Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Molecular Detection of *Salmonella enterica* serovars Enteritidis from Poultry Processing Environments at Wet Markets in Sulaymaniyah Province, Iraq

Khoshi A. Muhammed and Eman D. Arif*

Microbiology Department, Veterinary Medicine College, Sulaimani University, Sulaymaniyah, Iraq.

Abstract

SALMONELLA enterica affects both humans and animals and is categorized as an invasive gastrointestinal pathogen. *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) primarily affects individuals who consume poultry products. In this study, we aimed to isolate and detect the presence of *S.* Enteritidis in the poultry processing environment at the wet market in Sulaymaniyah Province using polymerase chain reaction (PCR), as well as to identify the special virulence gene, Hyperinvasive locus A (*hilA),* in this serovar. 200 samples were obtained from ceca, body swabs, knives, chopping boards, and cages, as well as the hands of workers. Culture-based and PCR-based techniques were used for the extraction and identification. Phylogenetic analysis was done by sequencing the *Salmonella* difference fragment I *(sdfI)* gene. Culture methods identified *Salmonella* in 50% of the samples, with the highest rate found in cecum samples (n = 39, 54.17%). PCR results confirmed that only (n = 2) 2% of the isolates were identified as *S.* Enteritidis using the *sdfI* gene, both of which were from chicken ceca. All remaining samples tested negative for the serovar, but the *hilA* gene was detected in both *S.* Enteritidis strains. Phylogenetic analysis revealed that the Iraqi isolate with accession number PV250091 belonged to a distinct subclade, indicating local genetic variation. The findings of this study revealed that poultry environments were intensely contaminated with *Salmonella*. Nevertheless, the low prevalence of *S.* Enteritidis and its virulence potential underscores the need for improved hygiene and surveillance in wet markets.

Keywords: *hilA gene, Poultry, Wet Markets, Salmonella Enteritidis, sdfI gene.*

Introduction

Salmonella enterica represents a category of invasive gastrointestinal pathogens that impact both human and animal populations. This organism comprises six distinct subspecies that occupy diverse ecological niches among a broad spectrum of host organisms. *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) is one of the most frequently encountered serovars associated with human salmonellosis [1, 2]. The incidence of foodborne outbreaks attributable to this microorganism is predominantly linked to the ingestion of contaminated poultry meat and associated poultry products, notably eggs. Among the numerous diseases that affect poultry, those related to the *Salmonella* genus are the most common, resulting in considerable economic losses for the poultry sector due to heightened mortality rates, impaired growth, and decreased egg production [3,4].

A range of contributing factors shapes the pathogenic potential of *Salmonella*. It relies on its capacity to adhere to and attack host cells, survive within epithelial cells and macrophages, and replicate inside them, facilitated by several virulence factors often found on plasmids. Numerous virulence genes are situated in particular areas of the genome referred to as "*Salmonella* pathogenicity islands" (SPIs), which are obtained via horizontal gene transfer [5]. *S.* Enteritidis can remain in the reproductive tissues of infected hosts, even in the presence of the host's innate and adaptive immune responses to the infection, as the bacteria are capable of circumventing the host's defense mechanisms. During infection, *S.* Enteritidis uses proteinaceous filamentous surface structures called fimbriae to adapt to host tissue by ingeniously creating a colonization niche. As protective and diagnostic antigens, these structures recognize and attach to host cells [2]. The *hilA*

*Corresponding authors: Eman Dhahir Arif, E-mail: eman.aref@univsul.edu.iq, Tel:009647702105528
(Received 04 July 2025, accepted 27 August 2025)
DOI: 10.21608/ejvs.2025.400694.2946

gene, known as the hyperinvasive locus A, codes for a transcriptional regulator belonging to the OmpR/ToxR family, which promotes the expression of genes that encode the type III secretion system and its related effector proteins, thereby participating in invasion due to environmental and genetic regulatory influences [6, 7].

Chicken meat is processed in extensive facilities, but wet markets continue to be a crucial channel for poultry distribution in Asian nations. These markets sell fresh fruit, meat, and seafood at room temperature, subject to environmental conditions [8]. Sulaymaniyah Province has numerous wet markets. All of the steps, including slaughtering, evisceration, scalding, rinsing, plucking, and packaging, are carried out by hand or with simple tools at these manual markets. The majority of the wet markets were generally in highly unsanitary conditions. The scalding areas and plucking boards were dirty, and the majority of the workers were not wearing gloves [9].

