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Antimicrobial resistance, virulence genes and genetic relatedness of *Salmonella enterica* serotype Enteritidis isolates recovered from human gastroenteritis in Tehran, Iran

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ABSTRACT

Objectives: *Salmonella enterica* serotype Enteritidis is a major serotype associated with human salmonellosis. The main objective of this study was to determine the antibiotic susceptibility patterns and the presence of virulence-associated genes among *S.* Enteritidis strains isolated from patients with gastroenteritis in Tehran, Iran.
Methods: Over a period of 14 months (May 2015 to July 2016), 44 *S.* Enteritidis isolates recovered from clinical sources were characterised for antimicrobial susceptibility and virulence genes. Possible genetic relatedness among the strains was also assessed using pulsed-field gel electrophoresis (PFGE).
Results: *Salmonella* Enteritidis isolates showed high rates of resistance to ciprofloxacin (90.9%) and nalidixic acid (77.3%). Of the 44 *S.* Enteritidis isolates, 30 (68.2%) were resistant to three or more antibiotics. Twenty-two different antimicrobial resistance patterns were detected among the isolates. The most frequent resistance type was antibiotype 14 (resistance to ciprofloxacin, cefuroxime and nalidixic acid), occurring in 8 (18.2%) of the isolates. Notably, all of the isolates carried *invA*, *sefA*, *sipA* and *sopE2* virulence genes. Furthermore, 17 virulence profiles were observed among the strains. The most common virulence profile was VP1 ($n = 17$; 38.6%), harbouring all of the virulence genes. Two distinct PFGE patterns were observed among 44 *S.* Enteritidis isolates. There was no association between virulence profiles or antibiotypes and PFGE clusters.
Conclusions: Overall, this study provides valuable information on the virulence gene content, antibiotic resistance and genetic diversity of *S.* Enteritidis isolated from human sources in Iran.

1. Introduction

Salmonella enterica serotype Enteritidis is not only a major serotype associated with human salmonellosis but it is also responsible for causing the largest number of foodborne cases related to outbreaks in Europe and the USA [1,2]. According to estimates from the US Centers of Disease Control and Prevention (CDC), non-typhoidal *Salmonella* causes ca. 1.2 million illnesses, 23 000 hospitalisations and 450 deaths each year in the USA [3]. In 2006, countries within the European Union reported 1729 outbreaks caused by *S.* Enteritidis leading to 13 853 illnesses [4]. Commercial poultry products, mainly undercooked eggs and meat, are recognised as important reservoirs for *S.* Enteritidis and vehicles for salmonellosis [5]. In humans, *S.* Enteritidis-induced salmonellosis is usually characterised by acute onset of fever, abdominal pain, diarrhoea, nausea and, sometimes, vomiting [6].

Gastroenteritis due to *Salmonella* is usually a self-limiting disease and does not require antibiotic therapy [7]. However, antibiotic treatment for salmonellosis may be lifesaving for patients with severe infections [8]. Of particular concern is the

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increasing prevalence and resistance of *S. enterica* to multiple antibiotics, with mostly animal-borne serotypes being multidrug-resistant (MDR) [9]. Indiscriminate use of antimicrobials in food animals for therapy, prophylaxis and growth enhancement has contributed to the emergence of antibiotic-resistant bacteria, including MDR *Salmonella* [10]. MDR *Salmonella* isolates can be transferred to humans by the food chain [11]. In addition, prolonged use of antimicrobials both in clinical and farm settings increases the virulence of resistant isolates [12]. These conditions limit the empirical therapeutic options available for clinical cases, making treatment of MDR isolates more difficult [9].

The pathogenicity of *Salmonella* isolates depends on a variety of virulence factors that help the pathogen in adhesion, invasion, intracellular survival, fimbrial expression, systemic infection, antibiotic resistance, toxin production, and Mg^{2+} and iron uptake [2,13]. Many *S.* Enteritidis virulence factors are linked to a virulence-associated plasmid (pSTV), chromosomal *Salmonella* pathogenicity islands (SPIs) and multiple fimbriae [13,14]. The SPI-1 and SPI-2 genes encode type 3 secretion systems (T3SS), promoting intestinal and reproductive tract colonisation [15]. For instance, the SPI-1-encoded genes such as *invA*, *sipA*, *sipD*, *sopA*, *sopB*, *sopD* and *sopE2* allow *S.* Enteritidis to invade phagocytic and non-phagocytic cells, whilst SPI-2-encoded genes such as *ssaR* and *ssrA* allow the survival and replication of *Salmonella* in host cells [16].

Given the paucity of published literature on the virulence genes and antimicrobial resistance of *S.* Enteritidis strains isolated from human sources in Iran, it is important to understand the molecular mechanisms of pathogenicity in this important pathogen for the implementation of intervention strategies. Therefore, the objectives of the present study were to evaluate the antibiotic susceptibility patterns and the presence of virulence-associated genes among *S.* Enteritidis strains isolated from patients with gastroenteritis. Possible genetic relatedness among the tested strains was also assessed by pulsed-field gel electrophoresis (PFGE).

