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Antibiotic resistance patterns, characteristics of virulence and resistance genes and genotypic analysis of *Salmonella* serotypes recovered from different sources

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Abstract

The present study evaluated the *Salmonella* isolates obtained from various origins in Iran. *Salmonella* strains previously recovered and stored in the veterinary microbiology laboratory were serotyped and subjected to antibiotic susceptibility test, detection of the virulence and resistance genes by polymerase chain reaction (PCR), and genotyping by enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR). All *Salmonella* isolates showed resistance to erythromycin and the most resistance rates were detected for trimethoprim (86.66%), ampicillin (75.00%), and sulfamethoxazole-trimethoprim (63.33%), respectively. In total, 86.33% of the isolates were known as multi-drug resistant and none of the isolates showed resistance to cefepime, nalidixic acid, imipenem, ceftriaxone, and polymyxin B. The virulence genes, *invA*, *sdiA*, and *hilA* besides the *tetA* resistance gene were identified in all 60 *Salmonella* strains. The most prevalent resistance genes were respectively *tetC* (70.00%), *sul2* (58.33%), and *ereA* (55.00%). Statistical analysis revealed a significant difference between *Salmonella* serotypes associated with the *sul1* resistance gene. In ERIC-PCR analysis, 14 distinct clusters were obtained. Statistically, there were significant relationships between the source and ERIC's genomic pattern and between the serotype of *Salmonella* isolates and genotypic pattern of ERIC. According to the results, *Salmonella* serotypes from non-human sources had considerable resistance to different antibiotics and carried significant virulence determinants and resistance genes. In addition, ERIC-PCR showed relevant results in discriminating *Salmonella* serotypes from other sources.

Introduction

Salmonella serotypes, Gram-negative rod-shaped bacteria of the *Enterobacteriaceae* family, carry resistance genes and virulence factors, leading to the durability and progression of these foodborne pathogens inside of their various hosts' bodies.[1] Infection caused by *Salmonella* spp. in humans and animals can be seen in different forms; but, the biggest impact of this bacterium is on the gastro-intestinal tract of humans and animals. Today, in many developed countries, salmonellosis is at the top of the list of infectious diseases.[2] *Salmonella* infections depend on virulence factors of the strain and the immunity of the host and *Salmonella* strains have the capacity to encounter diverse conditions in different organs when they commence an infection in their hosts. Furthermore, they possess the capability to exchange a variety of virulence and resistance genes, along with pathogenicity traits, being intricately linked to the genetic characteristics, including resistance and virulence plasmids.[3,4] Some antibiotics such as, sulfonamides, aminoglycosides, fluoro-quinolones, and β-lactams are the main choices for *Salmonella* infections in humans and animals and persistent utilization of these antibiotics in the farm animal has contributed to the emergence of resistant *Salmonella* strains.[5,6] Due to the wide distribution and high diversity of *Salmonella* serotypes in different regions of the world, it is necessary and inevitable to continuously and cautiously investigate the resistance and virulence characteristics of these strains and compare the other potential sources of *Salmonella* infections. For these reasons, the present study was conducted and *Salmonella* serotypes from various sources were compared for virulence properties, antibiotic resistance, their resistance gene contents, genotyping, and

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phylogenetic groups. Finally, statistical evaluation of the results was done to discriminate correspondences and varieties between the serotypes and sources.

Materials and Methods

***Salmonella* isolates, serotyping and DNA preparation.** In the current study, 60 *Salmonella* isolates were obtained from 10 different sources. Culture and isolation of *Salmonella* isolates were done during the years 2021 and 2022 in Mazandaran province, Iran, based on enrichment methods in Selenite F medium and cultivation on MacConkey and *Salmonella-Shigella* agar specific mediums (Oxoid Ltd., Basingstoke, UK).[7] The *Salmonella* isolates were serotyped at the *Salmonella* Research Center in Tehran University's Faculty of Veterinary Medicine, Tehran, Iran. The serotyping of the *Salmonella* strains was carried out using commercial anti-sera (Difco, Detroit, USA). Then, the results obtained were interpreted in accordance with the Kaufmann-White scheme, as described by Guibourdenche *et al*.[8] The extraction of DNA from *Salmonella* isolates was carried out utilizing a DNA extraction kit being specific for Gram-negative bacteria (Sinaclon, Tehran, Iran). Extracted DNA was preserved at a temperature of – 20.00 ˚C for sub-sequent experiments conducted as parts of the study. The strain of *Salmonella typhimurium* (ATCC 14028) was utilized as the reference strain for conducting antibiotic susceptibility tests and other bacteriological investigations.

