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Original Article

Clonal relatedness and antimicrobial susceptibility of *Salmonella* serovars isolated from humans and domestic animals in Iran: a one health perspective

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Abstract

Background: Salmonellosis is one of the most important zoonotic diseases in humans and animals worldwide. **Aims:** The main objective of this study was to report serovars, clonal relatedness, and antimicrobial resistance of *Salmonella* strains isolated from human, different animal hosts including pigeons, broilers, cattle, camel, parrots, and hamsters in different regions of Iran. **Methods:** Twenty-four *Salmonella* isolates were confirmed at the genus level by biochemical tests and polymerase chain reaction (PCR) by showing the presence of *invA* gene. Serovars were determined and their clonal relatedness was assessed by RAPD-PCR and antibiotic resistance profiles. **Results:** Overall, *Salmonella* Typhimurium was the most prevalent serovar (45.8%, 11/24), which was recovered from humans, pigeons, and camels. *Salmonella* Enteritidis (29.2%, 7/24) was the second common serovar that was recovered from cattle, broilers, humans, and hamsters. *Salmonella* Infantis (12.5%, 3/24) belonged only to broiler sources, and *Salmonella* Seftenberg (12.5%, 3/24) was isolated from eggs and a parrot. The major RAPD pattern was VI (33.3%) in which the two *S.* Typhimurium isolates (belonged to humans and pigeons) exhibited similarity in both RAPD pattern and resistance profile. Antimicrobial susceptibility test showed full resistance to tylosin and erythromycin (100%, 24/24). All isolates (100%, 24/24) were susceptible to ceftriaxone, cefixime, and gentamicin. In total, 75% of the isolates were multi-drug resistant (MDR) and revealed 15 different antimicrobial resistance profiles (R-type). **Conclusion:** This study supports the potential transmission of *Salmonella* serovars via animal contacts. Thus, it is necessary to establish a national systematic monitoring program with one health approach for controlling *Salmonella* infections.

Key words: Antimicrobial resistance, Iran, Molecular typing, One health, *Salmonella*

Introduction

Among more than 2500 *Salmonella* serovars that have been reported worldwide, 1500 serovars have been linked with human and animal diseases (Grimont and Weill, 2007). In this regard, this bacterium is an important zoonotic pathogen and one of the most frequent food poisoning microorganisms in developing countries (Cao *et al*., 2020; Dallal *et al*., 2020). Poultry products including meat and eggs have been the main sources of human salmonellosis which act as carriers of the pathogen in the human food chain (Gould *et al*., 2013).

Most *Salmonella* serovars have a wide host range including humans, livestock, poultry, rodents, reptiles, and birds which have varying levels of host specificity (Khan *et al*., 2020). Among these hosts, it seems that broilers are one of the main reservoirs of this pathogen in Iran (Peighambari *et al*., 2018). In addition, birds especially pigeons have always been mentioned as one of the most important carriers of *Salmonella* serovars (Pasmans *et al*., 2008; Madadgar *et al*., 2009; Hendriksen *et al*., 2011; Besharati *et al*., 2020). Importantly, *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis can infect various hosts including broilers and pigeons that can be transmitted to humans have an adverse impact on the marketing of poultry industries. Accordingly, these serovars of *Salmonella* are reported as the most widespread food-borne pathogens in Iran and most other countries (Hendriksen *et al*., 2011; Besharati *et al*., 2020).

To minimize the burden of Salmonellosis, it is always helpful to monitor and track *Salmonella* serovars sources in different geographical regions and different hosts. Moreover, in conjunction with determining serovars, it is very important to analyze the epidemiological relationships between the isolates by using a genetic typing method. In developing countries such as Iran, due to technological and economic constraints, the use of simple and cost-effective methods is a priority. Random amplification of polymorphic DNA (RAPD) analysis has been shown to be a simple, economic, and relatively reliable method in combination with antimicrobial susceptibility test for clonal studies and phylogenetic tracing of *Salmonella* isolates from both infected cases and carriers (Madadgar *et al*., 2008; Sabat *et al*., 2013; Salehi *et al*., 2013). In the RAPD method, a single short primer (8-12 nucleotides) is used in each reaction that can be randomly attached to several DNA sequences in the genome; by analyzing the differences in RAPD band patterns (the number and the positions of primer binding sites), clonal relationships can be evaluated in the epidemiological studies (Sabat *et al*., 2013).

