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Virulence Gene Properties and Phylogenetic Relationship of *Salmonella* Serotypes Isolated from Different Sources in North of Iran

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Abstract

Background: *Salmonella* species can cause various infections in humans and animals. The presence of certain genes determines the virulence of a *Salmonella* serotype.
Objectives: The current research endeavor was undertaken to assess the virulence characteristics and genotypic traits of *Salmonella* serotypes extracted from various sources within the geographical boundaries of Iran.
Methods: *Salmonella* isolates, previously retrieved and preserved in the veterinary microbiology laboratory, underwent serotyping and polymerase chain reaction (PCR) identification of nine virulence-associated genes. Genotyping was carried out using random amplified polymorphic DNA-PCR (RAPD-PCR).
Results: All *Salmonella* isolates showed the presence of *invA*, *sdiA*, *hilA*, and *iroB* virulence genes. There were a total of 17 different virulence gene patterns among *Salmonella* serotypes. The presence of *fliC* and *sefA* genes and their related patterns were significant among *S. typhimurium* and *S. enteritidis* serotypes, respectively ($P < 0.05$). In the RAPD-PCR fingerprinting, 11 distinct clusters were obtained, and 16 isolates (26.66%) were classified as untypeable strains. There was a significant association between RAPD genotypes and *Salmonella* serotypes ($P < 0.05$), while the association between these RAPD patterns and the source of the isolates was not significant ($P > 0.05$).
Conclusions: According to the results, *Salmonella* serotypes from non-human sources carry significant virulence determinants and show similar genotypic patterns with human isolates. These findings provide valuable insights into the virulence properties and genetic diversity of *Salmonella* serotypes in Iran, which could inform the development of effective control and prevention strategies for salmonellosis in the region.

Keywords: *Salmonella*, Virulence Factors, Genotype

1. Background

Salmonella, a genus of facultative anaerobic, non-spore-forming, gram-negative bacteria, has been identified as a pathogen capable of causing various infections in both human and animal populations. These infections can range from mild gastroenteritis to severe conditions such as typhoid fever and bacteremia, which can potentially be fatal (1). *Salmonella enterica* comprises over 2,600 serotypes, each characterized by unique antigenic properties based on their surface antigens. While many serotypes of *Salmonella* can cause disease, the severity of the illness often depends on the specific serotype involved (2).

One critical determinant of a *Salmonella* serotype's virulence is the presence of specific genes responsible for virulence factors. These genes can encode various factors that enable the bacteria to thrive in the host, including adhesins that facilitate bacterial attachment to host cells, toxins that damage host cells, and secretion systems that allow the bacteria to inject proteins into host cells (3). The expression of these virulence genes is often regulated by complex networks that respond to environmental cues and host signals (4).

Understanding the genetic basis of *Salmonella*

virulence and studying the phylogenetic differences among serotypes from various sources is crucial for developing effective strategies to prevent and treat infections caused by this pathogen. There is an ongoing and perpetual need for comprehensive investigations into the multitude of *Salmonella* serotypes. Additionally, it is essential to identify and elucidate the various genes responsible for encoding the virulent factors that play a crucial role in the pathogenicity of these bacterial pathogens. Furthermore, it is imperative to highlight and elucidate the most recent and cutting-edge discoveries concerning our overall understanding of these highly significant and clinically relevant microorganisms.

2. Objectives

From a specific perspective, this study involved the evaluation of *Salmonella* serotypes from various sources to assess their virulence gene characteristics, virulence gene profiles, as well as their DNA fingerprinting and phylogenetic classifications. Subsequently, the obtained results underwent statistical analysis to distinguish similarities and differences among the serotypes and sources examined in this investigation.

3. Methods

3.1. Salmonella Serotypes and Template DNA

This study utilized 60 *Salmonella* isolates, including 31 *S. enteritidis*, 16 *S. infantis*, 12 *S. typhimurium*, and 1 *S. typhi*, obtained from various sources (Table 1). These isolates had been previously obtained in the microbiology laboratory at the Faculty of Veterinary Medicine (unpublished data). The culture and isolation of *Salmonella* isolates were conducted between 2021 and 2022 using enrichment methods in Selenite F medium and cultivation on MacConkey and *Salmonella*-Shigella agar (SSA) specific media (5). The serotyping of *Salmonella* isolates was carried out at the *Salmonella* Research Center at Tehran University's Faculty of Veterinary Medicine. Commercial antisera (Difco, Detroit, Michigan, USA) were used for serotyping, and the results were analyzed following the Kaufmann-White scheme (6). *Salmonella typhimurium* (ATCC 14028) was a reference strain for other bacteriological examinations.