Antibiotic susceptibility assay. The susceptibility of the strains to anti-microbials was tested by the Kirby-Bauer disc diffusion procedure, according to the processes established by the Clinical and Laboratory Standards Institute.[9] The antibiotic disks utilized in the study were provided by PadtanTeb (Tehran, Iran). Each disk contained a specific concentration of the respective antibiotic as follows: Tetracycline at 30.00 µg, erythromycin at 15.00 µg, trimethoprim at 5.00 µg, gentamicin at 10.00 µg, ciprofloxacin at 5.00 µg, cefoxitin at 30.00 µg, ceftriaxone at 30.00 µg, cefepime at 30.00 µg, imipenem at 10.00 µg, sulfamethoxazole-trimethoprim at 1.25/23.75 µg, nalidixic acid at 30.00 µg, ampicillin at 10.00 µg, florfenicol at 30.00 µg, sulfonamide at 300 µg, and polymyxin B at 50.00 U. The Muller Hinton agar plates (HiMedia, Thane, India) were subjected to incubation at 37.00 ˚C for 24 hr. Subsequently, the measurement of inhibition zones' diameter was carried out and a comparison was made with the standard tables provided by the Clinical and Laboratory Standards Institute. Ultimately, the term multi-drug resistant (MDR) was defined as the state of being resistant to three or more classes of antibiotics.[10]

Molecular detection of the resistance genes. The *Salmonella* isolates were surveyed for the existence of 13 resistance genes, encompassed the *aac* gene associated with gentamycin resistance, $bla_{CTX\text{-}M}$, bla_{TEM}, bla_{VEB}, and bla_{SHV} associated with β-lactamases, *tetA*, *tetB*, and *tetC* associated with tetracycline, *Sul1* and *Sul2* associated with sulfonamides, *dfrA* linked to trimethoprim, *ereA* associated with erythromycin, and *qnrA* linked to quinolones. The polymerase chain reaction (PCR) reaction was conducted in 25.00 µL final volume. Each reaction included 12.50 µL of the PCR Master Mix (Sinaclon), 1.00 µL (0.50 µM) of the forward and reverse primers (Table 1), and 2.00 µL of template DNA. The PCR product was approved by agarose gel electrophoresis with 1.50% concentration, using a 100 bp marker (Sinaclon).

Detection of virulence determinants. The presence of three virulence-related genes was examined in *Salmonella* serotypes. The *hilA* gene, with the forward: 5`-CGTGAAGGGATTATCGCAGT-3` and the reverse: 5`- GT CCGGGAATACATCTGAGC-3` primers,[21] *sdiA* gene, with the forward: 5`-AATATCGCTTCGTACCAC-3` and the reverse: 5`-GTAGGTAAACGAGGAGCAG-3` primers,[22] and *invA* gene, with forward: 5`-ACAGTGCTCGTTTACGA CCTGAAT-3` and reverse: 5`-AGACGACTGGTACTGATC TAT-3` primers[23] with respectively 296, 274, and 244 bp PCR product length was subjected to PCR. The PCR reactions were conducted within a 25.00 µL final volume, encompassing 12.50 µL of a PCR Master Mix, 1.00 µL (0.50 µM) of each primer, and 2.00 µL of the template DNA. The resulting PCR product was assessed and validated through agarose gel electrophoresis in a 1.50% concentration, with the guidance of a 100 bp DNA ladder sourced from Sinaclon.

Genotyping by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). The ERIC-PCR was done in an ultimate volume of 25.00 µL comprising 1.50 µL of each primer with 2.00 pmol μL^{-1} concentration, 12.50 µL of Master Mix (Sinaclon), and 8.50 µL of deionized distilled water. Primer ERIC-1 with the sequence of 5`-ATGTAAGCTCCTGGGGATTCAC-3` and primer ERIC-2 with the sequence of 5`-AAGTAAGTGACTGGGGTGAGCG-3` were used in the ERIC reaction as formerly designated.[24] The ERIC-PCR images were loaded into the GelClust software for exploration.[25] Resemblance of the genetic patterns was computed through utilization of the Pearson correlation, wherein a 2.00% optimization tolerance and a 4.00% position tolerance shift were defined. Additionally, the dendrogram was established by employing the Dice correlation factor, alongside the implementation of the un-weighted pair group method with arithmetic averages.

