



Molecular detection of some virulence factors genes of salmonella spp. isolated from chicken's products and human in Wasit province, Iraq

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Abstract

A total of 300 samples (150 chicken products samples (eggs, livers and minced meat) from different local supermarkets and 150 human fecal samples from clinical cases) were collected for isolation and identification of *Salmonella enterica* from Wasit Province of Iraq from January to December 2022 by using PCR assay and sequencing for three virulence factors genes (*stn*, *avr A*, and *sop B*). The results of isolation for *S. enterica* showed 4.6% (7/150) in humans, and 8.66% (13/150) in the chicken products (6/50 eggs, 3/50 livers, and 4/50 minced meat); Also showed 7/7 of *S. typhi* in human, while in the eggs showed 3/6 of *S. typhi*, 2/6 of *S. typhimurium* and 1/6 of *S. enteritidis*. Also, the results showed 3/3 and 4/4 of *S. typhimurium* in both livers and minced meat, respectively. The results of virulence factors recorded that the *stn* gene was absent in human isolates, while 10/13 in chicken products isolates, also showed in human and chicken products isolates 7/7 and 13/13, respectively, for both *avr A* and *sop B* genes. The serovare *S. typhi* were 10/10 carried both *avr A* and *sop B* genes, but lacked the *stn* gene. While *S. typhimurium* and *S. enteritidis* were 9/9 and 1/1 respectively carried *stn*, *avr A* and *sop B* genes. Sequencing was done for some PCR products for three virulence factors that were registered in NCBI. The nucleotide sequencing showed many nucleotide substitutions (mutations) in the *sop B* and *avr A* genes while no substitution in the *stn* gene.

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Introduction

Salmonella enterica is the most common foodborne pathogen isolated from food-producing animals, responsible for zoonotic infections and food poisoning in humans and animal species such as birds (1). This bacterium can be transmitted to humans through food contamination, especially from animal origin, chicken products (eggs, livers, and minced meat), and other kinds of meat (2). There are more than 2600 serovars of *Salmonella* spp. Gram-negative, motile, rod-shaped bacteria belong to the *Enterobacteriaceae* family and are facultative anaerobes (3,4). Different serovars of *S. enterica* cause many diseases ranging from gastroenteritis (intestinal or diarrheal), which is caused by nontyphoidal *Salmonella* (NTS) (*S.*

typhimurium and *S. enteritidis*), to typhoid fever (enteric or systemic), that caused by *S. typhi* and *S. Paratyphi A*, and the effect of infection were vary depending on the type of the host (5,6). Human infections with *Salmonella* spp. are most commonly brought on by eating undercooked or semi-cooked poultry products such as meat and eggs. Moreover, food contamination could occur in various stages of the food chain such as production, distribution, and sale (7). The NTS causes high mortality in self-limiting diarrheal disease in immunocompromised adults especially in advanced AIDS infection which is most clearly connected to recurrent bacteremia (8). In poultry, there are many diseases: Pullorum disease caused by *S. enterica* serovar Pullorum; Fowl typhoid caused by *S. enterica* serovar Gallinarum; Paratyphoid disease caused by *S. typhimurium*, *S. enteritidis* and *S.*

enterica subsp. *Arizonae* (9,10). Bacterial contamination of poultry carcasses and cuts results from improper hygienic measures and improper cooking. The dissemination of infection throughout plants during processing occurs in the evisceration, cooling, packaging, and transport stages (11,12). Chicken meat is very essential due to its high-quality protein, low-fat content, and lower cholesterol and saturated fats than meat from other animals; therefore, it is usually considered healthier than red meat (13). Numerous virulence factors and determinants have been shown to play essential roles in the pathogenesis of *Salmonella* spp., which has been shown to colonize its hosts by invading, attaching, and bypassing the host's intestinal defense mechanisms, such as gastric acid. These factors included capsule, flagella, biofilm, adhesion systems, plasmids, and type III secretion systems (TTSS) encoded on the *Salmonella* pathogenicity island (SPI), types 1 and 2, and other SPIs (14). The virulence factors of bacteria are carried on the chromosome and others are carried on plasmids. Many *S. enterica* serovars have plasmids that carry genes for virulence, resistance to antibiotics, or transfer that enable them to adapt to different habitats (15). This bacterium has two TTSS encoded in SPI-1 and SPI-2 that transport virulence factors (effector proteins) to the host cell's cytoplasm to establish a replication place and suppress the immune system. Enterotoxin (*stx*) of *S. enterica* is a protein toxin that produces and targets the intestine, which is responsible for enzymatic activity and binding to the intestinal cells, and the enterotoxicity of *Salmonella* spp. (16). The virulence factor *avr A* is responsible for cell cycle progression, signal transmission, transcriptional regulation, receptor down-regulation, and endocytosis, which has a role in immunological response, malignant transformation, development, and programmed cell death (17). The virulence factor *sop B* is essential in invasion, Akt activation (Protein Kinase), formation of *Salmonella*-containing vacuoles (SCV), biogenesis, positioning, and fluid secretion; it causes acute inflammatory cell influx, intestinal fluid secretion, and enteritis that are associated with clinical diarrhea, which is also involved in epithelial cell adhesion, cytoskeletal rearrangements, and phagocytic and non-phagocytic cell invasion (18). In molecular biology, several infections have been identified and diagnosed using conventional PCR to detect a particular special identity *rRNA* gene for *S. enterica* and three genes coding for the virulence factors *stx*, *avr A*, and *sop B*. Also, studying sequencing for confirmation of the diagnosis of the bacteria and its virulence factors using species-specific oligonucleotide primers is considered an essential method for studying the phylogeny and taxonomy of the microorganisms (19).

Therefore, this study aimed to isolate and identify *S. enterica* from clinical samples of human and chicken products (eggs, livers, and minced meat) in the Wasit Province of Iraq. Also, PCR and sequences characterize *S. enterica* and detects its virulence factors molecularly.

Materials and methods

Ethical approve

The study was approved and carried out at the seventh session in the Council of the College of Veterinary Medicine, University of Baghdad, which held on the date 11/1/2022, with approval No. 297 in the date 2/2/2022.