3.2. DNA Extraction and Purification

DNA extraction from *Salmonella* isolates was performed using a DNA extraction kit designed for Gram-negative bacteria (CinaClone, Tehran, Iran), following the manufacturer's instructions. The purity and concentration of the DNA were determined through spectrophotometry at wavelengths of 260 and 280 nm (Nanodrop 1000; Thermo Scientific). The extracted and purified DNA samples were then stored at -20°C for future use.

3.3. Detection of Virulence Genes Through PCR

In this study, the isolates were examined for the presence of nine putative genes associated with *Salmonella* virulence (*invA*, *hilA*, *sdiA*, *iroB*, *fliC*, *pefA*, *sefA*, *sopB*, and *spv*) to identify potential differences among serotypes. PCR was conducted using specific primers (as detailed in Table 2) in a final volume of 25 μL, which included 12.5 μL of PCR master mix, 1 μL (0.4 μM) of both forward and reverse primers (7-14), and 2 μL of template DNA. All components were sourced from Sinaclon Corporation, Iran. Subsequently, the resulting PCR product was evaluated via electrophoresis in a 1.5% agarose gel. The DNA amplicons obtained were assessed using a 100 bp DNA marker (Sinaclon, Iran). Different virulence gene patterns were determined based on the presence of genes.

3.4. DNA Fingerprinting and Phylogenetic Analysis

The Random Amplification of Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) technique was executed using a 1254 random primer, with the sequence 5'-CCGCAGCCAA-3', as previously described (15). The thermal cycling device (MJ Mini, USA) employed the following program for the RAPD method: initial denaturation at 94°C for 5 minutes, followed by 34 cycles with denaturation at 94°C for 1 minute, annealing at 36°C for 1 minute, and extension at 72°C for 1 minute. The final extension occurred at 72°C for 6 minutes. PCR product visualization was achieved using gel electrophoresis on a 3% agarose gel. For analysis, the RAPD reaction images were processed with the GelClust software (16). Genetic similarity was computed using Pearson's correlation, and the dendrogram for the isolates was created using the Dice correlation coefficient, in addition to the unweighted pair group method with arithmetic averages (UPGMA). The final groupings were determined by applying a cut-off of 80%.

3.5. Statistical Analysis

The study's outcomes underwent analysis using SPSS version 22 software (IBM Armonk, North Castle, NY, USA). Mann-Whitney, Chi-square, and Kolmogorov-Smirnov tests were employed for statistical analyses, with a P-value of less than 0.05 considered statistically significant.

Table 1. Distribution of Virulence Genes Among *Salmonella* Serotypes

Source	No	Virulence Gene Distribution (%)								
		hilA	*invA*	*sdiA*	*iroB*	*fliC*	*pefA*	*sefA*	*sopB*	*spv*
Chicken meat	16	16 (100)	16 (100)	16 (100)	16 (100)	0 (0)	13 (81.25)	3 (18.75)	9 (56.25)	1 (6.25)
S. enteritidis	4	4	4	4	4	0	3	3	2	1
S. infantis	12	12	12	12	12	0	10	0	7	0
Poultry feces	8	8 (100)	8 (100)	8 (100)	8 (100)	4 (50)	3 (37.5)	5 (62.5)	7 (87.5)	1 (12.5)
S. enteritidis	4	4	4	4	4	0	2	4	3	1
S. typhimurium	4	4	4	4	4	4	1	1	4	0
Chicken skin	5	5 (100)	5 (100)	5 (100)	5 (100)	0 (0)	5 (100)	4 (80)	4 (80)	0 (0)
S. enteritidis	5	5	5	5	5	0	5	4	4	0
Chicken liver	2	2 (100)	2 (100)	2 (100)	2 (100)	0 (0)	1 (50)	1 (50)	2 (100)	1 (50)
S. enteritidis	1	1	1	1	1	0	0	1	1	0
S. infantis	1	1	1	1	1	0	1	0	1	1
Grilled chicken	3	3 (100)	3 (100)	3 (100)	3 (100)	0 (0)	3 (100)	3 (100)	3 (100)	0 (0)
S. enteritidis	1	1	1	1	1	0	1	1	1	0
S. infantis	2	2	2	2	2	0	2	2	2	0
Human	10	10 (100)	10 (100)	10 (100)	10 (100)	2 (20)	10 (100)	6 (60)	4 (40)	3 (30)
S. enteritidis	7	7	7	7	7	0	7	5	4	2
S. typhimurium	2	2	2	2	2	2	2	1	0	1
S. typhi	1	1	1	1	1	1	1	0	0	0
Cattle	10	10 (100)	10 (100)	10 (100)	10 (100)	5 (50)	6 (60)	4 (40)	7 (70)	3 (30)
S. enteritidis	5	5	5	5	5	0	3	2	3	2
S. typhimurium	5	5	5	5	5	5	4	2	4	1
Pigeon	4	4 (100)	4 (100)	4 (100)	4 (100)	0 (0)	4 (100)	3 (75)	3 (75)	0 (0)
S. enteritidis	3	3	3	3	3	0	3	3	2	0
S. infantis	1	1	1	1	1	0	1	0	1	0
Hamburger	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
S. typhimurium	1	1	1	1	1	1	1	1	1	1
Worker boots swab	1	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)
S. enteritidis	1	1	1	1	1	0	1	0	1	1
Total (%)	60	60 (100)	60 (100)	60 (100)	60 (100)	12 (20)	48 (80)	30 (50)	41 (68.33)	11 (18.33)