Statistical analysis. The data obtained from this investigation were subjected to analysis employing the SPSS Software (version 23.0; IBM Corp., Armonk, USA). The statistical examinations were performed utilizing the Mann-Whitney, Chi-square, and Kolmogorov-Smirnov procedures, employing a *p*-value of less than 0.05 to determine statistical significance.

Table 1. Sequences used as primers in polymerase chain reaction for identification of resistance genes among *Salmonella* serovars.

Target genes	Sequence (5' to 3')	Annealing temperature (˚C)	Product size (bp)	References
aac(3)	F: CTTCAGGATGGCAAGTTGGT R: TCATCTCGTTCTCCGCTCAT	55	286	11
bla$_{CTX-M}$	F: CATGTGCAGYACCAGTAA R: CCGCRATATCRTTGGTGGTG	42	544	12
bla$_{TEM}$	F: ATGAGTATTCAACATTTCCG R: CCAATGCTTAATCAGTGAGG	46	850	13
bla$_{VEB}$	F: CGACTTCCATTTCCCGATGC R: GGACTCTGCAACAAATACGC	51	643	14
Bla$_{SHV}$	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	52	768	14
tetA	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	50	210	15
tetB	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	50	659	16
tetC	F: CCTCTTGCGGGATATCGTCC R: GGTTGAAGGCTCTCAAGGGC	55	505	17
Sul1	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	47	822	18
Sul2	F: CGGCATCGTCAACATAACC R: GTGTGCGGATGAAGTCAG	51	720	18
dfrA	F: GGAGTGCCAAAGGTGAACAGC R:GAGGCGAAGTCTTGGGTAAAAAC	45	367	19
ereA	F: GCCGGTGCTCATGAACTTGAG R: CGACTCTATTCGATCAGAGGC	52	419	11
qnrA	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	50	516	20

Results

Antibiotic resistance. All *Salmonella* isolates were resistant to erythromycin. This is followed by trimethoprim (86.66%), ampicillin (75.00%), and sulfamethoxazole-trimethoprim (63.33%). None of the isolates showed resistance to cefepime, nalidixic acid, imipenem, ceftriaxone, and polymyxin B. Detailed results of the anti-biotic resistance of the *Salmonella* serotypes are listed in Table 2. A total of 83.33% (50/60) of *Salmonella* isolates were identified as MDR strains. Statistical analysis revealed a significant association between the source of *Salmonella* serotypes and antibiotic resistance against trimethoprim ($p < 0.05$). No significant association was identified among the *Salmonella* serotypes or ERIC genotypic pattern and resistance to an antibiotic ($p > 0.05$).

Detection of resistance and virulence factors. Three virulence-associated genes, *invA*, *sdiA*, and *hilA* were detected in all 60 *Salmonella* isolates. The *tetA* resistance-associated gene was also present in all isolates (100%). After that, among 60 *Salmonella* strains, the most prevalent resistance genes were *tetC* (70.00%), *sul2* (58.33%), and *ereA* (55.00%), respectively. The *tetB*, *blaVEB*, and *blaSHV* genes were not detected in any of the isolates. Table 3 shows the details of the presence of resistance genes in *Salmonella* isolates. A total of 48 resistance gene patterns were detected among 60 *Salmonella* isolates. The most prevalent pattern was *sul2*/*dfrA*/*ereA*/*tetA*/*tetC* (Table 4). Statistical analysis revealed a significant difference between *Salmonella* serotypes associated with the *sul1* resistance gene ($p < 0.05$). *Salmonella* Enteritidis serotype showed more presence of the gene. There was not significant association among the sources of isolates and occurrence of resistance genes.

Enterobacterial repetitive intergenic consensus polymerase chain reaction. The findings of the ERIC-PCR are presented in Figure 1 as a dendrogram linked to the origin and patterns of antibiotic resistance. Overall, the analysis of ERIC-PCR outcomes using GelClust Software yielded a total of 14 distinct clusters named C-1 to C-14 (Simpson's Index of Diversity = 0.07514; Table 5). Statistically, there was a significant relationship between the source and ERIC's genomic pattern ($p < 0.05$). Furthermore, a significant correlation was observed among the serotypes of *Salmonella* isolates and the genotypic pattern of ERIC ($p < 0.05$). The C-5 cluster was specific for the *S. enteritidis* serotype (Fig. 1). There was no significant relationship between ERIC-PCR clusters and antibiotic resistance or the presence of resistance genes ($p > 0.05$).