2. Materials and methods

2.1. Reagents and media

Mueller–Hinton agar and trypticase soy broth were purchased from Merck KGaA (Darmstadt, Germany), whilst antibiotics and Etest strips were obtained from Mast Diagnostics (Bootle, UK) and Liofilchem (Roseto degli Abruzzi, Italy), respectively. Other materials and reagents were purchased from Sigma (St Louis, MO).

2.2. Bacterial isolates

Over a period of 14 months (May 2015 to July 2016), a total of 44 *S.* Enteritidis strains were isolated from 2700 patients with gastroenteritis in Tehran, Iran. Stool specimens were collected from patients with diarrhoea as soon as attending one of two university hospitals (Imam Khomeini Hospital Complex and Children's Medical Centre) in the centre of Tehran. Patients had evidence of more than three episodes of watery, loose or non-bloody stools per day. All of the isolates were identified using routine biochemical and serological tests [17]. In addition, multiplex PCR was carried out to confirm *S.* Enteritidis isolates as described previously [18]. *Salmonella* Enteritidis isolates were stored in trypticase soy broth supplemented with 25% (v/v) glycerol and were maintained at −70 °C prior to use.

2.3. Antibiotic susceptibility testing

Antimicrobial susceptibilities for all of the *S.* Enteritidis isolates were determined by the disk diffusion method on Muller–Hinton agar according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [19]. All *S.* Enteritidis isolates were tested against a panel of 13 antibiotics. In this regard, at least one antimicrobial agent was selected for each class of antibiotics, as described previously [11,17,20]. The antibiotics tested were as follows: cefotaxime (30 μg); ceftazidime (30 μg); ceftriaxone

Table 1
Primers used in this study.

Gene	Sequences (5′ → 3′)	Amplicon size (bp)	Annealing temperature (°C)	Reference
invA	F-ACAGTGCTCGTTTACGACCTGAAT R-AGACGACTGGTACTGATCGATAAT	243	60	[21]
hilA	F-CGTGAAGGGATTATCGCAGT R-GTCCGGGAATACATCTGAGC	296	56.3	[22]
spvC	F-ACTCCTTGCACAACCAAATGCGGA R-TGTCTCTGCATTTCGCCACCATCA	447	56.3	[21]
sipA	F-CCATTCGACTAACAGCAGCA R-CGGTCGTACCGGCTTTATTA	449	56.3	[22]
sopE	F-CGAGTAAAGACCCCGCATAC R-GAGTCGGCATAGCACACTCA	362	58	[23]
stn	F-TTGTCTCGCTATCACTGGCAACC R-ATTCGTAACCCGCTCTCGTCC	617	59	[24]
pefA	F-TTGCACTGGGTGGTTCTGG R-TGTAACCCACTGCGAAAG	485	56.3	[25]
rck	F-AACGGACGGAACACAGAGTC R-TGTCCTGACGAAAGTGCATC	189	59	[23]
sipC	F-AGACAGCTTCGCAATCCGTT R-ATTCATCCCTTCGCGCATCA	446	60	This study
ssaR	F-GTTCGGATTTGCTTCGG R-TCTCCAGTGACTAACCCTAACCAA	1628	59	[26]
ssrA	F-CTTACGATTACGCCATTTACGG R-ATTTGGTGGAGCTGGCGGGAGT	706	58	[27]
sopB	F-CCTCAAGACTCAAGATG R-TACGCAGGAGTAAATCGGTG	1987	56.3	[28]
sefA	F-GCAGCGGTTACTATTGCAGC R-TGTGACAGGGACATTTAGCG	321	56.3	[18]
sopE2	F-TCAGGTGGAGCTGTGGA R-TCCAAAAACAGGAAACCACAC	642	56.3	[29]

(30 μg); cefepime (30 μg); cefuroxime (30 μg); streptomycin (10 μg); nalidixic acid (30 μg); ciprofloxacin (5 μg); tetracycline (30 μg); trimethoprim/sulfamethoxazole (25 μg); amoxicillin (20 μg); imipenem (10 μg); and chloramphenicol (30 μg). Isolates showing intermediate results were considered resistant. Furthermore, minimum inhibitory concentrations (MICs) of ciprofloxacin were determined using Etest strips for those *Salmonella* Enteritidis strains showing complete resistance or a decreased zone diameter in the disk diffusion assay. *Escherichia coli* ATCC 25922 was used as a quality control organism for susceptibility testing, and the results were interpreted in accordance with the CLSI guidelines [19].