Collection of samples

A total of 300 samples (150 chicken products samples (50 eggs, 50 livers, and 50 minced meats from different local supermarkets) and 150 human fecal samples from different clinical cases of different Hospitals) were collected from Wasit Province of Iraq from January to December 2022, for isolation and identification of *Salmonella enterica*. The samples were collected in a sterile condition, then labeled with the information of the cases, and transported by transport media in more excellent boxes to the microbiology laboratory of Al-Suwaira General Hospital for bacteria culture. According to the recommendation of the Global Foodborne Infections Network laboratory protocol (20), the isolation and identification of *Salmonella* spp. from stool specimens was performed as the following Suspended one g of feces in nine ml of buffered peptone water and incubated for 18-24 h. at 37 °C, then, one ml of the suspension was added to nine ml of Tetrathionate broth and incubated for 18-24 h. at 42 °C, after that, cultured by streaking on conventional media (MacConkey and blood agars), then streaked on selective media (Xylose Lysine Deoxycholate agar (XLD), *Salmonella-Shigella* Agar (S.S. agar), Hektoen Enteric Agar (HEA) and Chromogenic medium) (21). The plates were incubated at 37 °C for 18 - 24 h. Then, blood samples were taken from the same patients with positive results to confirm the diagnosis. Biochemical tests were done by using the Analytical Profile Index 20E (API 20E) and the Vitik 2- test. Serotyping test and PCR for *Salmonella* spp. isolates were performed in the Central Laboratory of the Iraqi Ministry of Health (22).

According to recommendations by the ISO 6579 standard (23), the isolation and identification of *Salmonella* spp. were performed in the chicken product samples. Each sample was weighed 25 g and mixed in a sterile flask with 225 ml of buffered peptone water, then incubated overnight at 37 °C for 18–24 h. After that, one ml of the suspension was added to nine ml of Tetrathionate broth, followed by incubation for 18–24 h at 42 °C. The culturing, diagnosis of the samples, serotyping, molecular identification, and sequencing of the isolates in human samples were done using the same steps mentioned above.

Molecular detection

The genomic DNA was successfully extracted from all isolates of *S. enterica*, and the bands of DNA were detected on Agarose gel and visualized under a U.V. transilluminator, showing 100% extracted genomic DNA. The purity and

concentration of extracted DNA were directly determined by the Nanodrop device. The extracted DNA purity ranged between 1.8 - 2 (24). PCR was used to detect *S. enterica* from clinical samples. Detection of three genes for the virulence factors of *Salmonella* spp., which were: *stn* gene 762 bp (25), *avr A* gene 422 bp (26), and *sop B* gene 1170 bp (27). All PCR assays were accomplished in the Central Laboratory of the Iraqi Ministry of Health. Forward and reverse primers provided by (Alpha DNA company, Canada) were used to amplify specific DNA fragments of *stn*, *avr A*, and *sop B* genes. Table 1 shows that the primers were tested in the NCBI Genbank database and used in the current study. The PCR products were separated according to the recommendation of (Genomic DNA Mini Kit, Geneaid, Thailand) using 1.5% agarose gel electrophoresis and visualized by ultraviolet light.

Sequencing and phylogeny analysis

A total of 10 PCR products of *S. enterica* (4 for a particular identity *rRNA* gene and 6 for the virulence factors genes from clinical human and chicken products samples) were sent to the Macrogen Company - Korea to perform sequencing. The sequence results were analyzed, and the similarity was achieved using the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI). The evolutionary history was inferred using the MEGA 11 method, and evolutionary analyses were conducted. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. By using the Maximum Composite Likelihood method, the evolutionary distances were detected in the units of the number of base substitutions per site.

Table 1: Primers used in the PCR assay in this study

Genes	Primer	Sequences	Expected size (bp)	References
<i>rRNA</i>	F	5'- CGATGCGTTGAGCTAACCGG -3'	865	(22)
	R	5'- CAGAAGCGATAACCACGTCGTC -3'		
<i>Stn</i>	F	5'- GGATCCTTGTTAATCCTGTTGTCTCG -3'	762	(25)
	R	5'- GTCGACTTACTGGCGTTTTTTTTGGCA -3'		
<i>avr A</i>	F	5'- CCTGTATTGTTGAGCGTCTGG -3'	422	(26)
	R	5'- AGAAGAGCTTCGTTGAATGTCC -3'		
<i>sop B</i>	F	5'- GATGTGATTAATGAAGAAATGCC -3'	1170	(27)
	R	5'- GCAAACCATAAAAACACTACTCA -3'		

Results

The results of a total of 300 samples obtained from the culture method and confirmed by PCR showed that the total percentage of isolation for *S. enterica* was 4.6% (7/150) in humans, and 8.66% (13/150) in chicken products (Table 2): (6/50 eggs, 3/50 livers, and 4/50 minced meat) (Table 3).

The results of identification and isolation by cultural media method of *S. enterica* isolates, microscopic examination, and conventional biochemical reactions done by API 20E and VITEK® 2 Test were similar to the

phenotypic characteristics of this bacterium (Figures 1 and 2).

The results of the serotyping test for all isolates of this bacterium isolated from human and chicken products showed that total *S. typhi* was 10/20, *S. typhimurium* was 9/20, and *S. enteritidis* was 1/20. Also, it showed 7/7 of *S. typhi* in humans, while in the chicken eggs, it was 3/6 of *S. typhi*, 2/6 of *S. typhimurium*, and 1/6 of *S. enteritidis*. Also, the bacteria *S. typhimurium* was 3/3 in chicken livers and 4/4 in the chicken minced meats (Table 4 and Figure 3).

