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Genetic diversity and virulence genes of *Salmonella enterica* subspecies *enterica* serotype Enteritidis isolated from meats and eggs

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ABSTRACT

Salmonella enterica subspecies *enterica* serotype Enteritidis (*S.* Enteritidis) is one of the leading causes of food-borne gastroenteritis associated with the consumption of contaminated food products of animal origin. Little is known about the genetic diversity and virulence content of *S.* Enteritidis isolated from poultry meats and eggs in Iran. A total of 34 *S.* Enteritidis strains were collected from different food sources of animal origin in Tehran from May 2015 to July 2016. All of the *S.* Enteritidis strains were serotyped, antimicrobial susceptibility tested, and characterized for virulence genes. Pulsed-field gel electrophoresis (PFGE) was also applied for comparison of genetic relatedness. All of the strains harbored *invA*, *hilA*, *ssrA*, *sefA*, *spvC*, and *sipA* genes. A high prevalence of resistance against certain antibiotics such as cefuroxime (79.4%), nalidixic acid (47%), and ciprofloxacin (44.2%) was also observed. Regarding PFGE, *S.* Enteritidis strains from different sources showed considerable overlap, suggesting the lack of diversity among these isolates. Moreover, no correlation between virulence profiles or antibiotypes and PFGE clusters was observed. In conclusion, our study provided valuable information on virulence gene content, antibiotic resistance, and genetic diversity of *S.* Enteritidis isolated from food sources.

1. Introduction

Salmonella enterica subspecies *enterica* serotype Enteritidis (*S.* Enteritidis) is one of the leading causes of food-borne infections associated with the consumption of contaminated food products of animal origin in humans. Poultry products and eggs are usually recognized as important reservoirs for this pathogen [1] Among more than 2500 known serotypes, *S.* Enteritidis is still by far the most common serotype reported from human isolates in most industrialized countries [2,3] In addition, a significant increase in the number of *Salmonella* infections has been observed in Iran over the past years [4–8].

S. Enteritidis pathogenicity has been related to a number of virulence factors that help the pathogen in adhesion and invasion mechanisms. Some of these virulence factors are well-documented and encountered more commonly than the other ones. Most of the virulence related genes are located on a virulence-associated plasmid (pSTV) and chromosomal *Salmonella* Pathogenicity Islands (SPIs). For instance, *spv* operon, which resides on a highly conserved 8 kb region within pSTV, potentiates the systemic spread of the pathogen and help in its replication in extra-intestinal sites. Furthermore, type 3 secretion systems (T3SS), which are encoded by SPI-1 and SPI-2, promote intestinal colonization. In this regard, the SPI-1-encoded genes such as *invA*, *sipA*, *sipD*, *sopA*, *sopB*, *sopD*, and *sopE* allow *S.* Enteritidis to invade phagocytic and non-phagocytic cells, while the SPI-2-encoded genes such as *ssaR* and *ssrA* enables *S.* Enteritidis to survive and replicate in host cells, macrophages in particular [9–11].

Although *S.* Enteritidis generally causes a relatively mild, brief, and self-limited gastroenteritis in humans, some cases may involve invasive infections, for which antimicrobial therapy is usually

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warranted [10,11]. On the other hand, antimicrobial agents are used widely in livestock and poultry to treat or prevent diseases and to promote growth. As a consequence, animal food sources can harbor antibiotic-resistant pathogens and function as a vehicle for spread of these strains to humans [4,12,13].

In this study, the objectives were to investigate frequencies of virulence genes and antibiotic resistance in *S.* Enteritidis strains isolated from food products of animal origin including poultry meats and eggs. Furthermore, we utilized pulsed-field gel electrophoresis (PFGE) to determine the clonal relatedness and to elucidate any epidemiological links between these strains.

2. Material and methods

2.1. Bacterial strains

A total of 34 *S.* Enteritidis strains recovered from meats and eggs were used in this study. Of these strains, 16 were isolated from poultry meat (14 from chicken meats and 2 from duck meats), 10 from table eggs, 4 from lamb meats, and 4 from beeves. These samples were collected from May 2015 to July 2016 in Tehran, Iran. Identification of *S.* Enteritidis strains was conducted according to routine biochemical and serological tests [14]. A multiplex PCR was also performed to confirm *S.* Enteritidis isolates as described previously [5].