Table 2. Number of *Salmonella* isolates (%) resistant to different antibiotics.

Sources	No.	ERY	TMP	AMP	SXT	TET	FFC	SSS	GEN	CIP	FOX	FEP	NA	IPM	CRO	PB
Chicken meat	16	16 (100)	15 (93.75)	12 (75.00)	10 (62.50)	10 (62.50)	9 (56.25)	6 (37.50)	2 (12.50)	1 (6.25)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	4	4	3	2	3	2	2	2	0	0	0	0	0	0	0	0
S. Infantis	12	12	12	10	7	8	7	4	2	1	0	0	0	0	0	0
Poultry feces	8	8 (100)	7 (87.50)	8 (100)	5 (62.50)	5 (62.50)	3 (37.50)	4 (50.00)	2 (25.00)	1 (12.50)	1 (12.50)	1 (12.50)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	4	4	4	4	2	3	1	2	0	1	1	1	0	0	0	0
S. Typhimurium	4	4	3	4	3	2	2	2	2	0	0	0	0	0	0	0
Chicken Skin	5	5 (100)	5 (100)	4 (80.00)	2 (40.00)	3 (60.00)	0 (0.00)	2 (40.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	5	5	5	4	2	3	0	2	0	0	0	0	0	0	0	0
Chicken Liver	2	2 (100)	2 (100)	2 (100)	2 (100)	1 (50.00)	1 (50.00)	1 (50.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0
S. Infantis	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
Grilled chicken	3	3 (100)	2 (66.66)	2 (66.66)	2 (66.66)	0 (0.00)	2 (66.66)	0 (0.00)	1 (33.33)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
S. Infantis	2	2	1	1	1	0	2	0	1	0	0	0	0	0	0	0
Human	10	10 (100)	10 (100)	8 (80.00)	7 (70.00)	8 (80.00)	2 (20.00)	3 (30.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	7	7	7	5	4	6	1	2	0	0	0	1	0	0	0	0
S. Typhimurium	2	2	2	2	2	1	1	1	0	0	0	0	0	0	0	0
S. Typhi	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Cattle	10	10 (100)	7 (70.00)	6 (60.00)	6 (60.00)	4 (40.00)	3 (30.00)	3 (30.00)	3 (30.00)	2 (20.00)	1 (10.00)	1 (10.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	5	5	3	3	4	1	1	2	2	2	1	1	0	0	0	0
S. Typhimurium	5	5	4	3	2	3	2	1	1	0	0	0	0	0	0	0
Pigeon	4	4 (100)	4 (100)	1 (25.00)	3 (75.00)	2 (50.00)	2 (50.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (25.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	3	3	3	0	2	2	2	0	0	0	1	0	0	0	0	0
S. Infantis	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Hamburger	1	1 (100)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Typhimurium	1	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0
Worker boots swab	1	1 (100)	0 (0.00)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	1	1	0	1	1	1	1	1	0	1	0	0	0	0	0	0
Total (%)	60	60 (100)	52 (86.66)	45 (75.00)	38 (63.33)	34 (56.66)	24 (40.00)	20 (33.33)	8 (13.33)	5 (8.33)	4 (6.66)	3 (5.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

ERY: Erythromycin; TMP: Trimethoprim; AMP: Ampicillin; SXT: Sulfamethoxazole-trimethoprim; TET: Tetracycline; FFC: Florfenicol; SSS: Sulfonamide; GEN: Gentamicin; CIP: Ciprofloxacin; FOX: Cefoxitin; FEP: Cefepime; NA: Nalidixic acid; IPM: Imipenem; CRO: Ceftriaxone; PB: Polymyxin B.

Table 3. Distribution of resistance genes (%) among *Salmonella* serotypes.