Table 2: Distribution of total *Salmonella enterica* isolates in human and chicken product samples

Source	No. of total samples	No. of total isolates	Percentage (%)	P-value
Human	150	7	4.66 %	0.16 NS
Chicken Products	150	13	8.66 %	

Table 3: Distribution of *Salmonella enterica* isolates in the chicken products samples

Source	No. of total samples	No. of total isolates	Percentage (%)	P-value
Eggs	50	6	12 %	0.55 NS
Livers	50	3	6 %	
Minced Meat	50	4	8%	

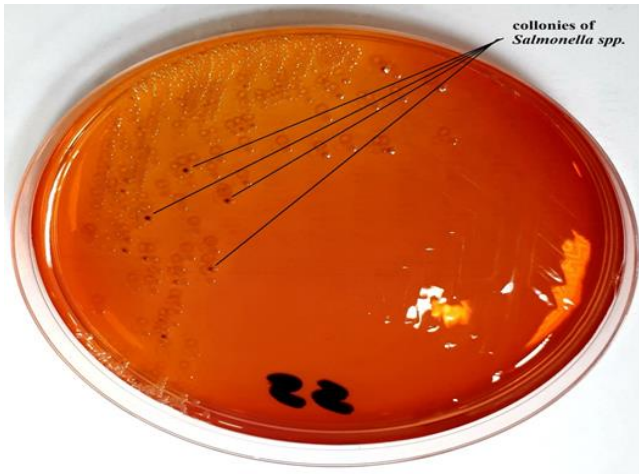


Figure 1: *Salmonella enterica* on SS agar.

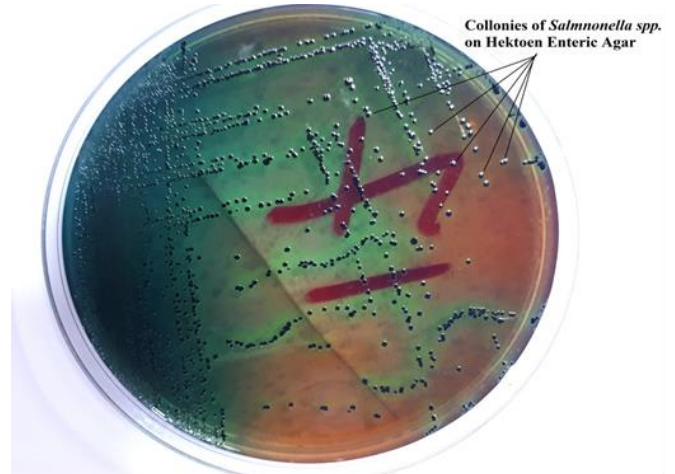


Figure 2: *Salmonella enterica* on Hektoen enteric agar.

Table 4: Total Percentages of *S. enterica* and their serotypes in Human and Chicken product isolates

Source	Samples	Isolates	<i>S. enterica</i>	<i>S. typhi</i>	<i>S. typhimurium</i>	<i>S. enteritidis</i>	χ^2	P value
Human	150	7	4.66%	100%	0%	0%		
Eggs	50	6	12%	50 %	33.33 %	16.66 %	17.37	<0.01
Livers	50	3	6%	0 %	100 %	0 %		
Meat	50	4	8%	0%	100 %	0 %		

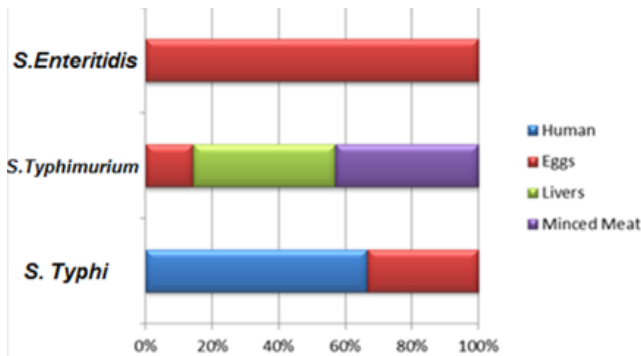


Figure 3: Percentages of *S. enterica* serotypes isolated from human and chicken products.

The results of molecular detection for virulence factors showed that the *stn* gene was absent in human isolates, while in the chicken products isolates was 10/13 (Table 5 and Figure 4). Also, the results showed 20/20 for each of *avr A* and *sop B* genes in both human and chicken products isolates (Tables 6 and Figure 5), (Table 7 and Figure 6). In all isolates of human and chicken products, the serovare *S. typhi* 10/10

carried each of *avr A* and *sop B* genes but lacked the *stn* gene, while both *S. typhimurium* and *S. enteritidis* 10/10 carried each of *stn*, *avr A* and *sop B* genes (Tables 8).

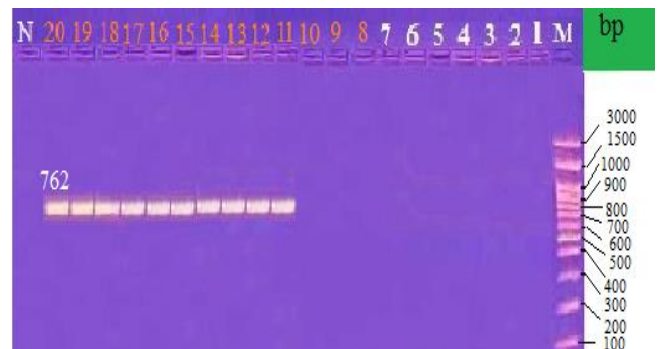


Figure 4: Gel electrophoresis (2% Agarose) of the *stn* gene (762 bp). Lane M: DNA ladder, Lane N: Negative control, Lanes 1-7: Human isolates (No DNA bands), Lanes 8-10: Chicken products (Eggs) isolates (No DNA bands), Lanes 11-20: DNA band of the *stn* gene of *S. enterica* isolated from chicken products.

Table 5: Percentage of the *stn* gene of *S. enterica* in human and chicken products isolates

Source	Samples	Isolates	No. of isolates carrying <i>stn</i> gene	Percentage	P-value
Human	150	7	0	0 %	
Chicken product	150	13	10	77 %	<0.01

Table 6: Percentage of the *avr A* gene of *S. enterica* in human and chicken products isolates

Source	Samples	Isolates	No. of isolates carrying <i>avr A</i> gene	Percentage	P-value
Human	150	7	7	100 %	<0.01
Chicken product	150	13	13	100 %	

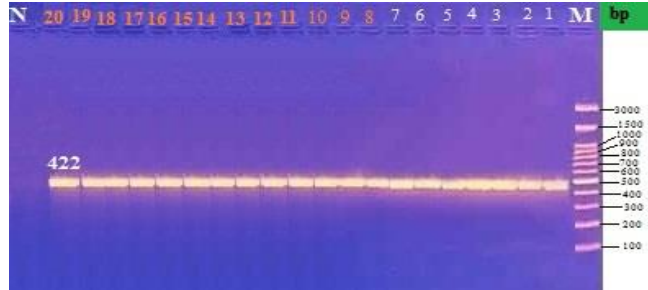


Figure 5: Gel Electrophoresis (2% Agarose) of *avr A* gene (422 bp). Lane M: DNA ladder, lane N: Negative control, Lanes 1-7: DNA bands of the *avr A* gene of *S. enterica* isolated from humans, Lanes 8-20: DNA bands of the *avr A* gene of *S. enterica* isolated from chicken products.

