Original Article

Plasmid Profile and Enterobacterial Repetitive Intergenic Consensus-polymerase Chain Reaction Characterization of *Salmonella* Enteritidis Isolates Recovered From Day-old Broilers

Farzaneh Jeldi, Seyed Mostafa Peighambari*, Jamshid Razmyar, Aazam Yazdani, Fattaneh Naderinezhad

Department of Avian Health and Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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How to Cite This Article Jeldi, F., Peighambari, S. M., Razmyar, J., Yazdani, A., & Naderinezhad, F. (2026). Plasmid Profile and Enterobacterial Repetitive Intergenic Consensus-polymerase Chain Reaction Characterization of *Salmonella* Enteritidis Isolates Recovered From Day-old Broilers. *Iranian Journal of Veterinary Medicine, 20*(4), 653-666. http://dx.doi.org/10.32598/ijvm.20.4.1005789

http://dx.doi.org/10.32598/ijvm.20.4.1005789

ABSTRACT

Background: Salmonellosis is widely recognized as an epidemic disease with public health significance. *Salmonella* Enteritidis has the ability to cause infections in humans and various animals, such as poultry. It is considered one of the most important and common serovars isolated in different regions of the world.

Objectives: The aim of this study was to characterize 100 *S.* Enteritidis isolates recovered from day-old broilers with respect to plasmid profile and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR).

Methods: All *Salmonella* isolates were recovered from cases referred to our laboratory. Standard bacteriological and molecular procedures were performed for the isolation and identification of *S.* Enteritidis. A commercial kit was used to extract the plasmids. Two primers, ERIC1R and ERIC2, were used for ERIC-PCR.

Results: Six plasmid profiles were identified among 100 isolates, and a plasmid with a molecular weight of greater than 10 kb was observed in all isolates. In ERIC-PCR, seven different profiles were identified with each primer. Using the ERIC1R primer, 80% of the isolates belonged to profiles A, B, and G, and the rest were distributed among the other four profiles. Additionally, using the ERIC2 primer, 85% of the isolates belonged to three profiles: D, F, and G, and the rest were distributed among the other four profiles. When the results of plasmid profiles and ERIC-PCR analysis using two different primers were combined, 100 *S.* Enteritidis isolates were divided into 43 groups, with seven groups comprising 54% of the isolates.

Conclusion: This study demonstrated that molecular techniques, such as ERIC-PCR and plasmid profile alone have limited power in differentiating between various isolates, and a combination of patterns obtained from multiple techniques will provide greater discriminatory power. This study presented genetic data related to *S.* Enteritidis isolates, which can be used for a broader epidemiological study at the national level.

Keywords: *Salmonella* Enteritidis, Public health, Poultry, Plasmid profile, ERIC-PCR

Article info:
Received: 20 Dec 2025
Accepted: 17 Feb 2026
Publish: 01 Jul 2026

*** Corresponding Author:**
Seyed Mostafa Peighambari, Professor.
Address: *Department of Avian Health and Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
Phone: *+98 (21) 61117150*
E-mail: *mpeigham@ut.ac.ir*

Introduction

Salmonella is a gram-negative, motile bacterium within the Enterobacteriaceae family, recognized as a significant pathogen capable of infecting a wide variety of animal hosts (Hawker et al., 2019). This bacterium is one of the most well-known causes of foodborne infectious diseases and is a major public health concern worldwide (El-Saadony et al., 2022). In the 1950s, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) identified *Salmonella* as a hazardous bacterium with zoonotic potential, posing significant economic consequences (Akinola et al., 2019; Marouf et al., 2022).

The primary reservoir of *Salmonella* is the intestines of humans and animals, although this organism has also been identified in reptiles and insects. These bacteria are excreted in the feces of humans and animals, leading to contamination of water, food, and the environment. Some serotypes are host-specific, while others can infect any warm-blooded animal. Approximately 50 *Salmonella* serovars are involved in causing disease in both humans and animals (Popa & Papa, 2021).

Poultry products are a major reservoir of *Salmonella*, posing risks to human health, poultry production, and food products (Sornplang et al., 2022; Boiko et al., 2024). In poultry farms, flocks can be infected through both vertical and horizontal transmission (Antunes et al., 2016; El-Saadony et al., 2022). Feces, bedding, water, feed, equipment, other infected birds, animals, rodents, and workers who carry *Salmonella* infection may all play a role in the horizontal spread of *Salmonella* among birds (Zamora-Sanabria & Alvarado, 2017; El-Saadony et al., 2022). Vertical transmission occurs through the ovaries or contamination of the egg shell after laying, representing the parent-to-offspring route of infection (Pande et al., 2016). Poultry salmonellosis causes high mortality, reduced flock performance, and increased susceptibility to other diseases, leading to economic losses (Kaonga et al., 2021).