4. Results

4.1. Distribution of Virulence-Associated Genes

Among the nine virulence genes studied, the most commonly observed genes were *invA*, *sdiA*, *hilA*, and *iroB* (100%), followed by *pefA* (80%) and *sopB* (68.33%). The comprehensive results of the presence of these nine virulence genes in the 60 studied *Salmonella* strains are shown in Table 1. In total, 17 different patterns of virulence gene presence were identified among *Salmonella* serotypes, as shown in Table 3. The most prevalent virulence gene pattern, in addition to the four genes with 100% prevalence, was $pefA^{+}/sefA^{+}/sopB^{+}$ with a frequency of 15 out of 60 (25%), followed by $pefA^{+}/sopB^{+}$ with a frequency of 12 out of 60 (20%).

4.2. Results of Phylogenetic Study

The utilization of the fingerprinting technique classified 73.33% of the isolates (44 out of 60). Additionally, an analysis of RAPD-PCR results using GelClust software

Table 2. Primers in PCR for Detection of Virulence Genes of *Salmonella* Isolates [z]

Target Gene	Sequence (5' to 3')	Annealing Temperature (°C)	PCR Product Size (bp)	Reference
hilA	F: CGTGAAGGGATTATCGCAGT	51	296	(7)
	R: GTCCGGGAATACATCTGAGC			
fliC	F: CCAGTCTGCGCTGTCGAG	53	349	(6)
	R: CACGTTCACGCCGTTGAAC			
invA	F:ACAGTGCTCGTTTACGACCTGAAT	55	244	(9)
	R: AGACGACTGGTACTGATCTAT			
iroB	F: TGCGTATTCTGTTTGTCGGTCC	55	606	(10)
	R: TACGTTCCCACCATTCTTCCC			
pefA	F: TGTTTCCGGGCTTGTGCT	53	157	(11)
	R: CAGGGCATTTGCTGATTCTTCC			
sdiA	F: AATATCGCTTCGTACCAC	52	274	(12)
	R: GTAGGTAAACGAGGAGCAG			
sefA	F: GCAGCGGTTACTATTGCAGC	51	330	(13)
	R: TGTGACAGGGACATTTAGCG			
sopB	F: TCAGAAGACGTCTAACCACTC	53	518	(14)
	R: TACCGTCCTCATGCACACTC			
spv	F: GCCGTACACGAGCTTATAGA	51	250	(10)
	R: ACCTACAGGGGCACAATAAC			
1254 (RAPD-PCR)	CCGCAGCCAA	36	Variable	(15)

Abbreviations: F, forward; R, reverse.

(UPGMA) led to the discovery of 11 distinct clusters, identified as R-1 to R-11 (SID = 0.1576). Sixteen isolates (26.66%) could not be serotyped by RAPD-PCR using a 1254 random primer. The most prevalent genotypes were R-1 and R-8, with frequencies of 16 out of 60 (26.66%) and 6 out of 60 (10%), respectively.

Figure 1 depicts the outcomes of the DNA fingerprinting analysis conducted on various *Salmonella* serotypes using RAPD-PCR in association with their virulence gene patterns. R3, R-5, R-6, R-7, R-9, R-10, and R-11 clusters (phylogenetic groups) were specific to the *S. enteritidis* serotype, and the R-2 cluster was specific to the *S. infantis* serotype. The highest variety of genotypic patterns (with 10 different patterns) and the most untypeable isolates were observed in the *S. enteritidis* serotype. The outcomes of disseminating the RAPD-linked configurations among *Salmonella* strains are itemized in Table 4.