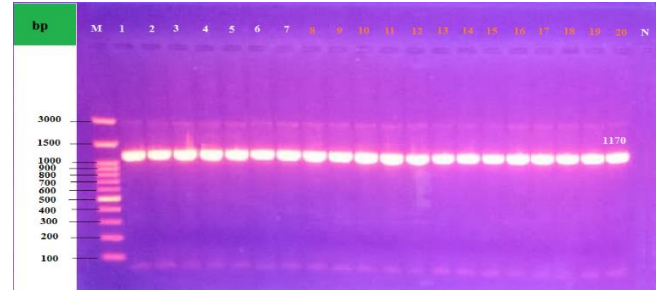


Figure 6: Gel Electrophoresis (2% Agarose) of *sop B* gene (1170 bp). Lane M: DNA ladder, lane N: Negative control, Lanes 1-7: DNA bands of the *sop B* gene of *S. enterica* isolated from humans, Lanes 8-20: DNA bands of the *sop B* gene of *S. enterica* isolated from chicken products.

Table 7: Percentage of the *sop B* gene of *S. enterica* in human and chicken product isolates

Source	Samples	Isolates	No. of isolates carrying <i>sop B</i> gene	Percentage	P-value
Human	150	7	7	100 %	<0.01
Chicken product	150	13	13	100 %	

Table 8: Presence of virulence factors genes in all isolates of *S. enterica* according to source and *Salmonella spp*

Source	Salmonella spp.	Total No.	Virulence Factors of Salmonella spp.		
			Stn	avr A	sop B
Human	<i>S. typhi</i>	7	-	+	+
	<i>S. typhimurium</i>	0	-	-	-
	<i>S. enteritidis</i>	0	-	-	-
Chicken eggs	<i>S. typhi</i>	3	-	+	+
	<i>S. typhimurium</i>	2	+	+	+
	<i>S. enteritidis</i>	1	+	+	+
Chicken livers	<i>S. typhi</i>	0	-	-	-
	<i>S. typhimurium</i>	3	+	+	+
	<i>S. enteritidis</i>	0	-	-	-
Chicken minced meat	<i>S. typhi</i>	0	-	-	-
	<i>S. typhimurium</i>	4	+	+	+
	<i>S. enteritidis</i>	0	-	-	-

Nucleotide Sequencing for some isolates of *Salmonella enterica*

To confirm the diagnosis of *S. enterica* and study the genetic characteristic features, sequencing was done for some PCR products of three virulence factors genes (*stn*, *avr A*, and *sop B*). All the PCR products that were sent for sequencing were registered in the NCBI for the first time under the following accession numbers: OQ131104,

OQ131105, OQ131106, OQ131107, OQ131108, and OQ131109 (Table 9).

The nucleotide sequencing for virulence factors showed three nucleotide substitutions in the *sop B* virulence factor gene, which were Guanine G>A to Adenine, Cytosine C>A to Adenine, and Cytosine C>T to Thymine (Figure 7), also one nucleotide substitution in the *avr A* virulence factor gene was Guanine G>T to Thymine (Figure 8); while no substitution in the *stn* virulence factor (Figure 9).

Table 9: Some PCR products of *S. enterica* serotypes that sent for sequences with their accession numbers and sources

No.	Accession No.	Source	Salmonella spp.
1	OQ131104	Human	<i>S. typhi</i>
2	OQ131105	Chicken eggs	<i>S. typhimurium</i>
3	OQ131106	Chicken eggs	<i>S. enteritidis</i>
4	OQ131107	Chicken livers	<i>S. typhimurium</i>
5	OQ131108	Chicken minced meat	<i>S. typhimurium</i>
6	OQ131109	chicken eggs	<i>S. enteritidis</i>

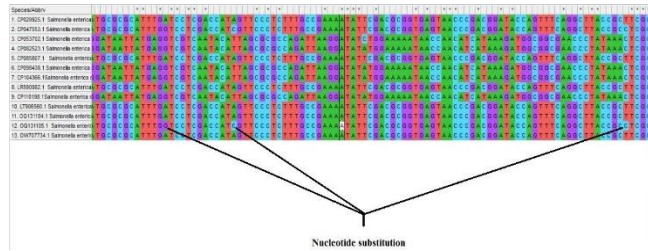


Figure 7: Nucleotide sequence of sense flanking the *sop B* virulence factor gene of *S. enterica* isolate using MEGA 11 program showed three nucleotide substitutions were Guanine G>A to Adenine, Cytosine C>A to Adenine, and Cytosine C>T to Thymine.

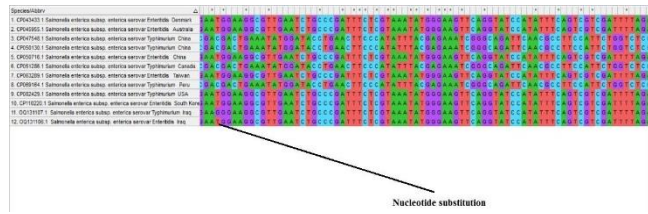


Figure 8: Nucleotide sequence of sense flanking the *avr A* virulence factor gene of *S. enterica* isolates using MEGA 11 program showed one nucleotide substitution (Guanine G>T Thymine).

