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Molecular characterization and genetic diversity of salmonella SPP

Ashwaq R. Nazzal[1]*, Hassan Nima Habib[2]

[1]*Department of Public Health, College of Veterinary Medicine University of Basra, Iraq*
[2]*University of Basrah, College of Agriculture, Animal Peoduction, Basrah, Iraq*

Abstract

This study investigated the molecular characterization and genetic diversity of Salmonella spp. isolated from various food sources between April and June 2025. A total of 90 samples (chicken, cheese, and beef) were collected, of which 70 (77.8%) tested positive for Salmonella spp. Biochemical identification was performed using conventional tests and the API 20E system, followed by species-level confirmation via the VITEK® 2 COMPACT system. Chicken samples showed the highest contamination rates (85–100%), followed by cheese (70–90%) and beef (55–75%). S. Typhimurium was the most prevalent serotype (42.8%), followed by S. arizona (25.7%) and S. paratyphi (17.3%). Molecular detection using conventional PCR and real-time PCR targeting the 16S rRNA gene, combined with sequencing and phylogenetic analysis (MEGA-X), revealed high genetic similarity (84–96%) among isolates, suggesting a common evolutionary origin. These findings underscore the significant public health risk posed by Salmonella contamination in animal-derived foods and highlight the necessity for rigorous food safety monitoring and targeted interventions to reduce pathogen transmission along the food chain.

Keywords: Salmonella SPP, Molecular characterization, Genetic diversity, VITEK 2 compact, PCR, Food safety.

Introduction

Infectious and foodborne diseases caused by Salmonella spp. represent a major global public health and food safety concern. According to the World Health Organization, approximately 10% of diarrheal diseases worldwide each year are attributable to Salmonella infections (Besser, 2018). Salmonella is a Gram-negative, facultatively anaerobic bacterium and one of the most prevalent foodborne pathogens of animal origin. Recent epidemiological reports indicate that more than 72% of bacterial food poisoning cases are specifically linked to Salmonella, posing a significant threat to both consumer health and the food industry. Among its numerous serovars, Salmonella enterica serovar Typhimurium (S. Typhimurium) is particularly associated with foodborne outbreaks and gastroenteritis.

The widespread distribution of Salmonella across regions including the Middle East, Asia, Europe, and the United States has been facilitated by its ability to persist in the food cold chain. Notably, refrigeration and conventional food sterilization processes often fail to eliminate these bacteria, largely due to genetic adaptations that confer enhanced tolerance to adverse environmental conditions. In addition to gastroenteritis, Salmonella is implicated in severe systemic diseases such as typhoid and paratyphoid fevers, typically transmitted via contaminated food or water. This underlines the importance of investigating its molecular characteristics and genetic diversity to better understand its epidemiology, evolution, and pathogenicity.

Recent research has emphasized molecular epidemiology approaches for Salmonella characterization, often employing 16S rRNA gene sequencing for phylogenetic analyses. The invA virulence gene is also widely used as a molecular marker for detection and strain differentiation. Over the past few decades, novel Salmonella serotypes have emerged, including monophasic S. Typhimurium variants, which exhibit unique genetic structures and altered flagellar expression. These variants frequently lack second-phase flagellar antigens due to mutations or deletions in the fljB gene and related loci (Zamperini et al., 2007). Some harbor IncHI2 plasmids carrying extended-spectrum β-lactamase (ESBL) and virulence genes, contributing to multidrug resistance (MDR) profiles against sulfonamides, tetracyclines, ampicillin, and streptomycin. For instance, a study in China

Correspondence: Ashwaq R. Nazzal, Department of Public Health, College of Veterinary Medicine University of Basra, Iraq, **e-mail:** drarn.molgen@gmail.com,
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identified the ASSuT resistance pattern as predominant among circulating strains.

Several epidemiological studies have revealed that pork and poultry are the primary reservoirs of antibiotic-resistant Salmonella strains, particularly in Europe and the United States. A notable example is Germany, where approximately 48% of pathogenic strains were isolated from pork between 2006 and 2008. In China, S. 4, [5],12: i: - was reported as the second most common serotype among clinical isolates, with a strong association to pork. Despite these observations, the molecular characteristics and evolutionary dynamics of S. 4, [5],12: i: - within individual pig farms remain poorly understood.