One of the recent public health global concerns about salmonellosis is the emergence and spread of resistant clones including the multiple drug resistance (MDR) strains in both humans and animals (Rodrigues *et al*., 2020; Yang *et al*., 2020). In Iran, poultry and livestock industries are rapidly growing and the use of antibiotics for therapeutic purposes can potentially increase the persistence and spread of resistant clones (Threlfall, 2002; Hu *et al*., 2017). As a result, poultry and livestock frequently carry MDR strains that can be transmitted to humans along with food chain through consumption of contaminated foods (Vaez *et al*., 2020). It has been shown that antibiotic resistance profile (R-typing) not only shows the resistance level but also in combination with a genotyping method like RAPD-PCR increases discriminatory power in epidemiological studies (Madadgar *et al*., 2008).

The main objective of this study was to report serovars, clonal relatedness, and antimicrobial resistance of *Salmonella* strains isolated from human and different animal hosts including pigeon, broiler, cattle, camel, parrot, hamster, and also from eggs in the cities of Tehran, Mashhad, Garmsar, Babol, and Gorgan, in Iran during the years 2009-2013. After confirming the isolates at genus level and reporting their serovars in humans and animal hosts, we defined the relatedness of *Salmonella* serovars isolated from different hosts and geographical origins to obtain a one health perspective.

Materials and Methods

Ethics approval and consent to participate

The study was carried out in accordance with relevant guidelines and regulations presented by Iran National Committee for Ethics in Biomedical Research. Accordingly, written or verbal informed consent was obtained from all participants for human experimentations and verbal informed consent was obtained from the owners of the companion animals. The authors approved that all protocols were conducted in accordance with the related guidelines and regulations (IR.1392.1236).

Salmonella isolates

A total of 31 fecal samples were collected from

Table 1: *Salmonella* serovars and their characteristics

Isolate	Origin	Location	Year	Serovar	RAPD Pattern [a]	Antibiotic Profile [b]
1	Cattle	Garmsar	2009	D (*S.* Enteritidis)	I	B
2	Pigeon	Garmsar	2011	B (*S.* Typhimurium)	II	A
3	Broiler	Gorgan	2010	C (*S.* Infantis)	IV	C
4	Broiler	Babol	2010	D (*S.* Enteritidis)	V	D
5	Broiler	Babol	2010	C (*S.* Infantis)	IV	C
6	Broiler	Babol	2010	D (*S.* Enteritidis)	V	E
7	Broiler	Babol	2010	C (*S.* Infantis)	V	F
8	Human	Garmsar	2013	B (*S.* Typhimurium)	VI	G
9	Human	Garmsar	2013	B (*S.* Typhimurium)	III	A
10	Human	Garmsar	2013	B (*S.* Typhimurium)	VI	A
11	Human	Mashhad	2013	B (*S.* Typhimurium)	V	H
12	Human	Mashhad	2013	B (*S.* Typhimurium)	VI	I
13	Human	Mashhad	2013	B (*S.* Typhimurium)	VI	I
14	Pigeon	Tehran	2011	B (*S.* Typhimurium)	IV	J
15	Camel	Tehran	2011	B (*S.* Typhimurium)	II	K
16	Egg	Tehran	2013	E (*S.* Senftenberg)	IV	L
17	Hamster	Tehran	2013	D (*S.* Enteritidis)	VI	M
18	Pigeon	Tehran	2011	B (*S.* Typhimurium)	VI	B
19	Cattle	Tehran	2009	D (*S.* Enteritidis)	III	N
20	Cattle	Tehran	2009	D (*S.* Enteritidis)	III	K
21	Cattle	Tehran	2009	D (*S.* Enteritidis)	VI	O
22	Egg	Tehran	2013	E (*S.* Senftenberg)	VII	A
23	Parrot	Tehran	2012	E (*S.* Senftenberg)	VIII	A
24	Pigeon	Tehran	2011	B (*S.* Typhimurium)	VI	A

[a] See Fig. 1 and Fig. 2, and [b] See Table 2

different hosts including humans (n=9), pigeons (n=5), broilers (n=8), cattle (n=4), camel (n=1), parrot (n=1), hamster (n=1), and eggs (n=2) which were previously isolated in the cities of Tehran, Mashhad, Garmsar, Babol, and Gorgan, in Iran over the years 2009 to 2013 (Table 1).