2.4. Virulence gene detection

Genomic DNA of the *S.* Enteritidis strains was extracted by the boiling method as follows. A loopful of bacterial cells was suspended in 200 μL of double-distilled water. The suspension was heated at 100 °C for 10 min and was then centrifuged at 8000 × *g* for 10 min. Then, 50 μL of clear supernatant was transferred to another microfuge tube and was used as a DNA template for subsequent PCR analysis. Table 1 outlines the sequences and predicted sizes of the amplified products. The PCR amplification reaction mixture consisted of 2.5 μL of 10× PCR buffer, 1 mg/μL $MgCl_2$, 200 mM of deoxynucleotide triphosphates (dNTPs), 0.5 U of *Taq* Polymerase, 10 pmol of each primer and 2 μL of sample DNA. Amplification was performed in a thermocycler (PEQLAB, Erlangen, Germany) using the following cycling programme: initial denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at different temperatures (Table 1) for 30 s and primer extension at 72 °C for 60 s; and a final extension period of 72 °C for 2 min. To ascertain the expected sizes of the amplicons, the reaction products were subjected to electrophoresis in a 1.0% agarose gel, were stained with DNA Green Viewer™ (GeneCopoeia, Rockville, MD) and were visualised under ultraviolet light.

2.5. Pulsed-field gel electrophoresis

PFGE was performed as described previously [30]. *Xba*I (Thermo Scientific, Waltham MA) was used for endonuclease restriction of *S.* Enteritidis genomic DNA. Digested DNA was separated using a CHEF-DR III Chiller apparatus (Bio-Rad Laboratories, Hercules, CA) in 1% agarose gel and was analysed using GelCompar II software v.6.6 (Applied Maths, Sint-Martens-Latem, Belgium). A band position tolerance of 1.5% was used to analyse the PFGE fingerprints. Clustering was carried out by the unweighted pair-group method with arithmetic mean (UPGMA) using the Dice coefficient.

3. Results

A total of 44 *S.* Enteritidis were isolated during the study period, of which 30 isolates (68.2%) were resistant to three or more antimicrobials. The isolates showed 22 different antimicrobial resistance patterns and the most frequent resistance type was antibiotype 14 (ciprofloxacin, cefuroxime and nalidixic acid), occurring in 8 (18.2%) of the isolates (Table 2). Of note, only one isolate was sensitive to all of the antibiotics tested. As evidenced in Fig. 1, *S.* Enteritidis isolates showed high rates of resistance to ciprofloxacin (90.9%), nalidixic acid (77.3%) and cefuroxime (72.2%). The range of ciprofloxacin MICs varied from 0.006 μg/mL to 0.25 μg/mL. According to the CLSI guidelines [19], the MICs of 40 isolates (90.9%) were interpreted as intermediate (0.125–0.5 μg/mL). However, based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [31], the MICs of these isolates were categorised as resistant (MIC ≥ 0.094 μg/mL).

Table 2
Antibiotic resistance profiles among 44 *Salmonella enterica* serotype Enteritidis isolates.

Antibiotype	Resistance pattern	*n* (%)
Ab1	CIP-CTX-CAZ-STR-CXM-FEP-CRO-NAL-TET-SXT-AMX	1 (2.3)
Ab2	CIP-CTX-CAZ-STR-CXM-FEP-CRO-NAL-SXT-AMX	1 (2.3)
Ab3	CIP-CTX-CAZ-STR-CXM-FEP-CRO-TET-AMX	1 (2.3)
Ab4	CIP-STR-CXM-NAL-TET-SXT-AMX	2 (4.5)
Ab5	CIP-STR-CXM-NAL-TET-SXT	1 (2.3)
Ab6	CIP-STR-CXM-TET-AMX-CHL	1 (2.3)
Ab7	CIP-CXM-FEP-NAL-SXT-AMX	1 (2.3)
Ab8	CIP-CXM-NAL-TET-SXT	1 (2.3)
Ab9	CIP-CXM-FEP-NAL	1 (2.3)
Ab10	CIP-CXM-NAL-SXT	1 (2.3)
Ab11	CIP-STR-CXM-NAL	7 (15.9)
Ab12	CIP-STR-CXM-TET	1 (2.3)
Ab13	CIP-CAZ-CXM-NAL	1 (2.3)
Ab14	CIP-CXM-NAL	8 (18.2)
Ab15	CIP-STR-NAL	2 (4.5)
Ab16	CIP-NAL	6 (13.6)
Ab17	CIP-CXM	2 (4.5)
Ab18	CIP-STR	1 (2.3)
Ab19	NAL	1 (2.3)
Ab20	CIP	1 (2.3)
Ab21	CXM	2 (4.5)
Ab22	Sensitive to all tested antibiotics	1 (2.3)

CIP, ciprofloxacin; CTX, cefotaxime; CAZ, ceftazidime; STR, streptomycin; CXM, cefuroxime; FEP, cefepime; CRO, ceftriaxone; NAL, nalidixic acid; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; AMX, amoxicillin; CHL, chloramphenicol.