Some Iraqi studies on *Salmonella* have primarily focused on product contamination, antimicrobial resistance, or selected virulence factors. One study analysed microbial quality in chicken breast meat from both approved and non-approved slaughterhouses during storage, but without serovar-specific characterization or virulence gene characterization [9]. Another study examined *Salmonella* isolates from imported chicken carcasses in relation to antimicrobial resistance and whole-genome sequencing, but did not investigate the *hilA* gene or perform *sdfI*-based phylogenetic analysis [10]. Moreover, a separate study examined certain virulence factors and carbapenemase genes in *S.* Enteritidis isolated from chicken meat and eggs in Iraq [11].

The objective of this research was to isolate and identify *Salmonella* from samples taken from the poultry processing environment at a wet market located in Sulaymaniyah Province. Determine the prevalence of *Salmonella enterica* serovar Enteritidis and detect the specific virulence gene, *hilA*, using polymerase chain reaction (PCR).

Material and Methods

Sampling

A total of 200 samples were randomly collected on different occasions from the small-scale processing facility located in the wet markets of Sulaymaniyah Province, Iraq, between October 2024 and March 2025. Fig. 1 showed the precise sampling locations. On each sampling date, markets were selected randomly from the list of active wet markets in the province. Further within the market, sampling points were also randomly chosen to avoid bias. Among the sources used for sampling were cutting boards (n = 29), knives (n = 32), broiler ceca following slaughter (n = 72), broiler body cavity and surface swabs (n = 27), hands of workers (n = 10), cages for poultry (n = 30). Sampling dates were therefore evenly distributed across the six months to counterbalance any potential time variations in contamination levels. Samples were taken using sterile cotton swabs that had been pre-soaked in 5 ml of buffered peptone water (Liofilchim, Italy). To preserve sample integrity, all samples were collected aseptically and sent right away in insulated cool boxes to the Research Center at the University of Sulaimani's College of Veterinary Medicine.

Culture method

After collecting the samples, they were placed in 10 ml of pre-enrichment broth (buffered peptone water) (Liofilchim, Italy). Each sample was cultured on the same day it was collected to ensure optimal microbial recovery. The samples were incubated for 24 hours at 37°C. Subsequently, the cultures were transferred to tetrathionate broth (Liofilchim, Italy), a selective enrichment medium for *Salmonella*, and incubated for an additional 48 hours at 37°C. A loopful of this cultured broth was then inoculated onto MacConkey agar (Liofilchim, Italy) and incubated for 24 hours. Any suspected colonies observed on this medium were further cultured on Xylose Lysine Deoxycholate (XLD) agar (Liofilchim, Italy), where *Salmonella* appeared as pink colonies with a black center. Following purification, the potentially implicated colonies were shifted to nutrient agar and incubated at 37 °C for 24 hours. They then underwent biochemical tests for urease and the Triple Sugar Iron agar (TSI) test. *Salmonella*, which was urease-negative, caused black precipitation on TSI agar, accompanied by an alkaline slant and an acid butt in TSI medium [12].

Extraction of DNA

DNA extraction from the samples was successfully performed using the boiling method, a commonly employed technique for extracting DNA from bacterial colonies. The boiling method was recognized as a rapid and efficient technique for extracting DNA from bacterial colonies [13]. The procedure began with the collection of a fresh single colony from each nutrient agar plate, which was then transferred into 1.5 ml Eppendorf tubes containing 100 µL of deionized water. The tubes were vortexed thoroughly to create a uniform cell suspension. Following this, the tubes were boiled at 100°C for 10 minutes using a thermo-shaker. After boiling, the

tubes were placed on an ice pack for 5 minutes. This rapid transition from high heat to cold effectively disrupted the cell walls, allowing the release of DNA from the bacterial cells. The bacterial suspension was then centrifuged for five minutes at 15,000 rpm. Following centrifugation, 50 µL of the supernatant, which contained the bacterial DNA, was carefully pipetted into individual sterile Eppendorf tubes. These tubes were stored at -20°C for future analyses. To measure the DNA's concentration and purity, a Nanodrop spectrophotometer (Blue-Ray Biotech, Taiwan) was used [14].

Molecular Method

Specific primers were used for each gene produced by Macrogen, South Korea, for the identification of *S.* Enteritidis. The *sdfI* gene was amplified using the *sdfI*-F (forward) and sdfI-R (reverse) primers, resulting in an amplicon of 304 bp. The *hilA* forward and reverse primers were also utilized to detect the virulence gene (Table 1).

