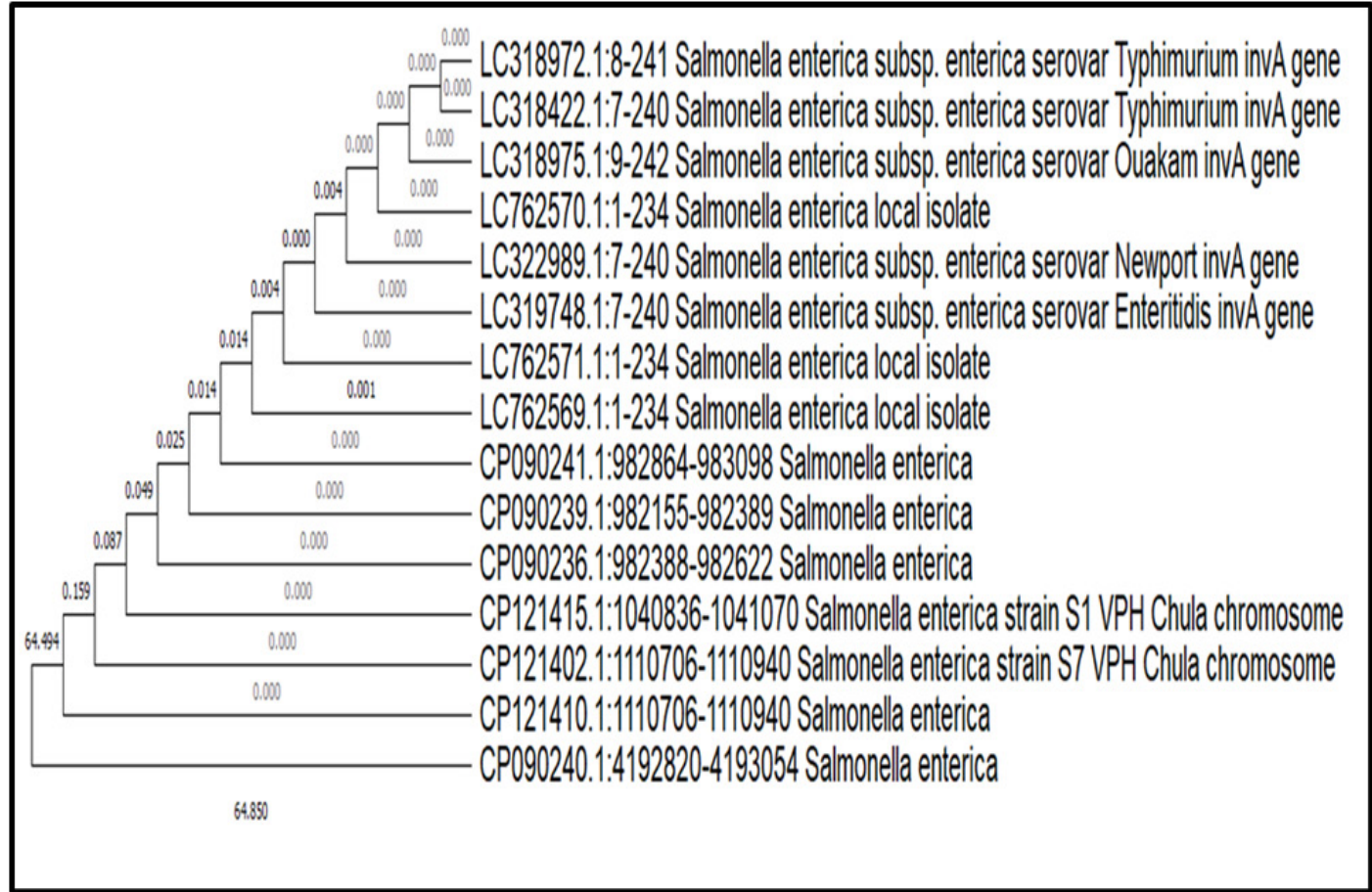


Figure 2: Using the Neighbor-Joining approach, the developmental history was collected, and the perfect tree emerged (moving on to the branches later). The developmental separations were calculated using the Most Extreme Composite Probability approach and were within the units of the number of base substitutions per place. This study included sixteen nucleotide configurations, including first, second, third, and noncoding codon positions. All ambiguous positions were eliminated for each arrangement combine (pairwise erasure alternative), resulting in 290 positions in the final dataset.