Objective

This study aims to comprehensively investigate the molecular characteristics and genetic diversity of Salmonella spp. isolated from food processing environments, utilizing 16S rRNA gene sequencing as a robust phylogenetic and taxonomic tool. By elucidating the genetic profiles and diversity patterns of these isolates, the research seeks to provide critical insights into their evolutionary relationships, potential pathogenicity, and implications for food safety management.

Methodology

A total of 90 samples were collected from food processing facilities, including cheese, beef, and chicken products (30 samples from each source). Sampling was carried out over a three-month period (April–June 2025), with ten samples collected monthly. Samples were randomly obtained from the designated sources, placed in sterile polyethylene bags, and immediately transported to the laboratory under refrigerated conditions (4 °C). All microbiological analyses were performed on the same day of collection to ensure sample integrity.

Bacterial colonies were purified by sub-culturing on MacConkey agar and nutrient agar, following a standardized streak plate method, and incubated at 37 °C for 24 h. Preliminary identification of bacterial isolates was based on morphological and cultural characteristics, including colony size, color, margin, elevation, lactose fermentation, mucus production, hemolysin production, and Gram reaction. Microscopic examination was performed after Gram staining, followed by a series of biochemical tests according to the protocols described by [10].

Identification of Salmonella spp. was initially performed through biochemical profiling (Table 1). Gram-negative isolates were subjected to oxidase testing, followed by genus-level confirmation using the API 20E system (Figure 1). Species-level identification was further achieved using the VITEK® 2 COMPACT system, and prevalence rates were calculated based on the number of confirmed isolates.

Confirmed Salmonella isolates [11] underwent molecular characterization using Polymerase Chain Reaction (PCR) targeting the 16S rRNA gene, a highly conserved genetic marker used for bacterial identification and phylogenetic studies (Table 2). Amplified products were subjected to sequencing, and phylogenetic relationships were analyzed using MEGA-X software to construct high-resolution phylogenetic trees incorporating both newly sequenced strains and reference sequences [12].

The phylogenetic analysis provided insights into the genetic diversity, evolutionary relationships, and possible geographical origins of the Salmonella isolates. Results revealed notable genetic heterogeneity among strains, contributing valuable data to the understanding of Salmonella population structure (ALKHARMAN, (2025)).

Table (1): Biochemical tests on the types of bacteria under study diagnosed in the laboratory

Test type	Type of bacteria		
	Gram negative		
	Klebsiella	*E. coli*	*salmonella*
Gram stine	–	–	–
Indol	–	+	–
Catalase	+	+	+
Coagulase	A + G+	A + G+	A+
CAMP	–	+	–
Oxidase	–	–	–
Urease	–	–	–
H2s	–	–	+
Methyl red	–	+	+

Table2: Primer sequences used for salmonella detection

Amplicon. Size	Sense	Sequence. 5'-3'	Targetreg.Ion	Primer
250.bp	+	AGA GTT TGA TCC TGG CTCAG	27 F	16 SrRNA
	-	GGT TAC CTT GTT ACG ACTT	1492 R	

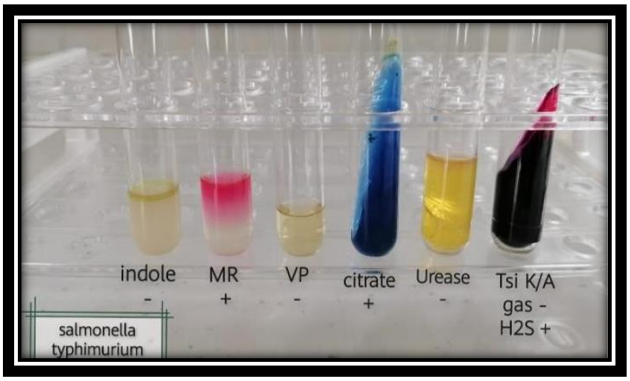

Figure (1) Biochemical tests for S. typhimurium bacteria

Statistical analysis

Statistical analyses were performed using SPSS software, version 25.0 (IBM Corp., Armonk, NY, USA). The distribution of results was summarized using descriptive statistics, and the proportion of positive and negative cases was calculated and presented as percentages. All analyses adhered to standard biostatistical practices to ensure the accuracy and reliability of the findings

Results and Discussion

Prevalence of salmonella contamination in the examined food sources

Following the completion of the biochemical identification procedures for Salmonella spp. (Figure 2) and the preliminary differentiation from other bacterial species, the isolates exhibited positive reactions for the H_2S and Methyl Red tests, while yielding negative results for Indole, Oxidase, and Urease tests (Table 1).