4.3. Results of Statistical Analysis

A significant relationship was found between the presence of the *fliC* gene and patterns related to it and the *S. typhimurium* serotype ($P < 0.05$). Similarly, a significant relationship was observed between the presence of the *sefA* gene and patterns related to it and *S. enteritidis* ($P < 0.05$). No other noteworthy correlations were observed between a specific cluster and a particular virulence gene. A specific RAPD cluster did not exhibit any significant

Table 3 . Virulence Gene Profiles of the *Salmonella* Serotypes

The Pattern of Resistance Genes	Number of Isolates				
	S. enteritidis	*S. infantis*	*S. typhimurium*	*S. typhi*	Total (%)
hilA/invA/sdiA/iroB	1	2			3 (5)
hilA/invA/sdiA/iroB/pefA	2	3		1	5 (8.33)
hilA/invA/sdiA/iroB/sefA	1				1 (1.66)
hilA/invA/sdiA/iroB/pefA/spv	1				1 (1.66)
hilA/invA/sdiA/iroB/fliC/pefA			1		1 (1.66)
hilA/invA/sdiA/iroB/pefA/sefA	4				4 (6.66)
hilA/invA/sdiA/iroB/fliC/sopB			3		3 (5)
hilA/invA/sdiA/iroB/sefA/sopB	4				4 (6.66)
hilA/invA/sdiA/iroB/pefA/sopB	3	9			12 (20)
hilA/invA/sdiA/iroB/pefA/sefA/spv	1				1 (1.66)
hilA/invA/sdiA/iroB/pefA/sopB/spv	1				1 (1.66)
hilA/invA/sdiA/iroB/fliC/sefA/sopB			1		1 (1.66)
hilA/invA/sdiA/iroB/fliC/pefA/sopB			2		2 (3.33)
hilA/invA/sdiA/iroB/fliC/pefA/sefA			2		2 (3.33)
hilA/invA/sdiA/iroB/pefA/sefA/sopB	13	2			15 (25)
hilA/invA/sdiA/iroB/fliC/pefA/sopB/spv			1		1 (1.66)
hilA/invA/sdiA/iroB/fliC/pefA/sefA/sopB/spv			2		2 (3.33)

correlation with virulence gene patterns. Statistically, the relationship between RAPD genotypes and *Salmonella* serotypes was significant ($P < 0.05$), while the relationship between these RAPD patterns and the source of isolates was not significant ($P > 0.05$). Furthermore, a significant relationship was found between R-1 and R-2 clusters and the *S. infantis* serotype ($P < 0.05$).

5. Discussion

A complicated interplay of different factors determines the virulence mechanism of *Salmonella*. These factors include the expression of various genes encoding proteins that enable the bacteria to colonize and invade host tissues, evade the host immune response, and cause damage to host cells (17). In this study, research was conducted on the presence of nine virulence genes in different serotypes of *Salmonella*, including the *invA* gene located in the *Salmonella* Pathogenicity Island-1, and the *sopB* gene, both of which code for the production of proteins from the type III secretion system, related to the invasion of *Salmonella* into eukaryotic host cells (18, 19). Additionally, the *Salmonella* plasmid virulence (*spv*) operon, which is important for the intracellular survival and replication of *Salmonella* and contributes to the systemic phase of the illness (20), was studied. Furthermore, the *sefA* and *pefA* genes related to fimbriae production (21), *hilA* and *sdiA* genes related to transcriptional regulation involved in the regulation of pathogenicity and quorum sensing (22), and the *iroB* gene that encodes an enzyme involved in the biosynthesis of salmochelins, siderophores that facilitate the acquisition of iron by the bacteria from the host (23) were analyzed.

One important virulence factor of *Salmonella* is its ability to produce a type III secretion system (T3SS), which is an injectisome that allows the bacteria to deliver effector proteins directly into host cells. These effector proteins, such as *invA*, manipulate host cell signaling pathways, promoting bacterial invasion and survival within host tissues (24). All studied *Salmonella* strains, regardless of their serotype, possessed the *invA*, *hilA*, *sdiA*, and *iroB* virulence genes. The presence of these genes in *S. enterica* serotypes makes them suitable candidates for determining and confirming this species. Although many other studies confirm the presence of these genes in *Salmonella* serotypes (25-27), it should be noted that some of these serotypes are considered non-pathogenic to humans, even though they carry pathogenicity islands and their important virulence genes.

Understanding the function and regulation of these genes is key to developing effective strategies for preventing and treating *Salmonella* infections. In