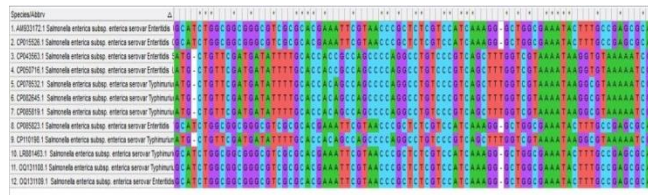


Figure 9: Nucleotide sequence of sense flanking the *stn* virulence factor gene of *S. enterica* isolate using the MEGA 11 program showed no nucleotide substitution.

The Phylogenetic tree of sense flanking for the virulence factors of *S. enterica* was drawn and compared with the related virulence factors documented in the Gene bank. Phylogenetic analysis was conducted using MEGA 11 (Figures 10-12).

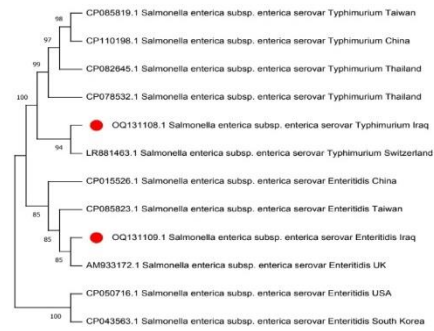


Figure 10: Phylogenetic tree of sense flanking the *stn* gene of *S. enterica* isolated from Iraq during 2022. Phylogenetic analysis was conducted using the MEGA 11 program.

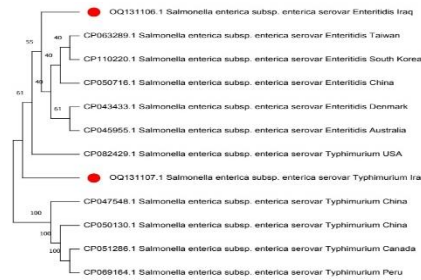


Figure 11: Phylogenetic tree of sense flanking the *avr A* gene of *S. enterica* isolated from Iraq during 2022. Phylogenetic analysis was conducted using MEGA 11 program.

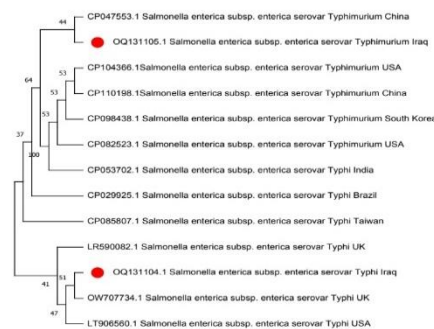


Figure 12: Phylogenetic tree of sense flanking the *sop B* gene of *S. enterica* isolated from Iraq during 2022. Phylogenetic analysis was conducted using the MEGA 11 program.

Discussion

The results of the isolation of *S. enterica* from humans and chicken products showed variation in the serotypes of *S. enterica*, and there are significant differences between these serotypes at p -value < 0.05 . The results of this study were agreed with Nader *et al.* (28), who isolated *S. enterica* from raw chicken meat and diarrheic patients and recorded 4% and 10%, respectively, from markets and Al-Yarmmok Hospital in Baghdad City of Iraq. Also, I agree with Kanaan *et al.* (27), who recorded 5% by isolating this bacterium from chicken meat and egg samples in Iraq. Additionally, this study agreed with Harb *et al.* (29), who isolated *S. enterica* in Iraq and reported 26% from fresh chicken meat samples.

The results are also consistent with that of Hasan *et al.* (13), who isolated 21.1% of this bacterium from chicken and their feed and drinking water in Iraq. This study's results were lower than those of Jaffer (30) who isolated *S. enterica* in Iraq from chicken eggs and recorded 30%. Also, they were lower than the results of Siddique *et al.* (31) who isolated *S. enterica* from poultry and its associated food products in Pakistan, which recorded 25.67%. The results of this study were considered higher than those Rahmani *et al.* (32) who isolated *Salmonella* spp. in Iran from birds and recorded 2.8%.

The variance in the percentages of the high prevalence of *S. enterica* in chicken products compared with that in humans may be due to the location of sampling, the timing of sample collection, geographic climate, age, immunity of humans or chickens, consumption of drugs, and hygienic restrictions, that may be linked to the relative differences in results between different places (33). The handling of raw poultry carcasses and ready-to-eat products afterward, cross-contamination from workers' hands, tools, and utensils, and consumption of improperly cooked poultry meat, as handling and contamination with the feces of chickens may be the most frequent sources of *Salmonella* infection in humans (3). The high prevalence of *Salmonella* spp. in the previous studies comparable to this study could be due to the low hygienic standards during the handling of slaughtering, scalding, de-feathering, evisceration and carcass cutting. These processes allow for the cross-contamination of healthy and clean birds with diseased ones or contaminated carcasses, and then with human beings. The lack of veterinary oversight could also slaughter sick chickens, and spread infections (34). Associations between the detection of *S. enterica* and different sample sources with additional factors were examined using an exact test, and a p -value < 0.05 was considered significant.

The results of serotyping in this study were agreed with Zubair *et al.* (35). Who recorded 4.85% of *Salmonella* spp. isolated from poultry eggs in Duhok/ Iraq was also recorded at 11.75%, 29.4%, and 58.8% for *S. typhi*, *S. typhimurium* and *S. enteritidis* respectively. Also, with the agreement of Kanaan *et al.* (27) who isolated *S. enteritidis* from chicken

meat and egg samples in Iraq and recorded 5.1% and 4.9% from eggs and chicken meats, respectively. Also, I agree with Yousif and Harab (36), who isolated *Salmonella* spp. from children in the Thi-Qar governorate of Iraq and recorded 11.17% but disagreed by recording 42.1% for *S. typhimurium* and 5.27% for *S. enteritidis*. *Salmonella* infection is transmitted by contamination between chickens, eggs, and humans. Fecal contamination is the primary source of transmission for this bacterium. The transmission of *Salmonella* infection may occur horizontally and vertically. The horizontal transmission occurs due to contamination during the handling of slaughtering, scalding, de-feathering, evisceration, and carcass cutting. These processes allow for the cross-contamination of healthy and clean birds with diseased ones or contaminated carcasses, and then with human beings. Additionally, the infection may occur through nutrition and water contamination that spreads the infection individually or to a whole chicken farm and human beings (34). In vertical transmission, this bacterium can pass from one generation of poultry to the next via the egg. *Salmonella* spp. spreads through the environment, even after the pathogenic bird has stopped shedding bacteria, the environment can still be infectious for a long time but becomes less infectious (37).