Approximately 99% of *Salmonella* infections (both systemic infections and enteritis) in humans and animals are caused by *Salmonella enterica* subspecies enterica serovars, while a small fraction of these serovars lead to typhoid fever in a limited range of humans and animal species. Paratyphoid serovars, such as *S. enterica* subspecies *enterica* serovar Enteritidis, have a broad host range, colonize the gastrointestinal tract of their hosts, and are zoonotic, causing acute enteritis or subclinical infections. Review of available data on the prevalence of salmonellosis in humans and animals across different countries shows variable rates. It appears that in poultry, there is a rotation between different serotypes, with one serotype replacing another during certain periods (Sever & Akan, 2019). Surveillance programs worldwide aim to control *Salmonella* and reduce its entry into the food chain (Gast et al., 2020). In the U.S., foodborne salmonellosis costs an estimated 4–11 billion USD annually in medical care, lost production, and premature deaths (Scharff et al., 2012).

Among more than 2,500 recognized *Salmonella* serovars, about 10% are found in poultry, with *Salmonella* Enteritidis (SE) and *S.* Typhimurium (ST) being the most prevalent worldwide (Shivaning Karabasanavar et al., 2020; Patra et al., 2021). SE infections in humans are often linked to the consumption of contaminated poultry products; especially eggs, while *S.* Typhimurium infections are mostly associated with the consumption of pork, poultry, beef, and even seafood (Antunes et al., 2016; Beshiru et al., 2019).

Given the health risks posed by *Salmonella*, especially SE for both humans and animals, it is of great importance to investigate the contamination of poultry flocks and study the various characteristics of related isolates, including their genotypic traits, from an epidemiological perspective and to determine the source of contamination. Using DNA-related techniques, researchers can now categorize isolates below the serotype level. These techniques include plasmid profiling, enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), pulsed-field gel electrophoresis (PFGE), etc.

This study compared 100 SE isolates obtained from day-old broiler chicks across Iran using two techniques, plasmid profiling and ERIC-PCR, to assess genetic diversity and the relationships between different isolates.

Materials and Methods

Bacterial isolates

In 2023-2024, a total of 100 SE isolates originated from day-old broiler chicks that were submitted to university or private partner laboratories from different regions of Iran were used in this study. The isolates were confirmed through simultaneous studies using standard culture methods and PCR (Piryaei et al., 2025). The isolates were preserved as pure cultures in brain heart infusion (BHI) broth containing 25% glycerol, stored at -20 °C in sterile plastic vials, with each vial labeled with a code and relevant information for each isolate.

Plasmid profile analysis

Plasmid DNA was extracted and purified from bacterial isolates using the FavorPrep™ Plasmid DNA Extraction Kit (Favorgen Biotech Corp., Taiwan). Plasmids were separated via gel electrophoresis (Paya Pajoohesh Pars, Iran) using a 0.7% agarose gel containing DNA Safe Stain® (SinaClon, Iran) in 1x TBE buffer. The gels were first run for 10 minutes at 100 volts and then for approximately 2 hours at 70 volts. After electrophoresis, the gels were exposed to ultraviolet light and photographed (Kiagene, Iran). A commercial DNA ladder (SinaClon) was used as molecular weight markers in each gel running.

ERIC-PCR

To extract bacterial DNA, 1 mL pure overnight culture of each SE isolate grown overnight at 37 ˚C for 16 h was transferred to a clean 1.5 mL microtube and centrifuged for 5 min at 10,000× g. The supernatants were carefully removed and discarded. The pellet was re-suspended in 300 µL sterile double distilled water by vortexing, incubated for 15 min at 100 ˚C, chilled on ice immediately, and centrifuged again for 5 min at 14,000×g at 4 ˚C. The supernatant was removed and used as template DNA. The concentration of DNA was determined by Biophotometer (Eppendorff, Germany) and adjusted to approximately 200 ng for each PCR reaction. The supernatant was stored at −20 ˚C for further use.