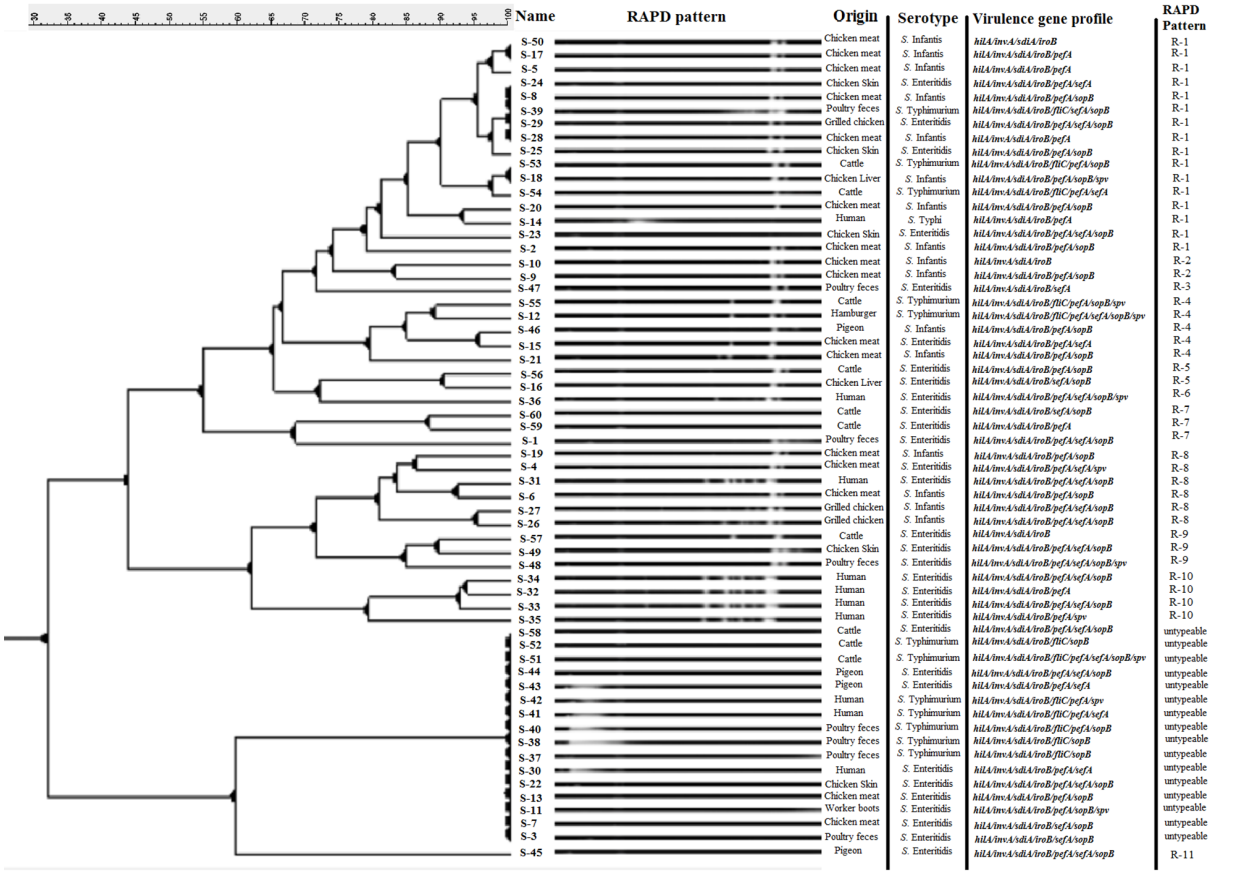

Figure 1. Dendrogram based on RAPD-PCR fingerprinting of *Salmonella* serotypes, in association with virulence gene profile, using the UPGMA analysis. RAPD-PCR assay resulted in 11 different clusters.

the case of *fliC* and *sefA*, these genes are expected to be present only in *Typhimurium* and *Enteritidis* serotypes, respectively (28-30). However, the *sefA* gene was also identified in *Typhimurium* isolates, and remarkably, the *sefA* gene-specific band with specific primers was observed in five *S. typhimurium* and two *S. infantis* isolates. According to the results, the most prevalent virulence gene profile was significantly associated with the *S. enteritidis* serotype ($P < 0.05$).

RAPD-PCR has been extensively used to study the epidemiology and taxonomy of Enterobacteriaceae, including important human pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella* spp. It has also been employed to investigate the genetic diversity of these bacteria in various environmental niches, such as soil, water, and food (31-33). The results of genotyping isolates using RAPD-PCR showed that, although when calculating the performance of the genotyping method using the 1254 primer, Simpson's index shows a relatively respectable result, there was a significant relationship between RAPD genotype and *Salmonella* serotype, and 16 isolates (26.66%) could not be successfully genotyped using this method, which can be significant. Notably, this lack of success is more significant in the case of *S. typhimurium* serotypes, where 7 out of 12 isolates (58.33%) were identified as untypeable. The most prevalent RAPD genotype, R-1, was found in all four serotypes, but there was no significant relationship between the source of isolates and the RAPD genotype. According to the results, *S. enteritidis* serotypes exhibited higher genotypic diversity (10 genotypes) than other serotypes, even though 29.03% (9 out of 31) isolates of this serotype were untypeable.