As shown in Table 3, Salmonella contamination was detected in 70 out of the 90 total bacterial isolates, representing an overall contamination rate of 77.8%. Chicken meat samples exhibited the highest contamination levels, reaching 100% in June and 85% in April. This seasonal variation could be associated with ambient temperature differences at the time of sample collection [13,25].

Cheese samples showed contamination rates of 90% in June and 70% in April. In contrast, beef samples had the lowest recorded contamination rates, with 75% in June and 55% in April. This marked difference between chicken meat and beef may be attributed to the enhanced ability of Salmonella to survive, proliferate, and colonize chicken meat, particularly within skin tissues. Previous studies have demonstrated that the surface layers of poultry skin provide a highly favorable microenvironment for Salmonella growth and dissemination [14].

These findings highlight the potential role of temperature, food type, and surface structure in influencing Salmonella contamination rates. Moreover, the significantly higher contamination levels in poultry products underline the need for stricter hygienic measures and targeted interventions during processing and storage to mitigate public health risks [15].

Table (3) The number of isolates carrying salmonella bacteria during the collection period of 3 months, where 10 samples were counted for each source

Source of isolates	Collection time, number and percentage of Salmonella positive samples collected					
	June	ratio%	May	ratio%	April	ratio%
Cheese	9	%90	7.5	%75	7	%70
Chicken	10	%100	9	%90	8.5	%85
Beef	7.5	%75	6	%60	5.5	%55
The Total	26.5	%88.3	22.5	%75	21	%70

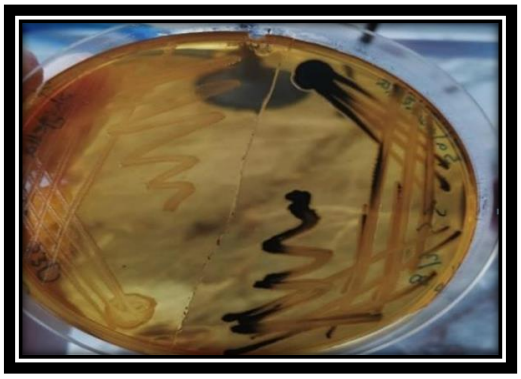

Figure (2) Salmonella bacteria on Ss agar medium

Calculating genotypes and identifying the types of Salmonella bacteria under study using the VITEK 2 Compact technique

Following the isolation of Salmonella spp. using the previously described methods, each isolate was identified using the VITEK® 2 COMPACT system in accordance with the manufacturer's instructions and protocols. The isolates obtained from various food sources were analyzed using this automated technology, revealing high similarity indices for several species: S. typhimurium (89%), S. arizona (92%), and S. paratyphi (90%), while other Salmonella species exhibited an overall similarity of 84% [16]. As shown in Table 4, S. typhimurium exhibited the highest prevalence, being detected in 30 out of 70 total samples (42.8%). S. arizona was identified in 18 samples (25.7%), followed by S. paratyphi in 12 samples (17.3%) [17]. The remaining Salmonella species collectively accounted for 10 samples (14.2%). Both Table 4 and Figure 3 clearly demonstrate that S. typhimurium was the most frequently detected species, regardless of the food source, followed by S. arizona and S. paratyphi, respectively [19, 18]. Statistical analysis indicated significant differences in the predominance of S. typhimurium compared to other Salmonella species. The high prevalence of S. typhimurium may be attributed to its remarkable adaptability and ability to proliferate under favorable food storage and preservation conditions [20], as well as the presence of multiple virulence determinants, such as biofilm-forming capabilities [21] and other pathogenicity-associated genes. Similar trends were observed for the other detected species, albeit at lower frequencies, which may be influenced by factors such as food source, preservation methods, and geographic variations. These findings are in agreement with several previous studies conducted worldwide [22,23], although differences in species distribution have been reported across regions due to variations in environmental and food-handling practices [24].