Two primers, ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'), were used separately (Versalovic et al., 1991; Louws et al., 1994). The primers were synthesized by Metabion (Munich, Germany). Amplification reactions for both primers were carried out in a 10 µL reaction volume containing 5.5 µL of master mix (Taq 2x Red Master Mix, Ampliqon, Denmark), 0.5 µL of primer (10 pmol/µL), 3 µL of nuclease-free water, and 1 µL of template DNA. Negative control (dH_2O instead of template DNA) was included in all PCR reaction sets. Amplifications were programmed in a thermocycler (SensoQuest, Germany) as follows: 5 cycles of 3 min at 94 ˚C, 1 min at 49 ˚C, and 2 min at 72 ˚C, followed by 35 cycles of 1 min at 94 ˚C, 1 min at 56 ˚C, and 2 min at 72 ˚C, with a final extension of 5 min at 72 ˚C (Elsayed et al., 2024).

The amplified products were detected by gel electrophoresis in 1.5% agarose gel containing Safe Stain® (SinaClon) at 70 V for 80 min in 1x TBE buffer and visualized under UV illumination. A commercial DNA ladder (ExcelBand™ 100 bp+3k DNA Ladder, SMOBiO, Taiwan) was used as a molecular weight marker for the PCR products in gel electrophoresis.

Results

Plasmid profile

Plasmid content analysis of 100 SE isolates exhibited six plasmid patterns, and all isolates contained one plasmid larger than 10 kb. Data showed that 79% of the isolates had only one plasmid, while 21% possessed two to four plasmids. Six different plasmid profiles (A to F) are demonstrated in Figure 1. The distribution of profiles among 100 isolates varied. Profiles A, B, C, D, E, and F were found in 79, 10, 4, 2, 3 and 2 isolates, respectively (Table 1). Two patterns of A and B were more frequent and included 89% of the isolates.

ERIC-PCR

ERIC1R

Using the ERIC1R primer, seven distinct ERIC profiles were identified among 100 SE isolates (Table 2). These profiles were designated as A to G. Bands with molecular weights ranging from 370 to 1200 bp were observed. All isolates contained a band with a molecular weight of 370 bp. The most prevalent profiles were A, B, and G.

ERIC2

Using the ERIC2 primer, seven distinct profiles were identified among the 100 isolates (Table 3). These profiles were designated as A–G. Bands with molecular weights ranging from 230 to 2500 bp were observed. All isolates contained a band with a molecular weight of 230 bp. The most prevalent profiles were D, F, and G.

Combination analysis

When the results of plasmid profiles and ERIC-PCR analysis using two different primers were combined, 100 SE isolates were divided into 43 groups, with 7 groups comprising 54% of the isolates (Table 4). Eleven percent of isolates belonged to group AGA, 9% of isolates belonged to group CFA. The remaining isolates from this study distributed among 41 profiles (Table 4).

Discussion

Poultry products have long been identified as one of the primary sources of non-typhoidal *S. enterica* serovars transmitted to humans. The ongoing prevalence of these infections is a significant public health concern

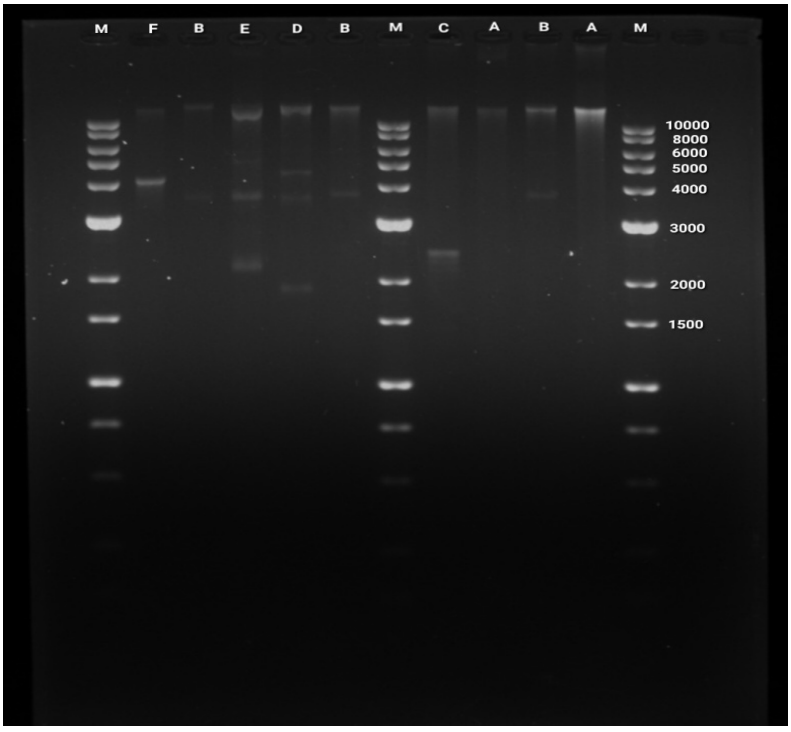

Figure 1. Plasmid profiles of *S.* Enteritidis designated as A to F

Notes: The letter "M" represents the commercial 1 kb plus DNA ladder.