A verbal or written informed consent was obtained from all human subjects participating in this study. For companion animals, verbal informed consent was received from their owners prior to sampling. The owner's consent was not required for commercial animals.

Biochemical and molecular identification

All samples were isolated using the standard protocol for isolation of *Salmonella* serovars as briefly described. The fresh fecal samples were transferred to Selenite F broth (Merck Co., Germany) and incubated at 37°C for 16 h. A loopful of the enriched samples were cultured on MacConkey agar (Merck Co., Germany) and XLD agar (Merck Co., Germany) and incubated at 37°C for 24-48 h. Suspected colonies were cultured into the TSI agar (Merck Co., Germany) and incubated at 37°C for 24 h. The lactose-negative and H_2S-positive isolates were examined using standard biochemical tests (Markey *et al*., 2013). Few samples were directly recovered from eggshells or tissues by conventional culture methods.

Isolates with typical *Salmonella* phenotypes were confirmed by PCR for the *invA* gene at the genus level, using the S139 (5´-GTG AAA TTA TCG CCA CTG TCG GGC AA-3´) and S141 (5´-TCA TCG CAC CGT CAA AGG AAC C-3´) primers after DNA extraction as previously described (Salehi *et al*., 2013). *Salmonella* Infantis (Collection isolate, University of Tehran), and *S.* Enteritidis (American Type Culture Collection (ATCC): 13076) were used as positive controls.

Salmonella serotyping

Salmonella serovars were characterized by the standard procedure to determine the Kauffman-White O- and H-types according to the manufacturer recommendations (Difco, USA) (Grimont and Weill, 2007).

Random amplified polymorphic DNA (RAPD) analysis

To assess clonal relatedness of the isolates, RAPD-PCR profiles were investigated using one primer, 1247 (5´-AAG AGC CCG T-3´) (Heuvelink *et al*., 1995). Amplification reactions were conducted in a 25 µL reaction volume containing 2.5 µL 10 × PCR buffer, 0.55 µL dNTP, 1 µL of primer, 0.2 µL of *Taq* polymerase DNA, 2.5 mM $MgCl_2$, 17.5 µL dH_2O, and 2 µL of template DNA. Amplification was programmed in a thermocycler (Techne, United Kingdom) as follows: 94°C for 4 min followed by 35 cycles of 94°C for 1 min, 40°C for 50 s, 72°C for 2 min, and a final extension at 72°C for 5 min (Fadl *et al*., 1995). The amplification products were electrophoresed on 2% agarose gel at 80 V for 2 h and visualized by GelDoc 1000 after staining with ethidium bromide (Vilber Lourmat, France). The RAPD patterns of individual strains were scored based on band presence or absence. To confirm the reproducibility, PCR was done at least twice on all the isolates. The primers and other materials used in PCR reaction were provided by Cinnagen Co. (Tehran, Iran). A *Salmonella* Typhimurium strain (ATCC: 14028) was used as the positive control and distilled water was used as the negative control.

Antimicrobial susceptibility test

According to Clinical and Laboratory Standards Institute (CLSI) Guidelines (Wayne, 2018), the susceptibility of *Salmonella* strains to a panel of 16 antimicrobial agents was performed by using the Kirby-Bauer disk-diffusion method. The following disc antibiotics and their respective concentrations, purchased from Padtan Teb Co. (Tehran, Iran), included: chlortetracycline (CTC; 30 µg), linco-spectin (LP; 15/200 µg), florfenicol (FLOR; 30 µg), doxycycline (DOX; 30 µg), gentamicin (GEN; 10 µg), neomycin (NEO; 30 µg), ceftriaxone (CRO; 30 µg), furazolidone (FUR; 100 µg), streptomycin (STR; 10 µg), tetracycline (TET; 30 µg), chloramphenicol (CHL; 30 µg), tylosin (TY; 30 µg), ampicillin (AMP; 10 µg), erythromycin (E; 15 µg), cefixime (CFM; 5 µg), and nalidixic acid (NAL; 30 µg). A multiple drug resistance isolate is defined as an isolate demonstrating resistance to three or more antibiotics belonging to different antibiotic classes. The *Escherichia coli* ATCC 25922 strain was used for quality control purposes.