5.1. Conclusions

In conclusion, the *invA*, *sdiA*, *hilA*, and *iroB* virulence genes were present in all *Salmonella* isolates, while *pefA* and *sopB* genes were also prevalent. A total of 17 different virulence gene patterns were identified. RAPD-PCR

Table 4. Distribution of RAPD-PCR Genotyping Patterns Among *Salmonella* Serotypes

Source	No	RAPD-PCR Pattern											
		R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8	R-9	R-10	R-11	Untypeable
Chicken meat	16												
S. enteritidis	4				1			1	1				1
S. infantis	12	7	2		1				2				
Poultry feces	8												
S. enteritidis	4			1						1			2
S. typhimurium	4	1											3
Chicken skin	5												
S. enteritidis	5	3								1			1
Chicken Liver	2												
S. enteritidis	1					1							
S. infantis	1	1											
Grilled chicken	3												
S. enteritidis	1	1											
S. infantis	2								2				
Human	10												
S. enteritidis	7						1		1		4		1
S. typhimurium	2												2
S. typhi	1	1											
Cattle	10												
S. enteritidis	5					1		2		1			1
S. typhimurium	5	2			1								2
Pigeon	4												
S. enteritidis	3											1	2
S. infantis	1				1								
Hamburger	1												
S. typhimurium	1				1								
Worker boots swab	1												
S. enteritidis	1												1
Total (%)	60	16 (26.66)	2 (3.33)	1 (1.66)	5 (8.33)	2 (3.33)	1 (1.66)	3 (5)	6 (10)	3 (5)	4 (6.66)	1 (1.66)	16 (26.66)

fingerprinting identified 11 distinct clusters. The study also found a significant correlation between *fliC* and *sefA* genes in *S. typhimurium* and *S. enteritidis* serotypes, respectively. Moreover, a significant relationship was found between RAPD genotypes and *Salmonella* serotypes.

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Footnotes

Authors' Contribution: Study design: H.K., R.K., and F.P.G.; data acquisition: S.M.H., and R.K.; data evaluation and preparation of the manuscript: H.K., and R.K.; data assessment: R.K., and F.P.G.; All authors read and approved the final manuscript.

Conflict of Interests: The authors declare no conflict of interests.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: This study was approved by the Research Ethics Committee of Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran (Code of Ethics: IR.IAU.AMOL.REC.1402.092).

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References

1. Cao ZZ, Xu JW, Gao M, Li XS, Zhai YJ, Yu K, et al. Prevalence and antimicrobial resistance of Salmonellaisolates from goose farms in Northeast China. *Iran J Vet Res*. 2020;**21**(4):287–93. [PubMed ID: 33584841]. [PubMed Central ID: PMC7871741].
2. Khan SA, Imtiaz MA, Sayeed MA, Shaikat AH, Hassan MM. Antimicrobial resistance pattern in domestic animal - wildlife - environmental niche via the food chain to humans with a Bangladesh