Sources	No.	*Sul1*	*Sul2*	*qnr*	*dfrA*	*ereA*	*aac*	*tetA*	*tetB*	*tetC*	bla_{TEM}	bla_{VEB}	bla_{CTX-M}	bla_{SHV}
Chicken meat	16	3 (18.75)	9 (56.25)	1 (6.25)	7 (43.75)	10 (62.50)	2 (12.50)	16 (100)	0 (0.00)	12 (75.00)	9 (56.25)	0 (0.00)	5 (31.25)	0 (0.00)
S. Enteritidis	4	1	3	0	3	4	0	4	0	3	2	0	1	0
S. Infantis	12	2	6	1	4	6	2	12	0	9	7	0	4	0
Poultry feces	8	3 (37.50)	4 (50.00)	2 (25.00)	2 (25.00)	2 (25.00)	4 (50.00)	8 (100)	0 (0.00)	5 (62.50)	5 (62.50)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	4	3	2	2	0	1	2	4	0	2	2	0	0	0
S. Typhimurium	4	0	2	0	2	1	2	4	0	3	3	0	0	0
Chicken Skin	5	3 (60.00)	2 (40.00)	0 (0.00)	2 (40.00)	4 (80.00)	1 (20.00)	5 (100)	0 (0.00)	3 (60.00)	2 (40.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	5	3	2	0	2	4	1	5	0	3	2	0	0	0
Chicken Liver	2	0 (0.00)	2 (100)	1 (50.00)	1 (50.00)	2 (100)	0 (0.00)	2 (100)	0 (0.00)	1 (50.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	1	0	1	0	1	1	0	1	0	1	0	0	0	0
S. Infantis	1	0	1	1	0	1	0	1	0	0	0	0	0	0
Grilled chicken	3	1 (33.33)	2 (66.66)	0 (0.00)	1 (33.33)	1 (33.33)	1 (33.33)	3 (100)	0 (0.00)	2 (66.66)	2 (66.66)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	1	1	1	0	1	1	0	1	0	1	0	0	0	0
S. Infantis	2	0	1	0	0	0	1	2	0	1	2	0	0	0
Human	10	4 (40.00)	7 (70.00)	1 (10.00)	5 (50.00)	5 (50.00)	0 (0.00)	10 (100)	0 (0.00)	7 (70.00)	4 (40.00)	0 (0.00)	1 (10.00)	0 (0.00)
S. Enteritidis	7	3	4	1	3	3	0	7	0	5	4	0	0	0
S. Typhimurium	2	1	2	0	1	2	0	2	0	1	0	0	1	0
S. Typhi	1	0	1	0	1	0	0	1	0	1	0	0	0	0
Cattle	10	0 (0.00)	5 (50.00)	2 (20.00)	5 (50.00)	6 (60.00)	3 (30.00)	10 (100)	0 (0.00)	7 (70.00)	5 (50.00)	0 (0.00)	4 (40.00)	0 (0.00)
S. Enteritidis	5	0	2	2	3	3	2	5	0	5	3	0	2	0
S. Typhimurium	5	0	3	0	2	3	1	5	0	2	2	0	2	0
Pigeon	4	1 (25.00)	3 (75.00)	0 (0.00)	3 (75.00)	2 (50.00)	0 (0.00)	4 (100)	0 (0.00)	3 (75.00)	2 (50.00)	0 (0.00)	1 (25.00)	0 (0.00)
S. Enteritidis	3	1	2	0	2	1	0	3	0	3	1	0	1	0
S. Infantis	1	0	1	0	1	1	0	1	0	0	1	0	0	0
Hamburger	1	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	1 (100)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
S. Typhimurium	1	0	0	0	0	1	0	1	0	1	0	0	0	0
Worker boots swab	1	1 (100)	1 (100)	1 (100)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)	1 (100)	1 (100)	0 (0.00)	0 (0.00)	0 (0.00)
S. Enteritidis	1	1	1	1	0	0	0	1	0	1	1	0	0	0
Total (%)	60	16 (26.66)	35 (58.33)	8 (13.33)	26 (43.33)	33 (55.00)	11 (18.33)	60 (100)	0 (0.00)	42 (70.00)	30 (50.00)	0 (0.00)	11 (18.33)	0 (0.00)

Table 4. Number of isolates according to the resistance gene profiles of the *Salmonella* serotypes.