Statistical analysis

The dendrogram in this study was constructed by using the SPSS computer software program (Version 26); showing the similarity index of *Salmonella* strains based on the available criteria such as RAPD-types, serovars, host, etc. (Fig. 1).

Results

Prevalence and serovar identification of *Salmonella* isolates

Based on standard biochemical tests and PCR reactions, out of 31 isolates tested, 24 (77.4%) isolates were confirmed as *Salmonella* spp. at the genus level. These isolates belonged to the cities of Tehran (n=11, 45.8%), Mashhad (n=3, 12.5%), Garmsar (n=5, 20.8%), Babol (n=4, 16.6%), and Gorgan (n=1, 4.1%) which were obtained from humans (n=6, 25%), pigeons (n=4, 16.6%), broilers (n=5, 20.8%), cattle (n=4, 16.6%), camel (n=1, 4.1%), parrot (n=1, 4.1%), hamster (n=1, 4.1%), and eggs (n=2, 8.3%) as listed in Table 1.

The conventional serotyping showed that 11 isolates (6 from humans, 4 from pigeons, and 1 from camel) were identified as *S.* Typhimurium (serogroup B), 7 isolates (4 from cattle, 2 from broilers, and 1 from hamster) were identified as *S.* Enteritidis (serogroup D), 3 isolates (broilers) were identified as *S.* Infantis (serogroup C),

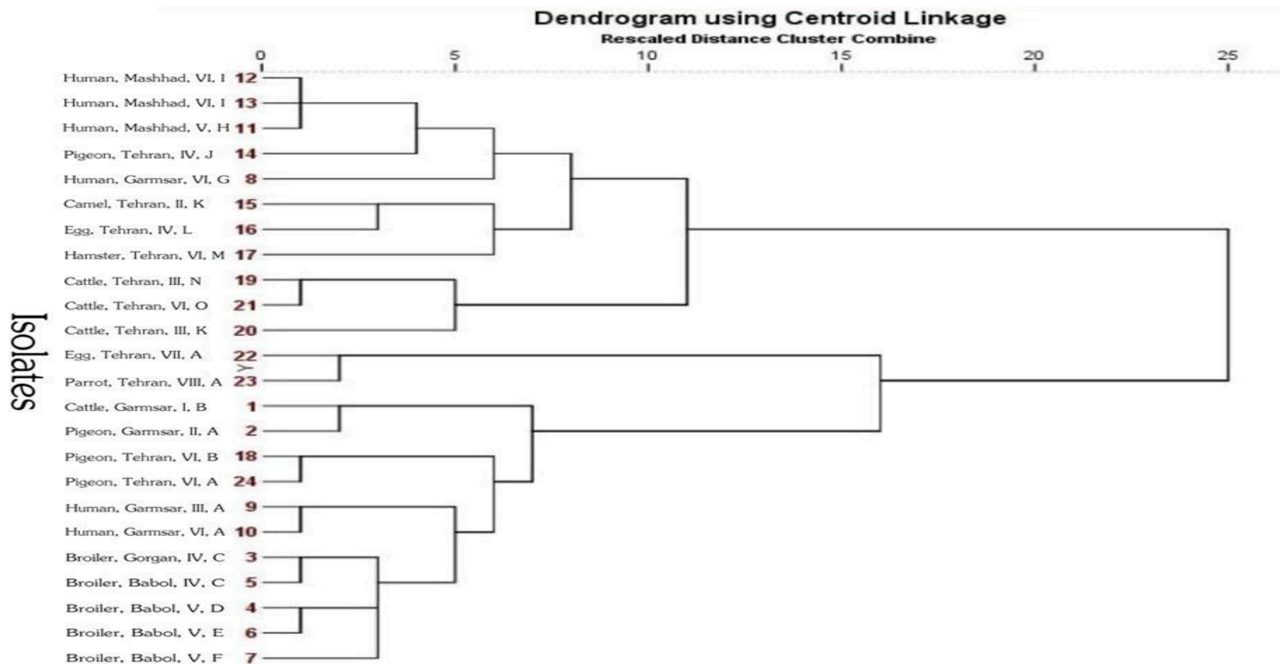

Fig. 1: Dendrogram showing the relatedness of *Salmonella* serovars based on their clonal and antimicrobial resistance profiles in regard to the different hosts and the cities