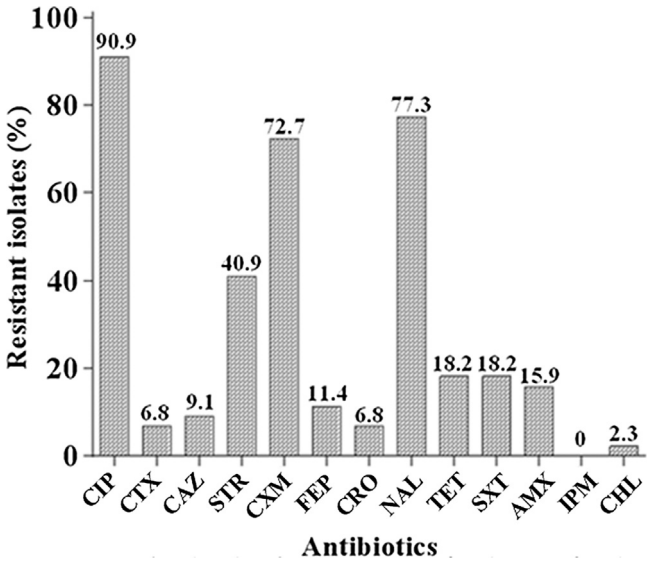

Fig. 1. Antimicrobial resistances of 34 *Salmonella enterica* serotype Enteritidis isolates. CIP, ciprofloxacin; CTX, cefotaxime; CAZ, ceftazidime; STR, streptomycin; CXM, cefuroxime; FEP, cefepime; CRO, ceftriaxone; NAL, nalidixic acid; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; AMX, amoxicillin; IPM, imipenem; CHL, chloramphenicol.

Resistance to chloramphenicol (2.3%), ceftriaxone (6.8%) and cefotaxime (6.8%) was observed less frequently (Fig. 1). Furthermore, all of the isolates were susceptible to imipenem.

Regarding virulence genes, all of the isolates carried *invA*, *sefA*, *sipA* and *sopE2* (Table 3). Of the other virulence genes, *hilA*, *stn*, *sipC* and *sopB* were each found in 43 isolates (97.7%), whilst *ssrA* and *sopE* were each detected in 40 isolates (90.9%) (Table 3). The least prevalent virulence gene was *ssaR* (*n* = 25; 56.8%). Seventeen virulence profiles were observed among the strains according to the combinations of virulence genes (Fig. 2). The most common virulence profile was VP1, which accounted for 17 (38.6%) of the isolates, followed by VP15 (*n* = 9; 20.5%).

Table 3
Distribution of virulence genes among 44 *Salmonella enterica* serotype Enteritidis strains isolated from patients with gastroenteritis.

Virulence gene	*n* (%)
invA	44 (100)
hilA	43 (97.7)
pefA	31 (70.5)
rck	34 (77.3)
stn	43 (97.7)
ssrA	40 (90.9)
ssaR	25 (56.8)
sefA	44 (100)
spvC	36 (81.8)
sipA	44 (100)
sipC	43 (97.7)
sopB	43 (97.7)
sopE	40 (90.9)
sopE2	44 (100)

Two distinct PFGE patterns were observed among 44 *S.* Enteritidis isolates, with all of the isolates categorised into two clusters. Most of the isolates ($n = 40$) were grouped in cluster B, whereas cluster A contained only 4 isolates. In addition, there was no association between virulence profiles or antibiotypes and PFGE clusters, i.e. isolates of the same PFGE pattern had different virulence profiles or antibiotypes, and vice versa (Fig. 3).

4. Discussion

Salmonella, as the most established zoonotic enteric pathogen in the global food chain, poses significant public health concerns worldwide [13,32]. Currently, *S.* Enteritidis is the most prevalent serotype isolated in Asian, African, European and Latin American countries [16]. *Salmonella* Enteritidis accounts for a large number of outbreaks and sporadic cases in humans [32]. Thus, epidemiological studies are not only important for elucidating contamination routes and improving monitoring, but also for implementing control programmes [16].

The findings of this study revealed that 68.2% of the *S.* Enteritidis isolates were resistant to three or more antibiotics, suggesting that the percentage of *S.* Enteritidis strains resistant to antimicrobials has increased over time in Iran. These results might be due to indiscriminate use of prescribed antibiotics together with horizontal transfer and clonal spread of resistance genes [12,17]. In this study, a high frequency of resistance to ciprofloxacin (90.9%) was observed, which is higher than rates reported in other studies from the USA [14], Italy [23], Ireland [33], Malaysia [12] and Iran [34]. The results of the current study are in accordance with a previous study performed in Iran where Morshed and Peighambari observed high levels of resistance to nalidixic acid (ca. 77%) among *S.* Enteritidis isolated from humans [35]. However, previous investigations from European countries such as Finland, France, Ireland, Lithuania, the Netherlands, Portugal, Spain and the UK demonstrated lower rates of resistance to nalidixic acid compared with the current study [33]. It is also believed that the wide usage of quinolones in treatment on poultry farms and in animal husbandry in Iran has an indicative role in the emergence and dissemination of nalidixic acid resistance in *Salmonella* in food animals, resulted in a high probability of transmission to humans [17]. Except for cefuroxime, isolates in the current study exhibited lower rates of resistance to third-generation cephalosporins such