Pattern of resistance genes	*S.* Enteritidis	*S.* Infantis	*S.* Typhimurium	*S.* Typhi	Total
tetA/tetC	1				1
ereA/tetA		1			1
***tetA/*bla_{TEM}**		1			1
aac/tetA/tetC		1			1
sul2/tetA/tetC	2		1		3
ereA/tetA/tetC	1		1		2
***tetA/tetC/*bla_{TEM}**		1			1
***ereA/tetA/*bla_{TEM}**			1		1
sul1/sul2/aac/tetA	1				1
sul2/qnr/tetA/tetC		1			1
sul2/qnr/ereA/tetA		1			1
sul2/dfrA/tetA/tetC				1	1
sul1/sul2/dfrA/tetA	1				1
dfrA/ereA/tetA/tetC	1				1
sul2/dfrA/ereA/tetA			1		1
***aac/tetA/tetC/*bla_{TEM}**			1		1
***sul1/tetA/tetC/*bla_{TEM}**	1				1
***sul1/ereA/tetA/*bla_{TEM}**	1				1

Table 4 (continued). Distribution of resistance genes (%) among *Salmonella* serotypes.

ereA/aac/tetA/bla$_{TEM}$				1										1
sul2/dfrA/tetA/bla$_{TEM}$				1					1					2
sul2/tetA/tetC/bla$_{TEM}$				1										1
ereA/tetA/tetC/bla$_{TEM}$				1					1					2
sul2/dfrA/tetA/bla$_{CTX-M}$									1					1
sul2/qnr/ereA/tetA/tetC				1										1
sul2/dfrA/aac/tetA/tetC									1					1
sul1/sul2/dfrA/tetA/tetC				2										2
tetA/tetC/bla$_{TEM}$/bla$_{CTX-M}$				1										1
sul2/dfrA/ereA/tetA/tetC				2		1			1					4
sul1/qnr/aac/tetA/bla$_{TEM}$				1										1
sul2/ereA/tetA/tetC/bla$_{TEM}$				1										1
sul1/ereA/tetA/tetC/bla$_{TEM}$						1								1
sul2/dfrA/ereA/tetA/bla$_{TEM}$						1								1
qnr/dfrA/tetA/tetC/bla$_{CTX-M}$				1										1
sul1/sul2/ereA/tetA/bla$_{CTX-M}$									1					1
sul2/tetA/tetC/bla$_{TEM}$/bla$_{CTX-M}$						1								1
sul1/sul2/dfrA/ereA/tetA/tetC				3										3
ereA/tetA/tetC/bla$_{TEM}$/bla$_{CTX-M}$						1								1
sul1/sul2/qnr/tetA/tetC/bla$_{TEM}$				1										1
qnr/ereA/aac/tetA/tetC/bla$_{TEM}$				1										1
sul1/qnr/ereA/tetA/tetC/bla$_{TEM}$				1										1
dfrA/ereA/aac/tetA/tetC/bla$_{TEM}$				1										1
sul2/dfrA/ereA/tetA/tetC/bla$_{TEM}$						2								2
sul2/dfrA/ereA/tetA/tetC/bla$_{CTX-M}$				1										2
sul1/dfrA/aac/tetA/bla$_{TEM}$/bla$_{CTX-M}$						1								1
ereA/aac/tetA/tetC/bla$_{TEM}$/bla$_{CTX-M}$									1					1
sul2/dfrA/ereA/tetA/bla$_{TEM}$/bla$_{CTX-M}$				1										1
sul1/sul2/dfrA/ereA/tetA/tetC/bla$_{TEM}$				1										1

Table 5. Distribution of ERIC-PCR genotyping patterns among *Salmonella* serotypes.

Sources	No.	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14
Chicken meat	16														
S. Enteritidis	4					2			1					1	
S. Infantis	12	1		1	1			4	4				1		
Poultry feces	8														
S. Enteritidis	4	1			1		1				1				
S. Typhimurium	4				2								2		
Chicken skin	5														
S. Enteritidis	5					2				1	1				1
Chicken liver	2														
S. Enteritidis	1								1						
S. Infantis	1								1						
Grilled chicken	3														
S. Enteritidis	1					1									
S. Infantis	2								1	1					
Human	10														
S. Enteritidis	7		5					1					1		
S. Typhimurium	2											2			
S. Typhi	1													1	
Cattle	10														
S. Enteritidis	5			1		1		2					1		
S. Typhimurium	5	3									1		1		
Pigeon	4														
S. Enteritidis	3	2										1			
S. Infantis	1		1												
Hamburger	1														
S. Typhimurium	1						1								
Worker boots swab	1														
S. Enteritidis	1						1								
Total (%)	60	7(11.66)	6(10.00)	2(3.33)	4(6.66)	6(10.00)	3(5.00)	7(11.66)	8(13.33)	2(3.33)	3(5.00)	3(5.00)	6(10.00)	2(3.33)	1(1.66)