Table 2: Antimicrobial-resistance patterns (R-types) of *Salmonella* serovars

Profile	Resistance pattern [a]	*S.* Typhimurium	*S.* Enteritidis	*S.* Infantis	*S.* Senftenberg	Total
A	E-TY	4	- [b]	-	2	6
B	E-TY-DOX	1	1	-	-	2
C	E-TY-AMP-STR-TET-DOX-NAL-LP-CTC-FUR-FLOR	-	-	2	-	2
D	E-TY-STR-NAL	-	1	-	-	1
E	E-TY-AMP-DOX-NAL-FLOR	-	1	-	-	1
F	E-TY-AMP-STR-TET-DOX-NAL-CHL-LP-CTC-FUR-FLOR	-	-	1	-	1
G	E-TY-STR	1	-	-	-	1
H	E-TY-DOX-NAL	1	-	-	-	1
I	E-TY-LP	2	-	-	-	2
J	E-TY-AMP-STR-TET-DOX-NAL-CHL-LP-CTC-FUR-FLOR-NEO	1	-	-	-	1
K	E-TY-TET-DOX-CTC	1	1	-	-	2
L	E-TY-AMP-TET-DOX-NAL-LP-CTC-FUR-FLOR	-	-	-	1	1
M	E-TY-AMP-TET-DOX-CHL-LP-CTC-FLOR	-	1	-	-	1
N	E-TY-TET-DOX-CTC-FLOR	-	1	-	-	1
O	E-TY-NAL	-	1	-	-	1
Total		11	7	3	3	24

[a] Key: Antimicrobial agents tested were AMP: Ampicillin, CFM: Cefixime, CRO: Ceftriaxone, CHL: Chloramphenicol, CTC: Chlortetracycline, DOX: Doxycycline, E: Erythromycin, FLOR: Florfenicol, FUR: Furazolidone, GEN: Gentamicin, LP: Linco-Spectin, NAL: Nalidixic acid, NEO: Neomycin, STR: Streptomycin, TET: Tetracycline, and TY: Tylosin. [b] No resistance pattern detected

and 3 isolates (2 from eggs, and 1 from parrot) were identified as *S.* Senftenberg (serogroup E). The most common serogroups were group B (45.8%, 11/24) and group D (29.1%, 7/24), as presented in Table 1.

Clonal relatedness based on RAPD patterns

Based on the results of RAPD-PCR (Fig. 2), these 24 isolates belonged to eight different RAPD patterns (I, II, III, IV, V, VI, VII, VIII) (Table 1). Among them, the predominant pattern was VI (33.3%, 8/24), followed by IV (16.6%, 4/24), V (16.6%, 4/24), III (12.5%, 3/24), II (8.3%, 2/24), I (4.1%, 1/24), VII (4.1%, 1/24), and VIII (4.1%, 1/24). Pattern VI was shared among 4 (66.6%), 2 (50%), 1 (100%), and 1 (25%) human, pigeon, hamster, and cattle isolates, respectively (Table 1). All five broiler isolates belonged to two patterns of IV (2 isolates) and V (3 isolates); also, all four cattle isolates belonged to three patterns of III (2 isolates), I (1 isolate), and VI (1 isolate) (Table 1). To determine the numerical similarity index of these isolates, a dendrogram was constructed by using the SPSS computer software program (Version 26) (Fig. 1).

Antimicrobial susceptibilities of *Salmonella* serovars

The isolates showed total resistance to tylosin (100%, 24/24), and erythromycin (100%, 24/24); and high resistance rates to doxycycline (54.1%, 13/24), tetracycline (37.5%, 9/24), chlortetracycline (37.5%, 9/24), and nalidixic acid (37.5%, 9/24). Also, all isolates (100%, 24/24) were susceptible to ceftriaxone, cefixime, and gentamicin. The isolates showed 15 different