In this study, the presence of *S. typhi* in the chicken eggs may be because the chicken considered a carrier or reservoir by this bacterium that presents inside and then transmitted to the eggs vertically or may be horizontally by fecal contamination of the chicken eggs during the presence of eggs on the floor, handling of raw poultry carcasses and eggs, cross-contamination from hands of workers by handling with the fecal contamination to the chicken eggs in different stages starting from collecting eggs in the egg production fields, as well as direct contact with eggs in commercial markets, and ending with preparing food inside homes and dealing with preparing egg dishes (38). The molecular results of this study agreed with Kanaan *et al.* (27), who isolated *S. enterica* from chicken meat and egg samples in Iraq, which recorded 22% of the *stn* gene of *S. enterica* but disagree by recorded 9% of the *sop B* gene. Also, I agree with Salem and Awadallah (39), who isolated *Salmonella* spp. from humans and chickens in Egypt, and recorded 100% for each *stn* and *avr A* gene in the examined *S. typhimurium* and *S. enteritidis* isolates. This study was similar to the results of Sadiq and Othman (40), who isolated *S. enterica* from chickens and humans in the Basrah and Baghdad governorates of Iraq, which recorded 98.29% of the *avr A* gene. There is no similarity in the *stn* gene between the serovar of *Salmonella* spp. such as; *S. typhi*, *S. typhimurium*, and *S. enteritidis*, this may explain the *stn* gene's presence in some serovars of *S. enterica* and its absence in other serovars of this bacterium (41). The sequencing and analysis for similarity using BLAST in NCBI compared with the global isolates of this bacterium showed some nucleotide

substitutions in some isolates and virulence factors that showed this bacterium's ability to produce mutations.

Conclusions

This study showed the presence of genetic mutations for *S. enterica* bacteria, which led to variations in the molecular characteristics, bacterial serotyping, and virulence factors; it also showed the relationship and route of bacterial infection of *S. enterica* and the method of transmission of the disease among these bacteria isolated from chicken products and human, that is by isolating the bacterial serovar that usually infects humans from chicken products.