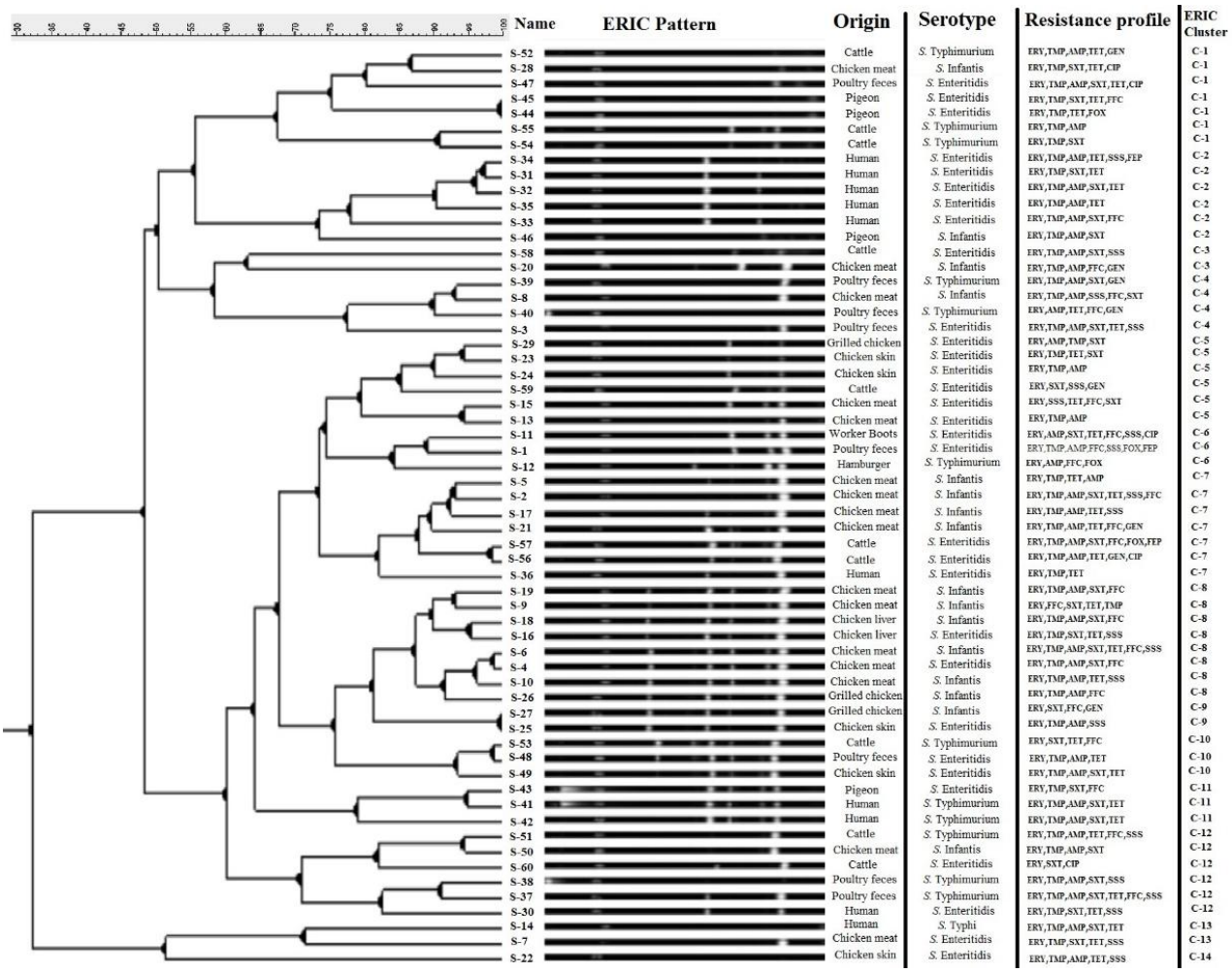

Fig. 1. Dendrogram based on enterobacterial repetitive intergenic consensus polymerase chain reaction fingerprinting of *Salmonella* serotypes using the un-weighted pair group method with arithmetic averages analysis, in association with resistance patterns and origin.