2.2. Antimicrobial susceptibility testing

The antibiotic susceptibility testing was performed by Kirby-Baur disc diffusion method on Mueller-Hinton agar (Oxoid Ltd, Basingstoke, UK) plates. The following antibiotics (Mast Diagnostics, Mast Group Ltd, Merseyside, UK) were tested as recommended by CLSI guidelines [15]: cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (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).

2.3. Detection of virulence genes

Genomic DNA was extracted from *S.* Enteritidis isolates by QIAamp DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions and used for subsequent Polymerase Chain Reaction (PCR) analysis. The name of virulence genes, primer sequences, and PCR annealing temperatures are shown in Table 1. Amplification reactions were performed in a PEQLAB thermal cycler (Germany) in a final volume of 25 μL containing 1X PCR buffer (50 mmol/L KCl, 10 mmol/L Tris, pH = 9), 2.5 mmol/L $MgCl_2$, 0.2 mmol/L of each primer with 1 U of Taq DNA polymerase, and 2 μL of sample DNA. The PCR conditions for amplification were as follows: 5 min of initial denaturation at 95 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at different temperatures (Table 1) for 30 s, and extension at 72 °C for 60 s, ending with

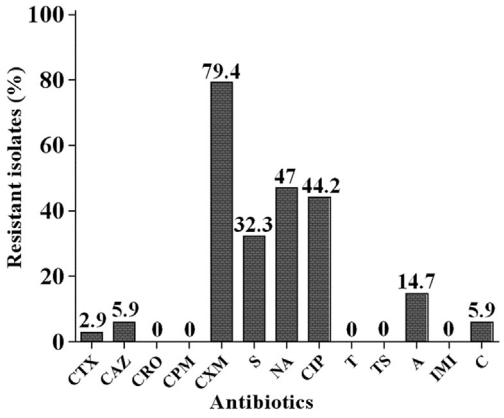

Fig. 1. Percentages of antimicrobial resistances in 34 *S.* Enteritidis isolates.
Abbreviation: CTX, Cefotaxime; CAZ, Ceftazidime; CRO, Ceftriaxone; CPM, Cefepime; CXM, Cefuroxime; S, Streptomycin; NA, Nalidixic acid; CIP, Ciprofloxacin; T, Tetracycline; TS, Trimethoprim/sulfamethoxazole; A, amoxicillin; IMI, imipenem; C, Chloramphenicol.

Table 1
Primers used in this study.

Primer	Nucleotide Sequences (5′ to 3′)	Amplicon size (bp)	Annealing temperature (°C)	Reference
invA-F	ACAGTGCTCGTTTACGACCTGAAT	243	56	[17]
invA-R	AGACGACTGGTACTGATCGATAAT			
hilA-F	CGTGAAGGGATTATCGCAGT	296	56	[18]
hilA-R	GTCCGGGAATACATCTGAGC			
spvC-F	ACTCCTTGCACAACCAAATGCGGA	571	56	[17]
spvC-R	TGTCTCTGCATTTCGCCACCATCA			
sipA-F	CCATTCGACTAACAGCAGCA	449	56	[18]
sipA-R	CGGTCGTACCGGCTTTATTA			
sopE-F	CGAGTAAAGACCCCGCATAC	362	58	[19]
sopE-R	GAGTCGGCATAGCACACTCA			
stnP1	TTGTCTCGCTATCACTGGCAACC	617	59	[20]
stnM13	ATTCGTAACCCGCTCTCGTCC			
pefA-F	TTGCACTGGGTGGTTCTGG	485	56	[21]
pefA-R	TGTAACCCACTGCGAAAG			
rck-F	AACGGACGGAACACAGAGTC	189	59	[22]
rck-R	TGTCCTGACGAAAGTGCATC			
sipC-F	AGACAGCTTCGCAATCCGTT	446	61	This study
sipC-R	ATTCATCCCTTCGCGCATCA			
ssaR-F	GTTCGGATTTGCTTCGG	1628	59	[23]
ssaR-R	TCTCCAGTGACTAACCCTAACCAA			
ssrA-F	CTTACGATTACGCCATTTACGG	706	58	[24]
ssrA-R	ATTTGGTGGAGCTGGCGGGACT			
sopB-F	CCTCAAGACTCAAGATG	1987	56	[19]
sopB-R	TACGCAGGAGTAAATCGGTG			
sef A-F	GCAGCGGTTACTATTGCAGC	321	55	[25]
sefA-R	TGTGACAGGGACATTTAGCG			