Acknowledgment

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

References

- Jajere SM. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Vet World*. 2019;12(4):504-521. DOI: [10.14202/vetworld.2019.504-521](https://doi.org/10.14202/vetworld.2019.504-521)
- Chaudhary A, Solanki S, Sharma D, Singathia R, Rathore K, Sain A, Devi D. Isolation, identification, molecular characterization and antibiogram studies of *Salmonella spp.* isolated from calf diarrhea in and around Udaipur (Rajasthan). 2022;11(2):1413-1417. [\[available at\]](#)
- Kanaan MG. Prevalence and antimicrobial resistance of *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from retail chicken meat in Wasit markets, Iraq. *Vet World*. 2023;16(3):455-463. DOI: [10.14202/vetworld.2023.455-463](https://doi.org/10.14202/vetworld.2023.455-463)
- Sharif YM, Tayeb BA. Estimation of limit of detection of *Salmonella typhimurium* in artificially contaminated chicken meat by cultured-based and polymerase chain reaction techniques. *Iraqi J Vet Sci*. 2021;35(4):621-625. DOI: [10.33899/ijvs.2020.127328.1496](https://doi.org/10.33899/ijvs.2020.127328.1496)
- Jaslow SL, Gibbs KD, Fricke WF, Wang L, Pittman KJ, Mammel MK, Thaden JT, Fowler VG Jr, Hammer GE, Elfenbein JR, Ko DC. Salmonella activation of STAT3 signaling by SarA effector promotes intracellular replication and production of IL-10. *Cell Rep*. 2018;23(12):3525-3536. DOI: [10.1016/j.celrep.2018.05.072](https://doi.org/10.1016/j.celrep.2018.05.072)
- Hanoun AT, Al-Samirae IA. Isolation and identification of *Escherichia coli* and *Salmonella typhimurium* from sheep in Baghdad city. *Iraqi J Vet Med*. 2019;43(1):124-129. DOI: [10.30539/iraqijvm.v43i1.482](https://doi.org/10.30539/iraqijvm.v43i1.482)
- Kumar M, Dahiya SP, Ratwan P. Backyard poultry farming in India: A tool for nutritional security and women empowerment, *Biol Rhythm Res*. 2021;52(10):1476-1491. [\[available at\]](#)
- Al-Juburi LI, AL-Sammarrae IA. The protective role of *Salmonella typhimurium*-whole sonicated killed antigen and *Syzygium aromaticum* extract on the histopathological changes against its infection in rabbits. *Iraqi J Vet Med*. 2023;46(2):12-19. DOI: [10.30539/ijvm.v46i2.1399](https://doi.org/10.30539/ijvm.v46i2.1399)
- Al-Samarrae IA. The immune response of rabbits immunized by *Salmonella typhimurium* and *Lactobacillus acidophilus*. *Iraqi J Vet Med*. 2018;42(1):28-34. DOI: [10.30539/iraqijvm.v42i1.27](https://doi.org/10.30539/iraqijvm.v42i1.27)
- Abdulwahid MT, Alobaidi DA, Hamood MF. Evaluation the some biochemical quality and bacterial load of the local and imported chicken meat. *Int J Sci Nat*. 2017;8(2):257-260. [\[available at\]](#)
- Sahib AM, Al-Khalisy AF, Abdulwahid MT. Association of TGF- β 2 gene polymorphism with growth rate in local chickens. *Iraqi J Vet Med*. 2021;45(1):9-16. [\[available at\]](#)
- Farhan WH, Ulaiwi AH. Histopathological and allergic study of evaporated essential oils in broiler chicken. *J Surv Fish Sci*. 2023;2964-2971. [\[available at\]](#)
- Hasan HJ, Abdulwahid MT, Ayyez HN. Molecular detection and phylogenetic analysis of *Citrobacter freundii* isolates from broilers in A.L. Diwanayah province of Iraq. *Int J Health Sci*. 2023;6(S9):4736-4752. DOI: [10.53730/ijhs.v6nS9.14026](https://doi.org/10.53730/ijhs.v6nS9.14026)
- Al- Khafaji MM. The inhibition activity of silver nanoparticles compared with D-Glycin and imipenem effect on the biofilm formation by food-origin Salmonella. *Iraqi J Sci*. 2017;58(2B):836-842. [\[available at\]](#)
- Aljahdali NH, Khajanchi BK, Weston K, Deck J, Cox J, Singh R, Gilbert J, Sanad YM, Han J, Nayak R, Foley SL. Genotypic and phenotypic characterization of incompatibility group FIB positive *Salmonella enterica* serovar typhimurium isolates from food animal sources. *Genes*. 2020;11(11):1307. DOI: [10.3390/genes11111307](https://doi.org/10.3390/genes11111307)
- Nikiema MM, Kakou-Ngazona S, Ky/Ba A, Sylla A, Bako E, Addablah AA, Ouoba JB, Sampo E, Gnada K, Zongo O, Traoré KA, Sanou A, Bonkougou IO, Ouédraogo R, Barro N, Sangaré L. Characterization of virulence factors of Salmonella isolated from human stools and street food in urban areas of Burkina Faso. *BMC Microbiol*. 2021;21(1):338. DOI: [10.1186/s12866-021-02398-6](https://doi.org/10.1186/s12866-021-02398-6)
- McGhie EJ, Brawn LC, Hume PJ, Humphreys D, Koronakis V. Salmonella takes control: Effector-driven manipulation of the host. *Curr Opin Microbiol*. 2009;12(1):117-24. DOI: [10.1016/j.mib.2008.12.001](https://doi.org/10.1016/j.mib.2008.12.001)
- Mahmoud MM, Megahed G, Yousef MS, Ali FZ, Zaki RS, Abdelhafeez HH. *Salmonella typhimurium* triggered unilateral epididymo-orchitis and splenomegaly in a Holstein bull in Assiut, Egypt: A case report. *Pathogens*. 2020;9(4):314. DOI: [10.3390/pathogens9040314](https://doi.org/10.3390/pathogens9040314)
- Khalefa HS, Ahmed ZS, Abdel-Kader F, Ismail EM, Elshafiee EA. Sequencing and phylogenetic analysis of the stn gene of Salmonella species isolated from different environmental sources at Lake Qarun protectorate: The role of migratory birds and public health importance. *Vet World*. 2021;14(10):2764-2772. DOI: [10.14202/vetworld.2021.2764-2772](https://doi.org/10.14202/vetworld.2021.2764-2772)
- WHO World Health Organization. Diet, nutrition and the prevention of chronic diseases. *Tech Rep Ser*. 2003;916:i-viii, 1-149.
- Markey BK, Leonard FC, Achambault M, Cullinana A, Maguire D. *Clinical veterinary microbiology*. 2nd ed. USA: Mosby Elsevier; 2014. 275-288 p. [\[available at\]](#)
- Kipper D, Hellfeldt RM, De Carli S, Lehmann FM, Fonseca AK, Ikuta N, Lunge VR. Salmonella serotype assignment by sequencing analysis of intergenic regions of ribosomal RNA operons. *Poult Sci*. 2019;98(11):5989-5998. DOI: [10.3382/ps/pez285](https://doi.org/10.3382/ps/pez285)
- ElSheikh M, Abdeen E, Ammar A. Molecular detection of some virulence genes of Salmonella serotypes isolated from poultry in Egypt. *J Curr Vet Res*. 2019;1(1):86-93. DOI: [10.21608/jcvr.2019.36570](https://doi.org/10.21608/jcvr.2019.36570)
- Mthembu TP, Zishiri OT, El Zowalaty ME. Detection and molecular identification of Salmonella virulence genes in livestock production systems in South Africa. *Pathogens*. 2019;8(3):124. DOI: [10.3390/pathogens8030124](https://doi.org/10.3390/pathogens8030124)
- Nakano M, Yamasaki E, Ichinose A, Shimohata T, Takahashi A, Akada JK, Nakamura K, Moss J, Hirayama T, Kurazono H. Salmonella enterotoxin (Stn) regulates membrane composition and integrity. *Dis Model Mech*. 2012;5(4):515-21. DOI: [10.1242/dmm.009324](https://doi.org/10.1242/dmm.009324)
- Huehn S, La Ragione RM, Anjum M, Saunders M, Woodward MJ, Bunge C, Helmuth R, Hauser E, Guerra B, Beutlich J, Brisabois A, Peters T, Svensson L, Madajczak G, Litrup E, Imre A, Herrera-Leon S, Mevius D, Newell DG, Malorny B. Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human