perspective; a systematic review. *BMC Vet Res*. 2020;**16**(1):302. [PubMed ID: 32838793]. [PubMed Central ID: PMC7445918]. https://doi.org/10.1186/s12917-020-02519-9.
3. Wang M, Qazi IH, Wang L, Zhou G, Han H. Salmonella Virulence and Immune Escape. *Microorganisms*. 2020;**8**(3). [PubMed ID: 32183199]. [PubMed Central ID: PMC7143636]. https://doi.org/10.3390/microorganisms8030407.
4. Sholpan A, Lamas A, Cepeda A, Franco CM. Salmonella spp. quorum sensing: an overview from environmental persistence to host cell invasion. *AIMS Microbiol*. 2021;**7**(2):238–56. [PubMed ID: 34250377]. [PubMed Central ID: PMC8255907]. https://doi.org/10.3934/microbiol.2021015.
5. Li Q, Yin J, Li Z, Li Z, Du Y, Guo W, et al. Serotype distribution, antimicrobial susceptibility, antimicrobial resistance genes and virulence genes of Salmonella isolated from a pig slaughterhouse in Yangzhou, China. *AMB Express*. 2019;**9**(1):210. [PubMed ID: 31884559]. [PubMed Central ID: PMC6935380]. https://doi.org/10.1186/s13568-019-0936-9.
6. Guibourdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemuhl J, Grimont PA, et al. Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. *Res Microbiol*. 2010;**161**(1):26–9. [PubMed ID: 19840847]. https://doi.org/10.1016/j.resmic.2009.10.002.
7. Wang YP, Li L, Shen JZ, Yang FJ, Wu YN. Quinolone-resistance in Salmonella is associated with decreased mRNA expression of virulence genes invA and avrA, growth and intracellular invasion and survival. *Vet Microbiol*. 2009;**133**(4):328–34. [PubMed ID: 18762392]. https://doi.org/10.1016/j.vetmic.2008.07.012.
8. Luna-Pineda VM, Ochoa SA, Cruz-Cordova A, Cazares-Dominguez V, Reyes-Grajeda JP, Flores-Oropeza MA, et al. Features of urinary Escherichia coli isolated from children with complicated and uncomplicated urinary tract infections in Mexico. *PLoS One*. 2018;**13**(10). e0204934. [PubMed ID: 30286185]. [PubMed Central ID: PMC6171886]. https://doi.org/10.1371/journal.pone.0204934.
9. Malorny B, Hoorfar J, Bunge C, Helmuth R. Multicenter validation of the analytical accuracy of Salmonella PCR: towards an international standard. *Appl Environ Microbiol*. 2003;**69**(1):290–6. [PubMed ID: 12514007]. [PubMed Central ID: PMC152403]. https://doi.org/10.1128/AEM.69.1.290-296.2003.
10. Pan TM, Liu YJ. Identification of Salmonella enteritidis isolates by polymerase chain reaction and multiplex polymerase chain reaction. *J Microbiol Immunol Infect*. 2002;**35**(3):147–51. [PubMed ID: 12380786].
11. Skyberg JA, Logue CM, Nolan LK. Virulence genotyping of Salmonella spp. with multiplex PCR. *Avian Dis*. 2006;**50**(1):77–81. [PubMed ID: 16617986]. https://doi.org/10.1637/7417.1.
12. Halatsi K, Oikonomou I, Lambiri M, Mandilara G, Vatopoulos A, Kyriacou A. PCR detection of Salmonella spp. using primers targeting the quorum sensing gene sdiA. *FEMS Microbiol Lett*. 2006;**259**(2):201–7. [PubMed ID: 16734780]. https://doi.org/10.1111/j.1574-6968.2006.00266.x.
13. Cortez AL, Carvalho AC, Ikuno AA, Burger KP, Vidal-Martins AM. Identification of Salmonella spp. isolates from chicken abattoirs by multiplex-PCR. *Res Vet Sci*. 2006;**81**(3):340–4. [PubMed ID: 16701750]. https://doi.org/10.1016/j.rvsc.2006.03.006.
14. Thung TY, Radu S, Mahyudin NA, Rukayadi Y, Zakaria Z, Mazlan N, et al. Prevalence, virulence genes and antimicrobial resistance profiles of Salmonella serovars from retail beef in Selangor, Malaysia. *Front Microbiol*. 2017;**8**:2697. [PubMed ID: 29379488]. [PubMed Central ID: PMC5770799]. https://doi.org/10.3389/fmicb.2017.02697.
15. Aslam M, Greer GG, Nattress FM, Gill CO, McMullen LM. Genetic diversity of Escherichia coli recovered from the oral cavity of beef cattle and their relatedness to faecal E. coli. *Lett Appl Microbiol*. 2004;**39**(6):523–7. [PubMed ID: 15548305]. https://doi.org/10.1111/j.1472-765X.2004.01620.x.
16. Khakabimamaghani S, Najafi A, Ranjbar R, Raam M. GelClust: a software tool for gel electrophoresis images analysis and dendrogram generation. *Comput Methods Programs Biomed*. 2013;**111**(2):512–8. [PubMed ID: 23727299]. https://doi.org/10.1016/j.cmpb.2013.04.013.
17. Stevens MP, Kingsley RA. Salmonella pathogenesis and host-adaptation in farmed animals. *Curr Opin Microbiol*. 2021;**63**:52–8. [PubMed ID: 34175673]. https://doi.org/10.1016/j.mib.2021.05.013.
18. Valdez Y, Ferreira RB, Finlay BB. Molecular mechanisms of Salmonella virulence and host resistance. *Curr Top Microbiol Immunol*. 2009;**337**:93–127. [PubMed ID: 19812981]. https://doi.org/10.1007/978-3-642-01846-6_4.
19. Zhao S, Xu Q, Cui Y, Yao S, Jin S, Zhang Q, et al. Salmonella effector SopB reorganizes cytoskeletal vimentin to maintain replication vacuoles for efficient infection. *Nat Commun*. 2023;**14**(1):478. [PubMed ID: 36717589]. [PubMed Central ID: PMC9885066]. https://doi.org/10.1038/s41467-023-36123-w.
20. Fluit AC. Towards more virulent and antibiotic-resistant Salmonella? *FEMS Immunol Med Microbiol*. 2005;**43**(1):1–11. [PubMed ID: 15607630]. https://doi.org/10.1016/j.femsim.2004.10.007.
21. Webber B, Borges KA, Furian TQ, Rizzo NN, Tondo EC, Santos LRD, et al. Detection of virulence genes in Salmonella Heidelberg isolated from chicken carcasses. *Rev Inst Med Trop Sao Paulo*. 2019;**61**. e36. [PubMed ID: 31340248]. [PubMed Central ID: PMC6648003]. https://doi.org/10.1590/S1678-9946201961036.
22. Zhang X, Liu B, Ding X, Bin P, Yang Y, Zhu G. Regulatory Mechanisms between Quorum Sensing and Virulence in Salmonella. *Microorganisms*. 2022;**10**(11). [PubMed ID: 36363803]. [PubMed Central ID: PMC9693372]. https://doi.org/10.3390/microorganisms10112211.
23. 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). [PubMed ID: 31405078]. [PubMed Central ID: PMC6789496]. https://doi.org/10.3390/pathogens8030124.
24. Naseer N, Egan MS, Reyes Ruiz VM, Scott WP, Hunter EN, Demissie T, et al. Human NAIP/NLRC4 and NLRP3 inflammasomes detect Salmonella type III secretion system activities to restrict intracellular bacterial replication. *PLoS Pathog*. 2022;**18**(1). e1009718. [PubMed ID: 35073381]. [PubMed Central ID: PMC8812861]. https://doi.org/10.1371/journal.ppat.1009718.
25. Siddiky NA, Sarker MS, Khan MSR, Begum R, Kabir ME, Karim MR, et al. Virulence and Antimicrobial Resistance Profiles of Salmonella enterica Serovars Isolated from Chicken at Wet Markets in Dhaka, Bangladesh. *Microorganisms*. 2021;**9**(5). [PubMed ID: 33924919]. [PubMed Central ID: PMC8145576]. https://doi.org/10.3390/microorganisms9050952.
26. Akhter S, Jung JH, Munkhbileg B, Jeong JH, Islam J, Rahman MM, et al. Detection of Salmonella genes in stool samples of children aged 5 years and younger in urban and rural areas of Bangladesh. *J Infect Dev Ctries*. 2021;**15**(4):506–15. [PubMed ID: 33956650]. https://doi.org/10.3855/jidc.12621.
27. Khaltabadi Farahani R, Ehsani P, Ebrahimi-Rad M, Khaledi A. Molecular detection, virulence genes, biofilm formation, and antibiotic resistance of Salmonella enterica serotype enteritidis isolated from poultry and clinical samples. *Jundishapur J Microbiol*. 2018;**11**(10). https://doi.org/10.5812/jjm.69504.
28. Xu J, Zhang P, Zhuang L, Zhang D, Qi K, Dou X, et al. Multiplex polymerase chain reaction to detect Salmonella serovars Indiana, Enteritidis, and Typhimurium in raw meat. *J Food Saf*. 2019;**39**(5). https://doi.org/10.1111/jfs.12674.
29. Alzwghaibi AB, Yahyaraeyat R, Fasaei BN, Langeroudi AG, Salehi TZ. Rapid molecular identification and differentiation of common Salmonella serovars isolated from poultry, domestic animals and foodstuff using multiplex PCR assay. *Arch Microbiol*. 2018;**200**(7):1009–16. [PubMed ID: 29627903]. https://doi.org/10.1007/s00203-018-1501-7.
30. Ogunremi D, Nadin-Davis S, Dupras AA, Márquez IG, Omidi K, Pope L, et al. Evaluation of a Multiplex PCR Assay for the Identification of