PCR amplification

Gene amplification was performed using 2× GS (GeneSand, China) Taq PCR mix. 0.2 mL PCR tubes were employed for this process. Each tube contained 1 µL (10 pmol) of forward primers (sdfI-F), 1 µL (10 pmol) of reverse primers (sdfI-R), 5 µL of DNA, and 10 µL of the PCR mixture. To achieve a total volume of 20 µL, three µL of diethylpyrocarbonate (DEPC)-treated water was added. The amplification was carried out using a PCR thermocycler program (Prime, UK). The thermal profile for *sdfI* started with an initial denaturation at 95 °C for 10 minutes. There were 30 cycles of denaturation at 94 °C for 1 minute, annealing at 60 °C for 90 seconds, and extension at 72 °C for 90 seconds, followed by a final extension at 72 °C for 5 minutes. The PCR cycle for *hilA* began with denaturation at 94 °C for 5 minutes. This was followed by 30 cycles of denaturation at 94 °C for 5 seconds, annealing at 55 °C for 45 seconds, extension at 72 °C for 1 minute, and a final extension step at 72 °C for 10 minutes.

The PCR result was then examined after nine µL of PCR product was loaded onto a 1% agarose gel (TransGene, China) in 1x Tris/Borate/EDTA (TBE) buffer (Addbio Inc., Korea). The gel was stained with ten µL of safe dye (Gel Red) (Genesand Biotech, China); electrophoresis was run for 60 minutes at 120 volts.

Sequencing of Salmonella Enteritidis and phylogenetic tree examination

A phylogenetic tree was generated using partial sequences of the *sdfI* gene to examine the genetic diversity and phylogenetic relationships among strains of *Salmonella enterica* subsp. *enterica* serovar Enteritidis. This gene is recognized as a distinctive genetic marker for *S.* Enteritidis and is widely utilized in molecular typing techniques due to its conserved presence in this serovar and its absence in other serovars [16]. The phylogenetic tree was constructed using the Maximum Likelihood (ML) technique in MEGA11 software. The Tamura–Nei model was selected for its ability to account for transition/transversion rate bias and base composition variations in bacterial evolution. A bootstrap analysis was performed to verify the stability of the deduced tree structure, with all internal branches showing 100% bootstrap values, indicating high confidence in each phylogenetic grouping [17].

Results

Cultural Results

Out of 200 samples collected from various sources within the poultry processing environment, 100 samples (50%) were positive for *Salmonella* based on culture results. The highest prevalence of bacteria was detected in the cecum of the chickens (54.17%), followed by cages (53.3%), chopping boards (51.72%), and other surfaces, as shown in Table 2. The lowest positivity was recorded from workers' hands (40%). The findings on MacConkey agar showed that it produced colorless colonies, which were typical for *Salmonella*, as lactose was not fermented. When XLD media was examined, pink colonies with a black center were observed. In the triple sugar iron agar test, an alkaline (red) slant was paired with an acid (yellow) butt, along with some blackening at the bottom. Moreover, the urea agar test revealed that the bacteria were urease-negative, as no color change was observed in the media.

PCR Results

One hundred *Salmonella* isolates were recovered from six different poultry-related environmental and biological samples. The PCR results showed that only 2 of the *S.* Enteritidis isolates, representing a rate of 2% among total isolates, were found in the cecum. All other samples were negative for this serovar. Furthermore, the results indicated that both *S.* Enteritidis isolates carried the *hilA* gene (n = 2) (Table 2). The gel electrophoresis results revealed that *S.* Enteritidis was positive for the *sdfI* gene, which was 304 bp in length, and the *hilA* gene, which was 312 bp in length *(*Fig. 2).

Sequence analysis results

The phylogenetic analysis of the partial *sdfI* gene sequence demonstrated a significant degree of

conservation across all *S.* Enteritidis strains. The tree exhibited a monophyletic cluster, with all eleven isolates closely linked, indicating the genetic homogeneity commonly seen in this serovar .The Iraqi isolate (PV250091) established a unique subclade, signifying minor sequence differences that distinguished it from other global reference strains. Despite the relatively minor evolutionary distance, its distinctive position within the phylogenetic tree indicated the establishment of a local microvariant or sub-lineage.

The complete topology of the tree was robustly endorsed by bootstrap values of 100%, highlighting the dependability and precision of the deduced phylogenetic relationships. No recombinant signals or long-branch attractions were detected, indicating that the partial *sdfI* gene fragment serves as a reliable molecular identifier for differentiating *S.* Enteritidis strains at the level of individual strains .The tree structure indicated that multiple strains from various countries were closely clustered, suggesting recent common ancestry or global dissemination of clonal lineages of *S.* Enteritidis, a phenomenon aligned with its extensively documented pandemic spread via the worldwide food supply chain (Fig. 3).