worldwide, mainly due to the widespread consumption of poultry and the persistence of contamination within the production chain (Schirone & Visciano, 2021). This study examined some of the genotypic characteristics of SE isolates, which could provide valuable data for future epidemiological studies. These findings, along with additional genotypic studies, will be more useful in comparative studies with isolates from various sources. Historically, *Salmonella* strains have been classified in epidemiological studies using various methods. Initially, biochemical techniques and serotyping were the main tools for strain identification. Later, plasmid profiling and PFGE were introduced to improve strain differentiation (Li et al., 2021).

The evolution of *S. enterica* is mainly influenced by the acquisition and recombination of various mobile genetic elements, such as genomic islands, transposons, integrons, and plasmids (Cosby et al., 2015). A detailed analysis of these factors will provide insights into the drivers of resistance, as well as the adaptations of *Salmonella* to its host and environment, and the sources of resistant infections (Li et al., 2021). Some plasmid types are highly associated with specific serotypes and sources; thus, plasmids provide important information for outbreak investigations (Zhao et al., 2020). Some *Salmonella* isolates may possess virulence plasmids that play an important role in the invasion and survival of *Salmonella* within the host. Virulence plasmids carry

Table 1. Plasmid profile of 100 *S.* Enteritidis isolates

Plasmid Profile	Molecular Weight (bp)	Number of Isolates
A	>10000	79
B	>10000, 4000	10
C	>10000, 2700	4
D	>10000, 5000, 4000, 2000	2
E	>10000, 6000, 4000, 2100	3
F	>10000, 4100	2

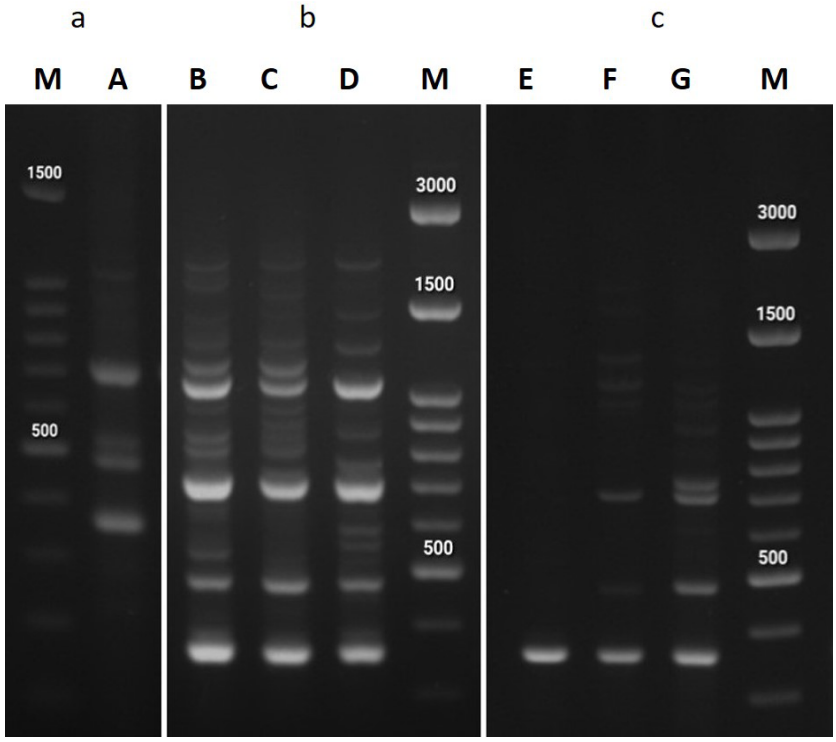

Figure 2. DNA fingerprints patterns of *S.* Enteritidis isolates by ERIC-PCR using the ERIC1R primer designated as A to G on three different gels

Notes: The letter "M" represents the commercial DNA ladder (ExcelBand™ 100 bp+3k DNA Ladder, SMOBiO).