Table 3: Antimicrobial-resistance of *Salmonella* serovars

Antimicrobial agent	Number of resistant strains (%)				Total number (%)
	S. Typhimurium (n=11)	*S.* Enteritidis (n=7)	*S.* Infantis (n=3)	*S.* Senftenberg (n=3)	
β-Lactam antibiotics					
Penam penicillins					
Ampicillin	1 (9.0)	2 (28.5)	3 (100.0)	1 (33.3)	7 (29.1)
Cephalosporins					
Cefixime	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftriaxone	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Aminoglysides					
Gentamicin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Neomycin	1 (9.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.1)
Streptomycin	2 (18.1)	1 (14.2)	3 (100.0)	0 (0.0)	6 (25.0)
Phenicols					
Chloramphenicol	1 (9.0)	1 (14.2)	1 (33.3)	0 (0.0)	3 (12.5)
Florfenicol	1 (9.0)	3 (42.8)	3 (100.0)	1 (33.3)	8 (33.3)
Tetracyclines					
Tetracycline	2 (18.1)	3 (42.8)	3 (100.0)	1 (33.3)	9 (37.5)
Chlortetracycline	2 (18.1)	3 (42.8)	3 (100.0)	1 (33.3)	9 (37.5)
Doxycycline	4 (36.3)	5 (71.4)	3 (100.0)	1 (33.3)	13 (54.1)
Quinolones					
Nalidixic acid	2 (18.1)	3 (42.8)	3 (100.0)	1 (33.3)	9 (37.5)
Macrolides					
Tylosin	11 (100.0)	7 (100.0)	3 (100.0)	3 (100.0)	24 (100.0)
Erythromycin	11 (100.0)	7 (100.0)	3 (100.0)	3 (100.0)	24 (100.0)
Others					
Furazolidone	1 (9.0)	0 (0.0)	3 (100.0)	1 (33.3)	5 (20.8)
Linco-spectin	3 (27.2)	1 (14.2)	3 (100.0)	1 (33.3)	8 (33.3)
Total	11 (23.8)	7 (32.1)	3 (70.8)	3 (29.1)	24 (32.7)

antimicrobial-resistance profiles (R-types) in which MDR patterns were observed in 18 isolates (75%) (Table 2). The details of phenotypic resistance to antimicrobials have been presented in Table 3.

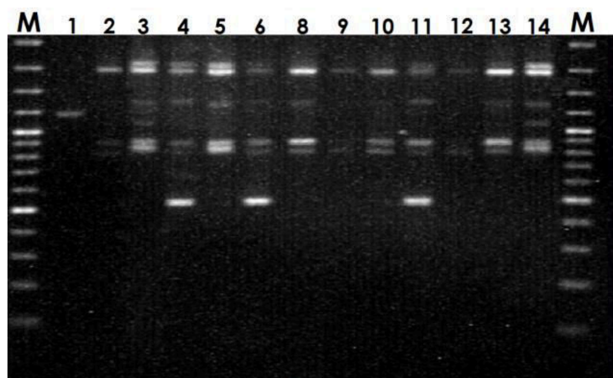

Fig. 2: The results of the selected RAPD-PCR patterns of *Salmonella* serovars in this study: M, Marker, 100 bp-plus; isolates number: 1-6 to 8-14

Discussion

A large number of *Salmonella* serovars infect both humans and most animal species which has led to the wide distribution of this pathogen (Wang *et al*., 2020). Our study showed that among 24 *Salmonella* strains, *S.* Typhimurium (45.8%, 11/24), and *S.* Enteritidis (29.1%, 7/24) had the highest frequency rate. These serovars were also found to be the predominant serovars in other studies in Iran (Emadi *et al*., 2009; Ranjbar *et al*., 2017). However, in some other studies in Iran, *S.* Infantis was the most common serovar (Fallah *et al*., 2013; Rahmani *et al*., 2013; Askari Badouei *et al*., 2021). Also, in studies conducted in Spain (Carramiñana *et al*., 2004), and China (Little *et al*., 2008), *S.* Enteritidis and *S.* Typhimurium constituted the most common serovars, respectively; which were in accordance with our study. Therefore, it seems that *S.* Typhimurium, and *S.* Enteritidis are among the most important *Salmonella* serovars regardless of the geographical regions.