Discussion

Poultry sales at Live Bird Markets (LBMs) have traditionally fallen outside the standardized industrial processing system, highlighting significant food safety concerns. Research from various countries has revealed high levels of *Salmonella* contamination in chicken meat found in retail markets [18]. A major contributor to this issue is cross-contamination that occurs during handling and storage between clean and contaminated food items. The presence of *Salmonella* is particularly alarming, as the bacteria can survive in various forms of meat, including whole birds and cuts, and under different storage conditions, such as fresh, chilled, or frozen. This enables them to be transmitted to humans through the food supply [19,20].

The study findings showed that 50% of all collected samples tested positive for the presence of *Salmonella* species, indicating a severe level of contamination within the local wet market setting. The highest rates were in the ceca (54.17%) this aligned with a previous study that *Salmonella* rate in non-vaccinated birds the prevalence was 64.2% vs. 38.3% in vaccinated [21], followed closely by cages (53.3%) and chopping boards (51.72%); this indicated that a combination of both biological and environmental factors can contribute to contamination. The findings aligned with previous reports from similar settings in Asia and the Middle East [1, 8], where inefficient hygienic measures, accompanied by the absence of protective measures and the risk of imminent cross-contamination, increase the chances of pathogen dispersal.

Using PCR to analyze these isolates, it was found that *S.* Enteritidis, identified by the *sdfI* gene, was present in only 2% of the isolates. A few cases of this serovar were observed in the local poultry supply, which may be attributed to geographical variation or recent developments in poultry vaccines against *S.* Enteritidis itself. These results aligned with a study conducted in Japan that analyzed 337 chilled chicken breast products. Researchers found an overall prevalence of 85.2% for *Salmonella*; however, a notable finding was the detection of only a single isolate of *S.* Enteritidis, which accounted for approximately 0.3% of the samples [22]. In contrast to meta-analyses conducted in China, Thailand, Cambodia, and other countries, which reported an overall *Salmonella* prevalence of 52.2%, *S.* Enteritidis was among the dominant serotypes. These disparities might be linked to differences in hygienic practices and the vaccination program [23].

In this study, both *S.* Enteritidis isolates detected in cecal samples harboured the *hilA* gene. The central virulence regulator associated with SPI-1. Having this gene means that these isolates could be invasive, posing a primary concern internationally, as *S.* Enteritidis is among the primary sources of non-typhoidal salmonellosis in humans. This finding was similar to another study in Brazil [24], where 100% of *S.* Enteritidis isolates from poultry tested were positive for *hilA*, further solidifying its position as a marker of invasiveness and systemic pathogenicity. The ubiquity of the *hilA* gene in that study demonstrated a tight correlation between its presence and the epithelial cell invasion capacity. However, a *hilA* transposon mutant of *S.* Enteritidis (strain SM6) was created and demonstrated impaired invasion of epithelial cells in vitro and altered colonization patterns in vivo [25]. Their findings thus implied that the expression of an active form of *hilA* was essential for full virulence and that disruption of *hilA* severely limited invasive capacity.

The phylogenetic analysis of the *sdfI* gene validated its utility as a serovar-specific and evolutionarily significant marker for *S.* Enteritidis. The tight clustering of isolates and consistently high bootstrap support highlighted the highly conserved characteristics of this gene throughout the serovar. The unique phylogenetic position of the Iraqi isolate (PV250091) indicated that even conserved loci, such as *sdfI*, could display sequence-level variations indicative of localized evolution. The unique

evolutionary position of the Iraqi strain suggested that it might have developed under divergent environmental or selective pressures. The preservation of the *sdfI* gene among all isolates substantiated its efficacy as a dependable molecular marker for identifying *S.* Enteritidis [16]. The genetic closeness, despite geographical differences, highlighted the worldwide dissemination and genetic stability of this serovar, a characteristic that enhanced its epidemiological success.

Conclusion

The study reveals key issues regarding the presence and molecular characterization of *Salmonella* in wet market poultry environments in Sulaymaniyah Province, Iraq, with a specific focus on *S.* Enteritidis and its virulence gene, *hilA*. Culture methods revealed *Salmonella* contamination rates at an overall value of 50% from various environmental and biological sources, with chicken ceca and poultry cages exhibiting the highest relative level of occurrence. In contrast, molecular examination through PCR with primers for the *sdfI* gene identified only 2% of the total *Salmonella* isolates as *S.* Enteritidis serovar; remarkably, the two isolates were both recovered from cecal samples .The *hilA* gene was detected in both *S.* Enteritidis isolates, although it was rare; this gene confers potential virulence through the invasion of epithelial cells. When studying the *sdfI* gene phylogenetically, the local isolate from Iraq formed an independent subclade, thus indicating it may have evolved locally as a microvariant.