- health in Europe. Foodborne Pathog Dis. 2010;7(5):523-35. DOI: [10.1089/fpd.2009.0447](https://doi.org/10.1089/fpd.2009.0447)
27. Kanaan MG, Khalil ZK, Khashan HT, 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. 2022;22(1):279. DOI: [10.1186/s12866-022-02696-7](https://doi.org/10.1186/s12866-022-02696-7)
28. Nader MI, Rasheed MN, Hamed HH. Molecular identification of *Salmonella typhimurium* from chicken, meat, and human by PCR. Int Conf Med Genet Cell Mol Biol Pharm Food Sci. 2015;1416-1422. [\[available at\]](#)
29. Harb A, O'Dea M, Abraham S, Habib I. Childhood diarrhoea in the eastern mediterranean region with special emphasis on nontyphoidal *Salmonella* at the human food interface. Pathogens. 2019;8(2):60. DOI: [10.3390/pathogens8020060](https://doi.org/10.3390/pathogens8020060)
30. Jaffer MR. Contamination of local laying hen's egg shell with *Salmonella* serotypes. Iraqi J Vet Med. 2013;37(1):13-16. DOI: [10.30539/iraqijvm.v37i1.318](https://doi.org/10.30539/iraqijvm.v37i1.318)
31. Siddique A, Azim S, Ali A, Andleeb S, Ahsan A, Imran M, Rahman A. Antimicrobial resistance profiling of biofilm forming non typhoidal *Salmonella enterica* isolates from poultry and its associated food products from Pakistan. Antibiotics. 2021;10(7):785. DOI: [10.3390/antibiotics10070785](https://doi.org/10.3390/antibiotics10070785)
32. Rahmani M, Peighambari SM, Yazdani A, Hojjati P. *Salmonella* infection in birds kept in parks and pet shops in Tehran, Iran. Int J Vet Res. 2011;5(3):145-148. [\[available at\]](#)
33. Gellatly SL, Hancock RE. *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. Pathog Dis. 2013;67(3):159-73. DOI: [10.1111/2049-632X.12033](https://doi.org/10.1111/2049-632X.12033)
34. Hassan AH, Salam HS, Abdel-Latef GK. Serological identification and antimicrobial resistance of *Salmonella* isolates from broiler carcasses and human stools in Beni-Suef, Egypt. Beni-Suef Univ J Basic Appl Sci. 2016;5:202-207. DOI: [10.1016/j.bjbas.2016.04.002](https://doi.org/10.1016/j.bjbas.2016.04.002)
35. Zubair AI, Al-Berfkani MI, Issa AR. Prevalence of *Salmonella* species from poultry eggs of local stores in Duhok. Int J Res Med Sci. 2017;5(6):2468. DOI: [10.18203/2320-6012.ijrms20172430](https://doi.org/10.18203/2320-6012.ijrms20172430)
36. Yousif AR, Harab AH. Isolation and serotyping of *Salmonella* species in diarrheal children. Univ Thi-Qar J Med. 2011;5(1):149-155. [\[available at\]](#)
37. Thomas ME, Klinkenberg D, Ejeta G, Van Knapen F, Bergwerff AA, Stegeman JA, Bouma A. Quantification of horizontal transmission of *Salmonella enterica* serovar *Enteritidis* bacteria in pair-housed groups of laying hens. Appl Environ Microbiol. 2009;75(19):6361-6. DOI: [10.1128/AEM.00961-09](https://doi.org/10.1128/AEM.00961-09)
38. Al-Zuhariy MT. Using T cell lymphokines of hyperimmunized chickens with *Salmonella pullorum* to protect layer hens against *Salmonella pullorum* infection. Iraqi J Vet Sci. 2022;36(1):223-227. DOI: [10.33899/ijvs.2022.136021.2556](https://doi.org/10.33899/ijvs.2022.136021.2556)
39. Salem L, Awadallah MA. Zoonotic importance of *Salmonellosis* in chickens and humans at Qualyobia province. Egypt J of Vet Sci. 2016;47(2):151-164. DOI: [10.21608/ejvs.2016.3585](https://doi.org/10.21608/ejvs.2016.3585)
40. Sadiq MS, Othman RM. Phylogenetic tree constructed of *Salmonella enterica* subspecies *enterica* isolated from animals and humans in Basrah and Baghdad governorates, Iraq. Iraqi J Vet Sci. 2022;36(4):895-903. DOI: [10.33899/ijvs.2022.132478.2096](https://doi.org/10.33899/ijvs.2022.132478.2096)
41. Beikzadeh B. Immunoinformatics design of multi-epitope vaccine using OmpA, OmpD and enterotoxin against nontyphoidal salmonellosis. BMC Bioinformatics. 2023;24(1):63. DOI: [10.1186/s12859-023-05183-6](https://doi.org/10.1186/s12859-023-05183-6)

التحري الجزيئي عن بعض جينات عوامل الضراوة لبكتيريا السالمونيلا المعزولة من منتجات الدجاج والإنسان من محافظة واسط، العراق

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الخلاصة

تم جمع 300 عينة (150 عينة من منتجات الدجاج (50 بيض، 50 كبد و 50 لحم مفروم) من الأسواق المحلية المختلفة و 150 عينة سريرية من براز الإنسان) لغرض عزل وتوصيف بكتيريا السالمونيلا انتركا من محافظة واسط في العراق للفترة من كانون الثاني الى كانون الأول لعام 2022 بواسطة تقنية سلسلة البلمرة وفحص تسلسل القواعد النيوتروجينية (النيوكليوتيدات) لثلاث جينات من عوامل الضراوة *avr A*، *sop B*، *stn*. بينت النتائج بان نسبة العزل الكلي لبكتيريا السالمونيلا انتركا في الإنسان كانت 8,6، 6، 6% في منتجات الدجاج وكالاتي: (12% في البيض، 6% في الكبد و 8% في لحم الدجاج المفروم). وكانت نسبة السالمونيلا تايفي في عينات الإنسان 100%. أما في عينات البيض فكانت النسبة 50% للسالمونيلا تايفي، 33,33% للسالمونيلا تايفيموريم و 16,66% للسالمونيلا انترابتيدس. وكذلك بينت النتائج بان نسبة السالمونيلا تايفيموريم كانت 100% في كل من عينات كبد الدجاج وعينات لحم الدجاج المفروم. وبينت نتائج عوامل الضراوة بعدم وجود جين *stn* في عينات الإنسان، بينما كانت نسبته 77% في عينات منتجات الدجاج. وكذلك كانت النسبة 100% لكل من الجينات *avr A* و *sop B* في جميع العينات البشرية ومنتجات الدجاج. وبينت النتائج بان النمط سالمونيلا تايفي كان يحمل الجينات *sop B* و *avr A* بنسبة 100% بينما يفتقد الى الجين *stn*. وكان النمط سالمونيلا تايفيموريم والنمط سالمونيلا انترابتيدس يحملان جميع الجينات *avr A*، *sop B*، *stn* بنسبة 100%. تم إجراء فحص تسلسل القواعد النيوتروجينية (النيوكليوتيدات) لستة من مستخلصات فحص تسلسل البلمرة لبعض عزلات البكتيريا من مصادر مختلفة لثلاثة من عوامل الضراوة والتي سجلت على موقع المركز الوطني لمعلومات التقانة الحيوية. بينت النتائج بان هنالك عدة طفرات وراثية في عوامل الضراوة *avr A* و *sop B* بينما لا توجد طفرات وراثية في عامل الضراوة *stn*.