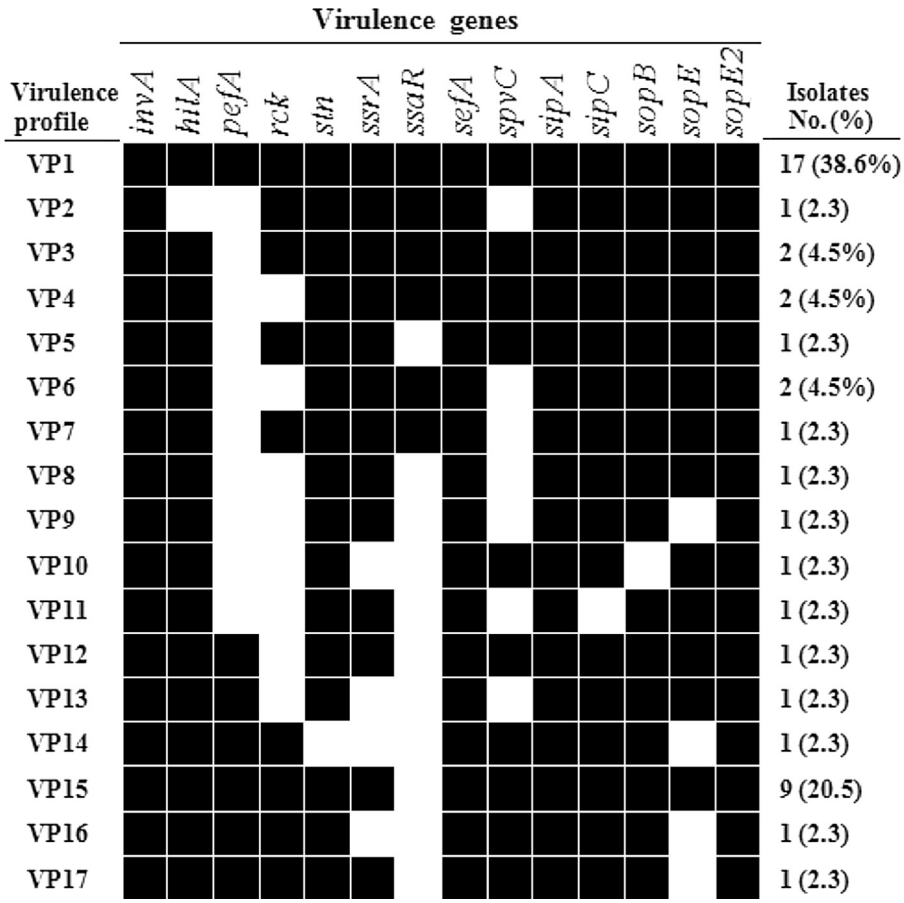

Fig. 2. Virulence profiles (virulotypes) of *Salmonella enterica* serotype Enteritidis isolates. Black and white square denotes the presence and absence of virulence genes, respectively.