Based on the findings, priority actions include worker hygiene training, strict cleaning and disinfection protocols, infrastructure upgrades, separation of processing zones, and regular microbiological surveillance. If these measures are implemented, they will significantly reduce contamination risks and enhance consumer safety. Future research should investigate the effectiveness of these interventions in reducing *Salmonella* prevalence and the antimicrobial resistance profiles of local isolates, as well as the involvement of other virulence genes in the pathogenicity of *S.* Enteritidis strains circulating in Iraq.

Acknowledgements

For assistance with this study, we would like to thank the College of Veterinary Medicine at Sulaimani University in Sulaymaniyah Province, Iraq. We are also grateful to the wet markets in Sulaymaniyah City for allowing us to collect samples.

Funding statement

This study didn't receive any funding support.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This research project has been reviewed and approved by the Ethics and Research Registration Committee at the College of Veterinary Medicine, University of Sulaimani, under the approval number VMUS.EC.Doc15-2025.

TABLE 1. Primers used for PCR-based molecular identification

Gene	Primer sequence (5′-3′)	Annealing Temp.	Length (bp)	Reference
sdfI	F: TGT GTT TTA TCT GAT GCA AGAGG R: TGA ACT ACG TTC GTT CTT CTGG	60 °C	304	(11)
hilA	hilA F: GACAGAGCTGGACCACAATAAGACA hilA-R: GAGCGTAATTCATCGCCTAAAC	55 °C	312	(15)

TABLE 2. Prevalence of *S*. Enteritidis and the *hilA* virulence gene in poultry processing environment. Using cultural and conventional PCR

Samples	Samples tested No.	Samples tested positive by culture No. (%)	*S*. Enteritidis No. (%)	*hilA* gene No. (%)
Cecum	72	39 (54.17)	2(5.13)	2(100)
Cages	30	16 (53.3)	0	0
Body swabs	27	12(44.4)	0	0
Knives	32	14(43.75)	0	0
Chopping Boards	29	15(51.72)	0	0
Workers'hands	10	4(40)	0	0
Total	200	100(50)	2(2)	2(100)

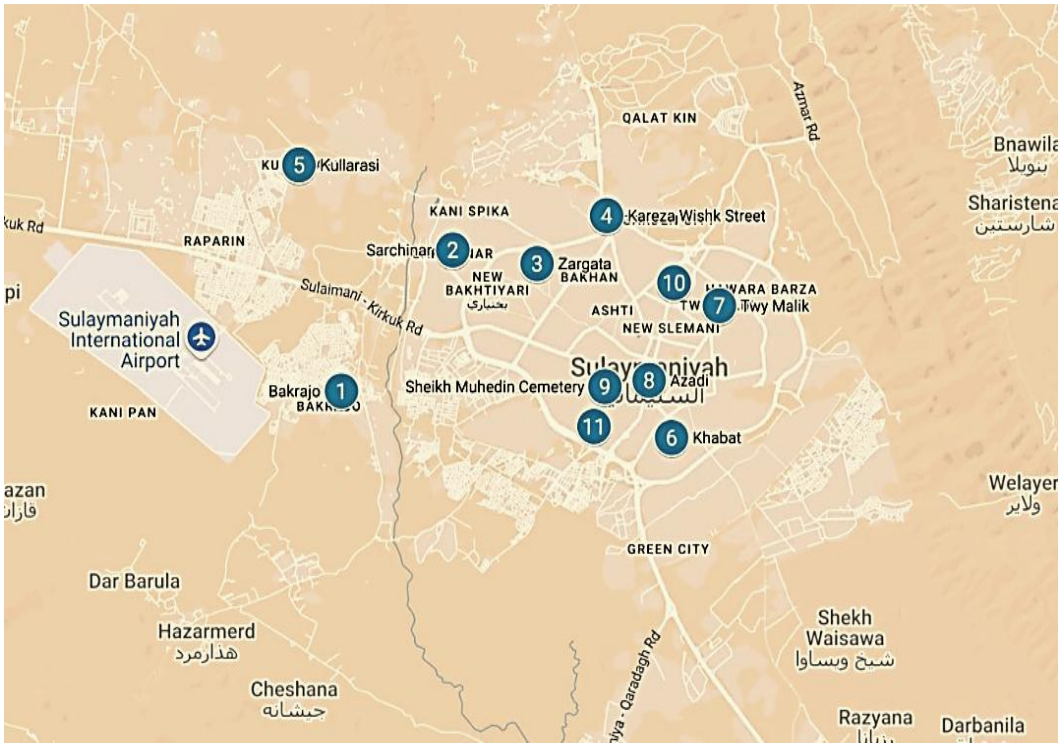

Fig. 1. illustrates various regions of wet market samples collected in Sulaymaniyah province.