SUBMISSION IN NCBI

InvA gene sequences were submitted in NCBI's Gene Bank and are available under accession numbers: LC762569, LC762570, and LC762571.

DISCUSSION

Investigating *S. enterica*'s genetic variants was the aim of the most recent study. To do this, 15 bacterial samples (designated S1 to S15) were collected from grill chicken, ROSE 308 faeces, and were analysed for the *InvA* sequences. Salmonella's invasion of intestinal epithelial cells depends on the *invA* gene, which codes for a protein in the bacterial inner cell wall (Al-Kaaby *et al.*, 2014; Sharma and Das, 2016; Shanmugasamy *et al.*, 2011). *Salmonella*'s *invA* gene is also utilised in the molecular diagnostics of the bacterium because it comprises a distinct sequence of this genus (Karim *et al.*, 2020).

One of the numerous chromosomal virulence genes that were utilised to detect Salmonella in poultry faecal samples was the *invA* gene. The PCR assay of Salmonella serovar also targeted the *invA* gene (Jamshidi *et al.*, 2009), and the *invA* gene was grouped in the islands of Salmonella species' pathogenicity (Daigle, 2008). The virulent *invA* gene, which encoded a protein present in the inner layer of the bacterial membrane and had sequences that were specific to all salmonella serovars, is what allowed the bacteria to infiltrate the host's epithelial cells (Daigle, 2008).

Salmonella-specific sequences can be found in the *invA* sequence of enteric bacteria. It has been found to be a good PCR target with possible diagnostic applications (Mohamed, 2013). Different distributions of the analysed samples were caused by the neighbouring places in the phylogenetic tree being attributed to the rate of variations within those samples. According to the *InvA* gene chromatograph file data, there are deletion mutations in several locations along this sequence, such as A35, T42, A69, A71, and 119del. Additionally, nucleotide insertion was found in a few different locations within the *InvA* gene, such as: A nucleotide in position 70 and G in the majority of samples; G nucleotide in position 91 of the S11 and S12 samples; and A nucleotide in position 119. The local isolate *S. enterica* (LC762570.1) exhibited a closed relationship and 100% similarity with reference isolates *S. enterica* subsp. *enterica* seovar *Typhimurium* (LC318972.1, LC318422.1) and *S. enterica* subsp. *Enterica* seovar Ouakam, as indicated by the phylogenetic tree of the *InvA* gene. The observed phylogenetic distances (tree scale 0.001) between the integrated organisms and local isolates within this clade are noteworthy since they clearly show that the incorporated bacterial genomes have progressive homology. To determine the relationship between Iraqi

isolates and the higher query cover, which exceeds 90% of national isolates, the maximum likelihood and minimum evolution methods were used to create the phylogenetic tree for each gene.

Taken together, the finding of this study highlight the emerging genetic variations in the *S. enterica* and may offer foundational information for improved diagnostics and vaccine preparation to contain the infection in future.

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NOVELTY STATEMENT

This study investigates the novel molecular characteristics of Salmonella isolated from poultry in Dhi Qar province. The research aims to determine its resistance to antibiotics and other emerging properties.

AUTHOR'S CONTRIBUTION

ZKH: Designed the study and conducted DNA sequence analysis using bioinformatics tools.

AAA: Performed laboratory experiments including DNA extraction and PCR amplification.

NHG: Contributed to statistical analysis and figure preparation.

AAA: Supervised the research, provided academic guidance, and reviewed the manuscript.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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