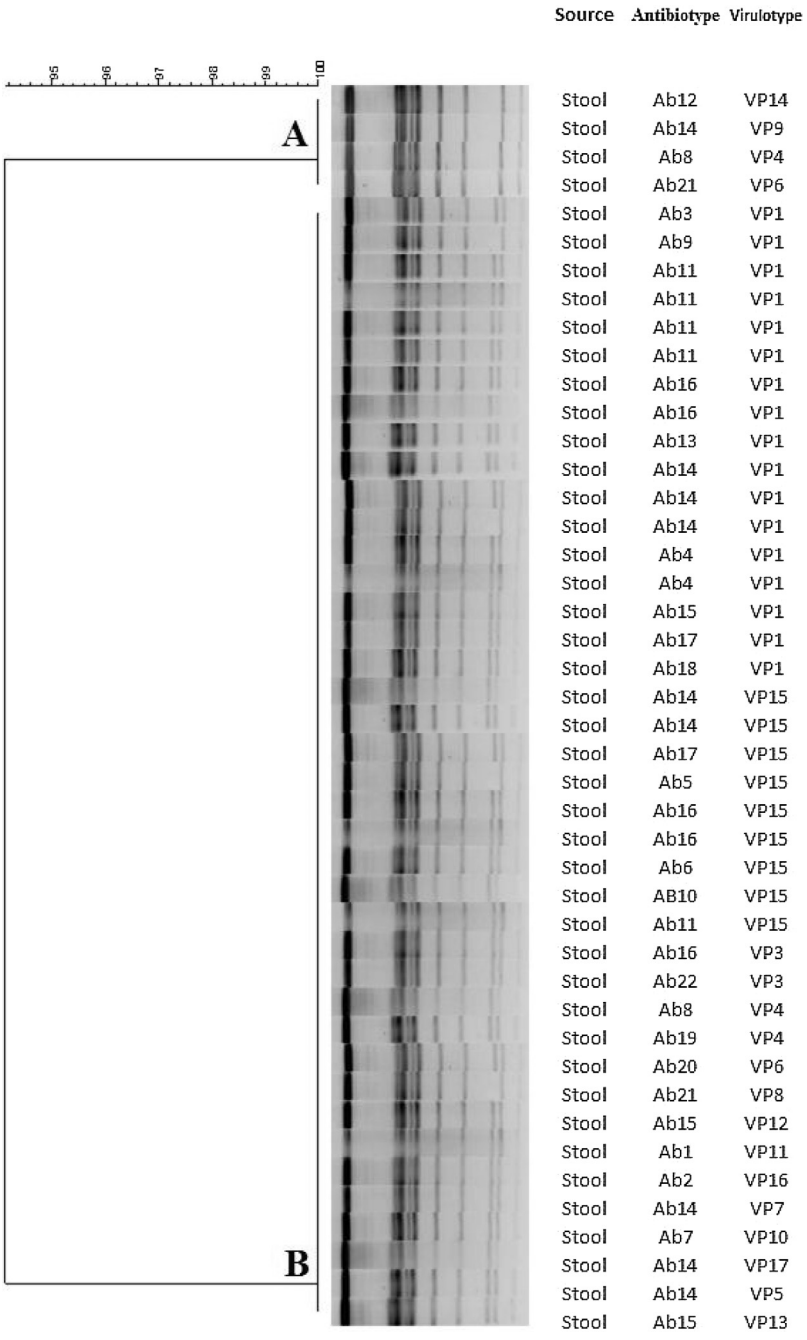

Fig. 3. Unweighted pair-group method with arithmetic mean (UPGMA) dendrogram representing the genetic relationship among *Salmonella enterica* serotype Enteritidis strains based on pulsed-field gel electrophoresis (PFGE) fingerprints. Similarity (%) between patterns was calculated from the Dice index.

as ceftazidime (9.1%) and cefotaxime (6.8%), which are higher than those of other studies from Austria [33], Italy [23] and Brazil [16]. No imipenem-resistant isolate was found in the present study, supporting the current scenario observed in Tehran in which no imipenem-resistant isolate was found among 42 *S.* Enteritidis isolated from humans [11]. This can be attributed to restricted prescription of carbapenems such as imipenem in Iran. Therefore, imipenem can be considered as an alternative drug for clinical cases where more resistance to extended-spectrum antimicrobials is encountered [17].

The virulence of bacteria is influenced both by the presence of virulence genes and antibiotic resistance [23]. In this study, all of the isolates were examined for the presence of 14 virulence genes in order to evaluate the potential factors that may contribute to the ability of *S.* Enteritidis to cause an infection. Based on virulence profiles, >38% of the *S.* Enteritidis isolates harboured 14 virulence genes, which highlights the pathogenic potential of the studied strains. Notably, all of the *S.* Enteritidis isolates were positive for *invA*, *sefA*, *sipA* and *sopE2* genes. In addition, >90% of the isolates harboured *hilA*, *stn*, *sipC*, *sopB*, *ssrA* and *sopE* genes, indicating that these virulence genes are widespread in *S.* Enteritidis. This is in agreement with the findings of previous studies [2,16], which showed that the majority of *S.* Enteritidis strains harboured all of the virulence genes tested. Many of these virulence factors, such as InvA, HilA and the SPI-1 effectors (including SipA, SopA, SopB, SopD and SopE2), are associated with human intestinal epithelial cell invasion and enterocolitis [15]. It should be noted that presence of the *stn* gene in the majority of clinical isolates

highlights the role of this virulence gene in production of enterotoxin, which is responsible for causing acute gastroenteritis [2]. In the present study, the *pefA* and *rck* genes were found in 70.5% and 77.3% of strains, respectively, which is lower than the rates reported in a previous study from Italy [23]. However, the prevalence of *spvC* in this study (81.8%) was much higher than that reported in Italy [23]. To our knowledge, there is a paucity of surveillance data regarding the prevalence of virulence genes among *S.* Enteritidis strains isolated from humans in Iran. For instance, in a study from Iran, Amini et al. identified the *spvC* gene in 90% of *S.* Enteritidis isolated from humans [36], which was higher than in the current study. It is worth noting that the *spvC*, *pefA* and *rck* genes are located on plasmids and contribute to adhesion and systemic infection against host cells [37].

PFGE is still regarded as the 'gold standard' fingerprinting method used to assess relatedness among *S.* Enteritidis isolates from different sources and for outbreak investigations [38]. In the current study, indistinguishable PFGE patterns were observed in most of the *S.* Enteritidis isolates. The low genetic diversity among *S.* Enteritidis is also shown in some previous studies, suggesting that this serotype is highly clonal [39,40]. However, this may also be due to a confined geographical coverage and the limited number of isolates in this study. Therefore, for these organisms the best discrimination can be achieved by using a combination of different typing methods such as enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and multiple-locus variable-number tandem-repeat analysis (MLVA) [41,42].