Fig. 2. Agarose gel electrophoresis showing PCR amplification of *S.* Enteritidis-specific and virulence gene.
Lane M: DNA ladder (molecular weight marker, 500 bp indicated); Lane 1: Negative control (PCR mix without DNA); Lane 2: Amplified product of the *sdfI* gene specific for *S.* Enteritidis (304 bp); Lane 3: Amplified product of the *hilA* virulence gene (312 bp).

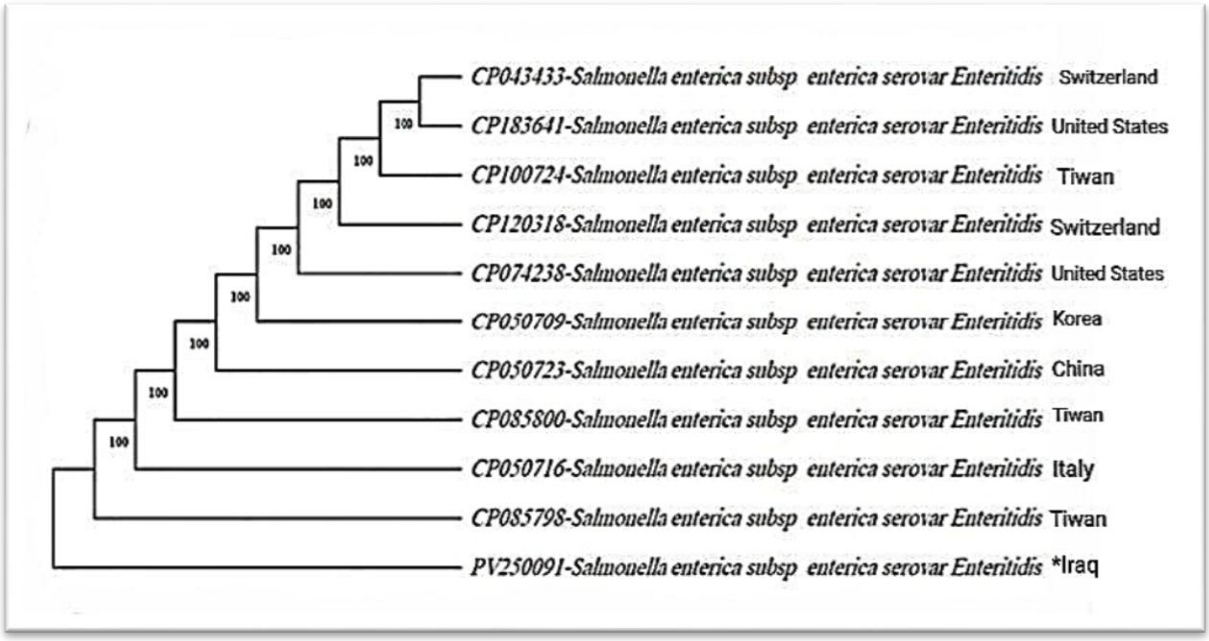

Fig. 3. The phylogenetic tree illustrates the relationships of *Salmonella enterica* serovar Enteritidis from Iraq (*) with strains from different geographical origins.
The tree was created employing the Maximum Likelihood approach based on the Tamura-Nei model using MEGA11 software, and a bootstrap analysis was conducted with 1000 resampling iterations. Partial DNA sequences from the concatenated partial *sdfI* gene served as the input data.

References

1. Dar, M.A., Ahmad, S.M., Bhat, S.A., Ahmed, R., Urwat U., Mumtaz, P.T., Bhat, S.A., Dar, T.A. and Shah, R.A., Ganai, N.A. *Salmonella* typhimurium in poultry: a review. *World's Poult. Sci. J.,* **73**, 345–354 (2017).

2. Quan, G., Xia P., Zhao, J., Zhu, C., Meng, X., Yang, Y., Wang, Y., Tian, Y., Ding, X. and Zhu, G. Fimbriae and related receptors for *Salmonella* Enteritidis. *Microb. Pathog*., **126**, 357–362 (2019).

3. Saravanan, S., Purushothaman, V., Murthy, T.R., Sukumar, K., Srinivasan, P., Gowthaman, V., Balusamy, M., Atterbury, R. Kuchipudi, S.V. Molecular epidemiology of Nontyphoidal *Salmonella* in poultry and poultry products in India: implications for human health. *Indian J. Microbiol*., **55**, 319–326 (2015).