Salmonella Serovars Enteritidis and Typhimurium Using Retail and Abattoir Samples. *Journal of Food Protection*. 2017;**80**(2):295–301. https://doi.org/10.4315/0362-028x.Jfp-16-167.

31. Lozano-Villegas K, Rodriguez-Hernandez R, Rondon-Barragan I. Effectiveness of six molecular typing methods as epidemiological tools for the study of Salmonella isolates in two Colombian regions. *Vet World*. 2019;**12**(12):1998–2006. [PubMed ID: 32095053]. [PubMed Central ID: PMC6989315]. https://doi.org/10.14202/vetworld.2019.1998-2006.
32. Fazel F, Jamshidi A, Khoramian B. Phenotypic and genotypic study on antimicrobial resistance patterns of E. coli isolates from bovine mastitis. *Microb Pathog*. 2019;**132**:355–61. [PubMed ID: 31096003]. https://doi.org/10.1016/j.micpath.2019.05.018.
33. Saadatian Farivar A, Nowroozi J, Eslami G, Sabokbar A. RAPD PCR profile, antibiotic resistance, prevalence of armA Gene, and detection of KPC enzyme in Klebsiella pneumoniae isolates. *Can J Infect Dis Med Microbiol*. 2018;**2018**:6183162. [PubMed ID: 29623139]. [PubMed Central ID: PMC5829425]. https://doi.org/10.1155/2018/6183162.