In the present study, all isolates from human clinical samples (100%, 6/6) were identified as *S.* Typhimurium. It indicates the importance of this serovar in causing the disease in humans that might be resulted from the widespread prevalence of this serovar (Khaltabadi *et al*., 2019). Another important point in this study was the isolation of *S.* Typhimurium with a high frequency rate in pigeons (100%, 4/4). It suggests that the pigeons can be a potential carrier of zoonotic *Salmonella* serovars and a potential risk for human infection (Pasmans *et al*., 2008; Madadgar *et al*., 2009). Besides, the high frequency rate of *S.* Enteritidis in cattle (100%, 4/4) and *S.* Infantis in broilers (60%, 3/5) indicate that these serovars are important in livestock and poultry farms, respectively (Dallal *et al*., 2020; Askari Badouei *et al*., 2021). However, in other studies conducted by Ezatpanah *et al*. (2013) and Asadpoor *et al*. (2014) in Iran, *S.* Enteritidis had the highest rate (45.3% and 75%, respectively) in broiler farms. Based on the mentioned reports, *S.* Enteritidis is important in both cattle and broilers as a non-host adapted serovar. Therefore, it is necessary to establish a national surveillance program to control *Salmonella* contamination in dairy and poultry products and also in game or ornamental birds like pigeons.

Among different genotyping tools, RAPD-PCR is an

easy, cost-effective, and relatively reliable method that can be performed for investigations of strain origin, clonal relatedness, and epidemiological surveillance of *Salmonella* serovars (Madadgar *et al*., 2008; Sabat *et al*., 2013). In this study, RAPD analysis of 24 isolates revealed eight different RAPD patterns in which 66.6% of isolates belonged to three patterns: VI, IV, and V. In accordance with our study, Fadl *et al*. (1995) found seven RAPD patterns among 33 *Salmonella* isolates obtained from fecal samples in humans and different animal sources (poultry, mouse, and cat) in the USA; similarly, Madadgar *et al*. (2008) reported six RAPD patterns in thirty *Salmonella* isolates collected from fecal samples of different animals (cat, chicken, cow, sheep, dove, parrot, canary, sparrow, and pony) in Iran. As shown in the current study, *S*. Typhimurium was the only detected serovar in human and pigeon isolates. Additionally, we detected a high similarity between the RAPD patterns of these isolates including pattern VI that was prevalent in 66.6% and 50% of human and pigeon isolates, respectively. For the cattle and broiler isolates, no significant relation was found. In fact, the cattle isolates were distributed into three patterns, III (50%), VI (25%), and I (25%); and broiler isolates into two patterns, V (60%) and IV (40%); this may be due to differences in geographical regions of these samples; however, using a more robust genotyping technique might be necessary to discriminate these isolates more precisely.

Antimicrobial resistance of *Salmonella* serovars remains one of the important challenges to control *Salmonella* infection in Iran (Fallah *et al*., 2013). Our results showed that, all *Salmonella* isolates (100%) were resistant to tylosin, and erythromycin; also, all isolates (100%) were susceptible to ceftriaxone, cefixime, and gentamicin. In the study of Peighambari *et al*. (2018) resistance to tylosin, and erythromycin was similarly reported in 100% of cases; moreover, sensitivity to ceftriaxone, cefixime, and gentamicin was reported in 100%, 100%, and 72.8% of cases, respectively (Peighambari *et al*., 2018) which was in agreement with the present study. As shown in the results of resistance profiles, the most common resistance pattern (25%, profile A) was against two antibiotics including tylosin, and erythromycin. Accordingly, resistance to macrolide antibiotics should be considered a common trait among *Salmonella* serovars.

Based on our results, antibiotic profiles related to RAPD pattern IV had the highest resistance rate which includes samples of broilers, pigeons, and eggs. Also, in the isolates No. 12 and No. 13, a similar RAPD pattern and antibiotic profile were observed, indicating a clonal relatedness between these two isolates which was probably due to their similar host (human) and region (Mashhad) and time of sampling. Furthermore, the major RAPD pattern in this study was VI (33.3%), in which the most isolates were *S*. Typhimurium (75%) obtained from human and pigeon samples; and because of the similarity in RAPD pattern and antibiotic profile in two isolates (isolates No. 10 from human and No. 24 from pigeon), pigeons could be considered important carriers in the epidemiology of salmonellosis in Iran (Pasmans *et al*., 2008; Madadgar *et al*., 2009).

As a final conclusion, this study confirmed the transmission of *Salmonella* serovars especially the MDR clones via animal contacts which has a negative impact on public health. We also showed that R-typing in combination with RAPD-PCR discriminated *Salmonella* serovars that can be used in studies with a one health perspective, where more complicated methods are not available.

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Conflict of interest

None to declare.

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