4. Bahramianfard, H., Derakhshandeh, A., Naziri, Z. and Khaltabadi Farahani, R. Prevalence, virulence factor and antimicrobial resistance analysis of *Salmonella* Enteritidis from poultry and egg samples in Iran. *BMC Vet. Res.*, **17**, 196 (2021).

5. Nazari Moghadam, M., Rahimi, E., Shakerian A. and Momtaz, H. Prevalence of *Salmonella* Typhimurium and *Salmonella* Enteritidis isolated from poultry meat: virulence and antimicrobial-resistant genes. *BMC Microbiol*., **23**, 168 (2023).

6. Boddicker, J.D., Knosp, B.M. and Jones, B.D. Transcription of the *Salmonella* invasion gene activator, *hilA*, requires HilD activation in the absence of negative regulators. *J. Bacteriol.,* **185**, 525–533 (2003).

7. Hamed, S., Shawky, R.M., Emara, M., Slauch, J.M. and Rao, C.V. HilE is required for synergistic activation of SPI-1 gene expression in *Salmonella enterica* serovar Typhimurium. *BMC Microbiol*., **21**,1–9 (2021).

8. Nidaullah, H., Abirami, N., Shamila-Syuhada, A.K., Chuah, L.O., Nurul, H. and Tan, T.P. Prevalence of *Salmonella* in poultry processing environments in wet markets in Penang and Perlis, Malaysia. *Vet. World*, **10**, 286–292 (2017).

9. Mawlood, M.H. and Khidhir, Z.K. Microbiological assessment of chicken breast meat from unlicensed and licensed slaughterhouses during refrigeration and freezing storage. *Basrah J. Vet. Res*., **17**,1-15 (2018).

10. Harb, A., Habib, I., Mezal, E. H., Kareem, H. S., Laird, T., O'Dea, M. and Abraham, S. Occurrence, antimicrobial resistance and whole-genome sequencing analysis of *Salmonella* isolates from chicken carcasses imported into Iraq from four different countries. *Int. J. Food Microbiol.*, **284**, 84–90 (2018).

11. Kanaan, M.H., Khalil, Z.K., Khashan, H.T. and Ghasemian, A. Occurrence of virulence factors and carbapenemase genes in *Salmonella enterica* serovar Enteritidis isolated from chicken meat and egg samples in Iraq. *BMC Microbiol*., **22**, 279 (2022).

12. Fujihara, M., Hayashi, M., Hara, K., Sakazume, N., Tsukuda, T. and Tagaino, Y. Verification of different methods used for isolating *Salmonella enterica* serovar Dublin from cattle feces. *J. Vet. Med. Sci*., **85**, 1077–1082 (2023).

13. Dashti, A.A., Jadaon, M.M., Abdulsamad, A.M. and Dashti, H.M. Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques. *Kuwait Med. J*., **41**, 117–122 (2009).

14. Dimitrakopoulou, M.E., Stavrou, V., Kotsalou, C. and Vantarakis, A., Boiling extraction method vs commercial kits for bacterial DNA isolation from food samples. *J. Food Sci. Nutr. Res*., **3**, 311–319 (2020).

15. Ramatla, T., Khasapane, N.G., Mlangeni, L.N., Mokgokong, P., Ramaili, T., Ndou, R., Nkhebenyane, J.S., Lekota, K. and Thekisoe, O. Detection of *Salmonella* Pathogenicity Islands and Antimicrobial-Resistant Genes in *Salmonella enterica* Serovars Enteritidis and Typhimurium Isolated from Broiler Chickens. *Antibiotics*, **13**, 458 (2024).

16. Andesfha, E., Indrawati, A., Mayasari, N., Rahayuningtyas, I. and Jusa, I. Detection of *Salmonella* pathogenicity island and *Salmonella* plasmid virulence genes in *Salmonella* Enteritidis originated from layer and broiler farms in Java Island. *J. Adv. Vet. Anim. Res*., **6**, 384–393 (2019).

17. Tamura, K., Stecher, G. and Kumar, S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol*., **38**, 3022–3027 (2021).

18. Ubeyratne, J.K., Jayaweera, M.D., Lokugalappatti, L.G., Wickramasinghe, S. and Tun. H.M. Market characteristics, *Salmonella* prevalence and associated risk practices in poultry processing environments in wet markets in Sri Lanka. *Sri Lanka Vet. J.*, **69**, 9-21 (2022).

19. Yang, B., Xi M., Wang, X., Cui S., Yue, T., Hao, H., Wang, Y., Cui, Y., Alali, W.Q., Meng, J. and Walls, I. Prevalence of *Salmonella* on raw poultry at retail markets in China. *J. Food Prot*., **74**,1724–1728 (2011).