Discussion

The prevalence of *Salmonella* in different sources of this bacterium, especially food sources and poultry meat and eggs, is high in developing countries, and the increasing contact between humans and farm animals increases the spread of *Salmonella* serotypes in societies.[26-29] This study did not investigate the prevalence of *Salmonella* in the north of Iran. However, many studies in recent years have reported the high prevalence of this bacterium from different sources in Iran.[30,31] The noteworthy point in the investigation of drug resistance among *Salmonella* serotypes was the high prevalence of MDR strains. In studies in other countries also a high prevalence of MDR strains has been stated.[26,32,33] The studied strains showed high resistance to macrolides, anti-folates, β-lactams, and tetracycline. Perhaps this level of resistance is not far from expectation considering the amount and improper use of antibiotics in veterinary medicine in Iran.[34,35] Maybe some of these *Salmonella* serotypes are not very pathogenic; but, they have shown relatively high resistance.

The presence of transferable resistance genes being located on plasmids like *incC*, *incF*, and *incHI* in different strains is responsible for many of these drug resistances. Genes such as, tetracycline resistance, β-lactamase-related, and sulfonamide resistance genes are from this category.[36] According to the results, among the *Salmonella* isolates, 32 strains showed the simultaneous presence of more than four resistance genes, being a significant and worrying amount. In the present study, *sul2*, *tetA*, and bla_{TEM} genes were respectively dominant in relation to sulfonamides, tetracycline, and β-lactamases, and other studies have also shown such a pattern.[10,37-39] However, Chen *et al*. have shown a higher prevalence of the *Sul1* gene in *Salmonella* serotypes in their study.[10] Despite the ubiquitous presence of plasmids in *Salmonella* across diverse organisms, the distribution of such plasmids across *Salmonella* serotypes exhibits remarkable heterogeneity. It is pertinent to note that novel plasmids have the potential to emerge within any given *Salmonella* serotype. Although certain serotypes are statistically more predisposed to contain certain plasmids in comparison with others, plasmids may be

found in any serotype without limitations to any specific incompatible groups correlated only to one serotype.

One of the applicable genotyping methods for the *Enterobacteriaceae* family is the ERIC-PCR method, being used in various epidemiological studies to differentiate and identify isolates from various origins, especially in the case of *Escherichia coli* and *Salmonella*.[40-42] In this study, the ERIC-PCR method showed good results in the recognition and differentiation of *Salmonella* serotypes, being confirmed by statistical findings. So, the relationship between the source of isolates and ERIC-PCR pattern and also between the serotype and ERIC pattern was significant. Accordingly, the ERIC cluster, C-2, was associated with human *S.* Enteritidis, clusters C-7 and C-8 were associated with chicken meat *S.* Infantis, C-11 was associated with human *S.* Typhimurium, C-8 pattern was associated with the chicken liver source, and C-1 pattern was in relation with cattle *S.* Typhimurium. The results of this genotyping method were significant and much better compared to other PCR-based methods, *e.g.,* random amplified polymorphism DNA being used in the study (unpublished data). Other similar studies have also emphasized the reliability, appropriate discrimination power, and availability of this method, making ERIC-PCR the main choice of low-cost genotyping methods for *Salmonella* in epidemiological studies.[37,42,43] In the present study, the occurrence of three virulence genes was also inspected. The *hilA* gene is a transcriptional activator of the OmpR/ToxR family being encoded in SPI1,[21] the *sdiA* gene is a quorum-sensing related activator gene,[22] and *invA* is an invasion gene of the genus. All three studied virulence genes were detected in all *Salmonella* serotypes from different sources. Similarly, other previous studies have reported the 100% presence of the genes among *Salmonella* strains.[2,3,44] The presence of these genes in all serotypes makes them a suitable option for molecular identification of food, animal, and human *Salmonella* spp.

In conclusion, the results of the investigation showed that MDR strains of *Salmonella* serovars were frequently found in both animal and human samples. Significant plasmid resistance genes were found in many isolates, suggesting a plausible source of resistance transfer to human serotypes. In particular, most of the isolates, irrespective of their origin, showed resistance to erythromycin and trimethoprim. The ERIC-PCR genotyping of the *Salmonella* serotypes showed a reasonable discrimination and identification potential, which can be used to identify the source of infections and outbreaks in both human and animal populations.

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Conflict of interest

The authors have no conflict of interest to declare. All co-authors have seen and agreed with the contents of the manuscript and there is no financial interest to report.

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