In conclusion, this study provides valuable information on the virulence gene content and antibiotic resistance in *S.* Enteritidis isolated from human sources in Iran. However, future studies should examine the relationship between these virulence genes and the severity of salmonellosis. In addition, the findings of this study suggest that *S.* Enteritidis isolates were derived from a limited number of clones that undergo minor genetic changes over the course of time. To gain more insight into the genetic diversity of *S.* Enteritidis isolates, additional strains from a variety of regions and combination of other genotyping methods should also be considered.

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Competing interests

None declared.

Ethical approval

Not required.

References

[1] McKelvey JA, Yang M, Jiang Y, Zhang S. *Salmonella enterica* serovar Enteritidis antimicrobial peptide resistance genes aid in defense against chicken innate immunity, fecal shedding, and egg deposition. Infect Immun 2014;82:5185–202.
[2] Zou M, Keelara S, Thakur S. Molecular characterization of *Salmonella enterica* serotype Enteritidis isolates from humans by antimicrobial resistance, virulence genes, and pulsed-field gel electrophoresis. Foodborne Pathog Dis 2012;9:232–8.
[3] US Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States. Atlanta, GA: CDC; 2013 https://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf. [Accessed 10 July 2016].
[4] European Food Safety Authority. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Unionin 2006. EFSA J 2007;130:1–352.
[5] Retamal P, Fresno M, Dougnac C, Gutierrez S, Gornall V, Vidal R, et al. Genetic and phenotypic evidence of the *Salmonella enterica* serotype Enteritidis human–animal interface in Chile. Front Microbiol 2015;6:464.
[6] World Health Organization. *Salmonella* (non-typhoidal). Geneva, Switzerland: WHO; 2016. Fact sheet no. 139, revised December 2016. http://www.who.int/mediacentre/factsheets/fs139/en/. [Accessed 20 January 2018].
[7] Crum-Cianflone NF. Salmonellosis and the gastrointestinal tract: more than just peanut butter. Curr Gastroenterol Rep 2008;10:424–31.
[8] Cordeiro NF, Yim L, Betancor L, Cejas D, García-Fulgueiras V, Inés Mota M, et al. Identification of the first bla_{CMY-2} gene in *Salmonella enterica* serovar Typhimurium isolates obtained from cases of paediatric diarrhoea illness detected in South America. J Glob Antimicrob Resist 2013;1:143–8.
[9] Khumalo J, Saidi B, Mbanga J. Evolution of antimicrobial resistance of *Salmonella enteritidis* (1972–2005). Onderstepoort J Vet Res 2014;81:e1–6.
[10] Ahmed AM, Younis EE, Ishida Y, Shimamoto T. Genetic basis of multidrug resistance in *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from diarrheic calves in Egypt. Acta Trop 2009;111:144–9.
[11] Firoozeh F, Zahraei-Salehi T, Shahcheraghi F, Karimi V, Aslani MM. Characterization of class I integrons among *Salmonella enterica* serovar Enteritidis isolated from humans and poultry. FEMS Immunol Med Microbiol 2012;64:237–43.
[12] Ngoi ST, Thong KL. Molecular characterization showed limited genetic diversity among *Salmonella* Enteritidis isolated from humans and animals in Malaysia. Diagn Microbiol Infect Dis 2013;77:304–11.
[13] Elemfareji OI, Thong KL. Comparative virulotyping of *Salmonella typhi* and *Salmonella enteritidis*. Indian J Microbiol 2013;53:410–7.
[14] Han J, Gokulan K, Barnette D, Khare S, Rooney AW, Deck J, et al. Evaluation of virulence and antimicrobial resistance in *Salmonella enterica* serovar Enteritidis isolates from humans and chicken- and egg-associated sources. Foodborne Pathog Dis 2013;10:1008–15.
[15] Hur J, Kim JH, Park JH, Lee YJ, Lee JH. Molecular and virulence characteristics of multi-drug resistant *Salmonella* Enteritidis strains isolated from poultry. Vet J 2011;189:306–11.
[16] Campioni F, Moratto Bergamini AM, Falcão JP. Genetic diversity, virulence genes and antimicrobial resistance of *Salmonella* Enteritidis isolated from food and humans over a 24-year period in Brazil. Food Microbiol 2012;32:254–64.
[17] Fardsanei F, Nikkhahi F, Bakhshi B, Salehi TZ, Tamai IA, Soltan Dallal MM. Molecular characterization of *Salmonella enterica* serotype Enteritidis isolates from food and human samples by serotyping, antimicrobial resistance, plasmid profiling, (GTG)5-PCR and ERIC-PCR. New Microbes New Infect 2016;14:24–30.
[18] Mirzaie S, Hassanzadeh M, Ashrafi I. Identification and characterization of *Salmonella* isolates from captured house sparrows. Turk J Vet Anim Sci 2010;34:181–6.
[19] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 26th ed. Document M100S, 26th ed. Wayne, PA: CLSI; 2016.
[20] Lu Y, Zhao H, Sun J, Liu Y, Zhou X, Beier RC, et al. Characterization of multidrug-resistant *Salmonella enterica* serovars Indiana and Enteritidis from chickens in Eastern China. PLoS One 20149:e96050.
[21] Chiu CH, Ou JT. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, *invA* and *spvC*, by an enrichment broth culture–multiplex PCR combination assay. J Clin Microbiol 1996;34:2619–22.
[22] 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;133:328–34.
[23] Capuano F, Mancusi A, Capparelli R, Esposito S, Proroga YT. Characterization of drug resistance and virulotypes of *Salmonella* strains isolated from food and humans. Foodborne Pathog Dis 2013;10:963–8.
[24] Prager R, Fruth A, Tschäpe H. *Salmonella* enterotoxin (*stn*) gene is prevalent among strains of *Salmonella enterica*, but not among *Salmonella bongori* and other Enterobacteriaceae. FEMS Immunol Med Microbiol 1995;12:47–50.
[25] Heithoff DM, Shimp WR, Lau PW, Badie G, Enioutina EY, Daynes RA, et al. Human *Salmonella* clinical isolates distinct from those of animal origin. Appl Environ Microbiol 2008;74:1757–66.
[26] Hu Q, Coburn B, Deng W, Li Y, Shi X, Lan Q, et al. *Salmonella enterica* serovar Senftenberg human clinical isolates lacking SPI-1. J Clin Microbiol 2008;46:1330–6.
[27] Kutsukake K, Nakashima H, Tominaga A, Abo T. Two DNA invertases contribute to flagellar phase variation in *Salmonella enterica* serovar Typhimurium strain LT2. J Bacteriol 2006;188:950–7.
[28] Raffatellu M, Wilson RP, Chessa D, Andrews-Polymenis H, Tran QT, Lawhon S, et al. SipA, SopA, SopB, SopD, and SopE2 contribute to *Salmonella enterica* serotype Typhimurium invasion of epithelial cells. Infect Immun 2005;73:146–54.
[29] Hopkins KL, Threlfall EJ. Frequency and polymorphism of *sopE* in isolates of *Salmonella enterica* belonging to the ten most prevalent serotypes in England and Wales. J Med Microbiol 2004;53:539–43.
[30] US Centers for Disease Control and Prevention. Standard operating procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*. Atlanta, GA: CDC; 2013.