Table (4) Identification of salmonella SPP bacteria species using the API 20 E system and VITEK 2 Compact technology

Gram stain	Percentage	Number of infected samples Salmonella sp	Match percentage according to VITEK 2	Type of pathogenic bacteria
Gr -	%42.80	30	%89	S. typhimurium
Gr -	%25.70	18	%91	S.arizona
Gr -	%17.30	12	%90	S.paratyphi
Gr -	%14.20	10	%94	Salmonella sp.
	%100	70		The Total

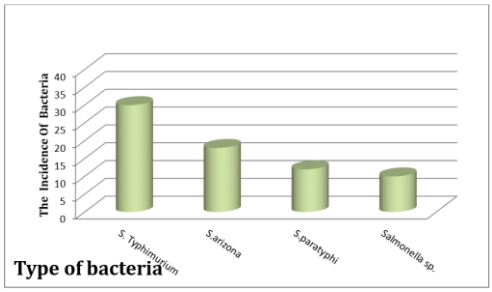

Figure (3) Frequency of bacterial species of the genus Salmonella sp. in the study

Molecular Diagnosis and Genetic Profiling of Salmonella spp. via 16S rRNA Gene Sequencing for Epidemiological Surveillance

In this study, molecular techniques were employed to identify and characterize Salmonella spp. isolates through partial genetic profiling using the 16S rRNA gene. Ten Salmonella isolates, previously recovered from the analyzed food sources, were subjected to both conventional polymerase chain reaction (PCR) and real-time PCR (qPCR) assays. The amplification

reactions were performed using specific primers (Table 2), following the manufacturer's protocols for the PCR System 9700 (GENE Amp, USA) without deviation from the standardized procedures.

Amplification products were analyzed by gel electrophoresis (Figure 3), with clear band patterns observed in lanes 1–8, corresponding to isolates 1, 3, 4, and 6–8, with an approximate size difference of 100 base pairs (Figure 4). Subsequent sequencing of the amplified 16S rRNA gene fragments allowed precise species-level identification. The sequence data were analyzed using MEGA-X software to construct a phylogenetic tree (Figure 5), revealing the evolutionary relationships, genetic divergence, and clustering patterns among the studied isolates. This approach provided valuable insights into the genetic diversity and phylogenetic proximity of Salmonella strains, enhancing the understanding of their epidemiological distribution.

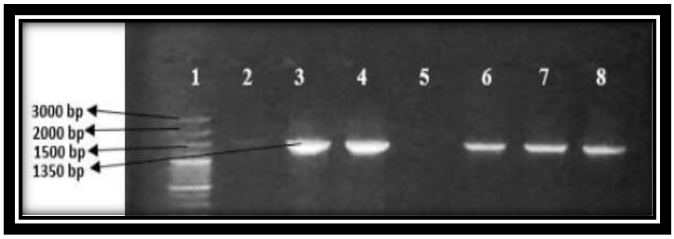

Figure 4: PCR gel electrophoresis of colonies for detection of 16S rRNA using universal primer 27F and 1492R lanes 1 and 8, 100 base pairs plus DNA ladder (0.5 µg/lane)

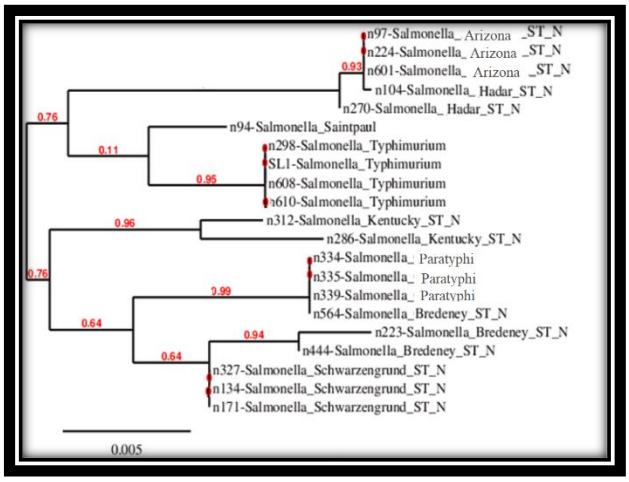

Figure (5) The genetic tree shows the extent of convergence, origin and evolution between the bacterial species under study

Conclusion

This study demonstrated that Salmonella species of diverse origins were frequently detected in food samples, with an overall prevalence of approximately 70%. Among the examined sources, chicken exhibited the highest contamination rate, followed by cheese and beef. S. Typhimurium emerged as the most dominant and recurrent serotype across the investigated food matrices. Molecular characterization revealed a high degree of genetic relatedness, with Salmonella spp. and S. enterica serotypes sharing nearly 90% genetic similarity, suggesting a common ancestral origin. These findings highlight the potential public health risks associated with contaminated animal-derived food products and underscore the need for stringent food safety monitoring, targeted intervention strategies, and continuous molecular surveillance to mitigate the spread of pathogenic Salmonella strains in the food chain.

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