20. NidaUllah, H., Omar, A.M., Rosma A., Huda, N. and Sohni, S. Analysis of *Salmonella* contamination in poultry meat at various retailing, different storage temperatures and carcass cuts—A literature survey. *Int. J. Poult. Sci*., **15**,111 (2016).

21. Dórea, F.C., Cole, D.J., Hofacre, C., Zamperini, K., Mathis, D., Doyle, M.P., Lee, M.D. and Maurer, J.J. Effect of *Salmonella* vaccination of breeder chickens on contamination of broiler chicken carcasses in integrated poultry operations. *Appl. Environ. Microbiol*., **76**, 7820–7825 (2010).

22. Sasaki, Y., Ikeda, T., Momose, Y., Yonemitsu, K., Uema, M. and Asai, T. Geographical variation of antimicrobial resistance of *Salmonella* in Japanese chicken. *Food Saf*., **12**, 59–66 (2024).

23. Tan, S.J., Nordin, S., Esah, E.M. and Mahror, N. *Salmonella* spp. in chicken: prevalence, antimicrobial resistance, and detection methods. *Microbiol. Res*., **13**, 691–705 (2022).

24. Borges, K.A., Furian, T.Q., Borsoi, A., Moraes, H.L. and Salle, C.T. Nascimento V.P., Detection of virulence-associated genes in *Salmonella* Enteritidis isolates from chicken in South of Brazil. *Pesqui. Vet. Bras.*, **33**, 1416–1422 (2013).

25. Lv, S., Si W., Yu, S., Li Z., Wang, X. and Chen, L. Characteristics of invasion-reduced *hilA* gene mutant of *Salmonella* Enteritidis in vitro and in vivo. *Res. Vet. Sci*., **101**, 63–68 (2015).

الكشف الجزيئي عن السالمونيلا المعوية المصلية Enteritidis من بيئات تجهيز الدواجن في الأسواق الرطبة في محافظة السليمانية ، العراق

خوشي ئارى محمد و ايمان ظاهر عارف

قسم الأحياء المجهرية، كلية الطب البيطري، جامعة السليمانية، السليمانية، العراق.

الملخص

تصيب السالمونيلا المعوية (*Salmonella enterica*) كلاً من البشر والحيوانات، وتُصنف كممرض معوي غازي. تصيب السالمونيلا المعوية المصلية (*S.* Enteritidis) بشكل أساسي الأفراد الذين يستهلكون منتجات الدواجن. في هذه الدراسة، هدفنا إلى عزل وكشف وجود بكتيريا *S.* Enteritidis في بيئة معالجة الدواجن في السوق الرطب بمحافظة السليمانية باستخدام تقنية تفاعل البوليميراز المتسلسل (PCR)، بالإضافة إلى تحديد جين الضراوة الخاص، وهو (*hilA*) Hyperinvasive locus A، في هذا النمط المصلي. تم الحصول على 200 عينة من الأعور، ومسحات من أجسام الدواجن، والسكاكين، وألواح التقطيع، والأقفاص، بالإضافة إلى أيدي العمال. استُخدمت تقنيات قائمة على الثقافة وتفاعل البوليميراز المتسلسل (PCR) للاستخلاص والتعريف. تم إجراء التحليل الوراثي (Phylogenetic analysis) عن طريق تسلسل جين قطعة السالمونيلا المختلفة (*sdfI*) *Salmonella* difference fragment I. حددت طرق زراعة السالمونيلا في 50٪ من العينات، مع أعلى معدل موجود في عينات الأعور (ن = 39، 54.17٪). أكدت نتائج تفاعل البوليميراز المتسلسل (PCR) أن2% فقط (ن = 2) من العزلات تم تعريفها على أنها *S.* Enteritidis باستخدام جين *sdfI*، وكلاهما من أعور الدجاج. كانت جميع العينات المتبقية سلبية لهذا النمط المصلي، ولكن تم الكشف عن جين *hilA* في كلتا سلالتي *S.* Enteritidis. كشف التحليل الوراثي أن العزلة العراقية برقم الوصول PV250091 تنتمي إلى سلالة فرعية مميزة، مما يشير إلى وجود تنوع وراثي محلي. كشفت نتائج هذه الدراسة أن بيئات الدواجن ملوثة بشدة بالسالمونيلا. ومع ذلك، فإن انخفاض انتشار *S.* Enteritidis وإمكانية ضراوتها يؤكد على الحاجة إلى تحسين النظافة والمراقبة في الأسواق الرطبة.

الكلمات الدالة : جين الـ*hilA* ، دواجن ،الأسواق الرطبة، السالمونيلا إنترتيديس، جين الـ *sdfI*