[31] European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.0, 2017. EUCAST; 2017 http://www.eucast.org/clinical_breakpoints/. [Accessed 9 February 2018].
[32] Nair A, Rawool DB, Doijad S, Poharkar K, Mohan V, Barbuddhe SB, et al. Biofilm formation and genetic diversity of *Salmonella* isolates recovered from clinical, food, poultry and environmental sources. Infect Genet Evol 2015;36:424–33.
[33] European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2012. EFSA J 2014;12:3590.
[34] Eshraghi S, Soltan Dalall MM, Fardsanei F, Zahraii Salehi T, Ranjbar R, Nikmanesh B, et al. *Salmonella enteritidis* and antibiotic resistance patterns: a study on 1950 children with diarrhea. Tehran Univ Med J 2010;67:876–82.
[35] Morshed R, Peighambari SM. Drug resistance, plasmid profile and random amplified polymorphic DNA analysis of Iranian isolates of *Salmonella* Enteritidis. New Microbiol 2010;33:47–56.
[36] Amini K, Zahraei Salehi T, Nikbakht G, Ranjbar R, Amini J, Ashrafganjooei SB. Molecular detection of *invA* and *spv* virulence genes in *Salmonella* Enteritidis isolated from human and animals in Iran. Afr J Microbiol Res 2010;4:2202–10.
[37] Huehn S, La Ragione RM, Anjum M, Saunders M, Woodward MJ, Bunge C, et al. Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. Foodborne Pathog Dis 2010;7:523–35.
[38] Wattiau P, Boland C, Bertrand S. Methodologies for *Salmonella enterica* subsp. *enterica* subtyping: gold standards and alternatives. Appl Environ Microbiol 2011;77:7877–85.
[39] Ozdemir K, Acar S. Plasmid profile and pulsed-field gel electrophoresis analysis of *Salmonella enterica* isolates from humans in Turkey. PLoS One 20149:e95976.
[40] Abbassi-Ghozzi I, Jaouani A, Aissa RB, Martinez-Urtaza J, Boudabous A, Gtari M. Antimicrobial resistance and molecular analysis of non-typhoidal *Salmonella* isolates from human in Tunisia. Pathol Biol (Paris) 2011;59:207–12.
[41] Bertrand S, De Lamine de Bex G, Wildemauwe C, Lunguya O, Phoba MF, Ley B, et al. Multi locus variable-number tandem repeat (MLVA) typing tools improved the surveillance of *Salmonella enteritidis*: a 6 years retrospective study. PLoS One 201510:e0117950.
[42] Campioni F, Pitondo-Silva A, Bergamini AM, Falcão JP. Comparison of four molecular methods to type *Salmonella* Enteritidis strains. APMIS 2015;123:422–6.