genes that contribute to the ability of *Salmonella* to cause disease. Some plasmids are high molecular weight plasmids responsible for resistance to antimicrobial agents (Peighambari et al., 2013; Emond-Rheault et al., 2020). Plasmid profiles have been frequently used for characterization of *Salmonella* typhimurium isolates due to their plasmid diversity, but for SE, this technique initially had little value but gained importance after the year 2000. The number of plasmids and the resulting profiles vary among SE isolates (Fernandes et al., 2003; Bakeri et al., 2003; Liebana et al., 2004; Redondo-Salvo et al., 2020). In a study, Fernandes et al. (2003) analyzed 105 SE isolates from human and non-human sources and identified seven distinct plasmid profiles. Although 96% of the strains carried a 36 MDa plasmid, the frequency of additional plasmids was very low, limiting the utility of plasmid profiling for epidemiological discrimination. In a study on *S. enterica* outbreaks, whole-genome sequencing was used to investigate plasmid diversity among isolates from various sources, including poultry products

Table 2. ERIC-PCR profiles of 100 *S.* Enteritidis isolates using ERIC1R the primer

Profile	Molecular Weight (bp)	Number of Isolates
A	1100, 700, 500, 490, 370	26
B	1100, 700, 490, 370	29
C	1100, 700, 600, 560, 490, 370	4
D	1200, 1100, 700, 500, 490, 370	7
E	370	7
F	750, 700, 490, 370	2
G	1100, 850, 700, 490, 370	25

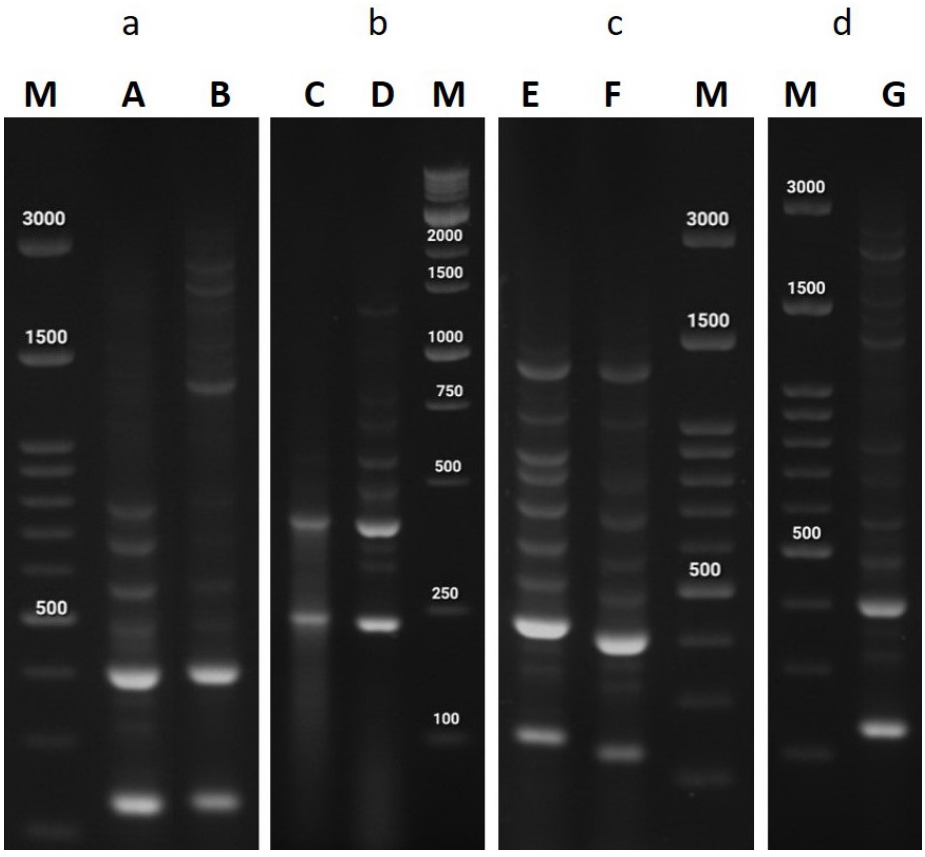

Figure 3. DNA fingerprints patterns of *S.* Enteritidis isolates by ERIC-PCR using the ERIC2 primer designated as A to G on four different gels

Notes: The letter "M" represents the commercial DNA ladder (ExcelBand™ 100 bp+3k DNA Ladder, SMOBiO) for "a", "c" and "d" and the commercial 1 kb plus DNA ladder (SinaClon) for "b".

and zoonotic cases. While most outbreaks showed no or only one plasmid profile, variation in plasmid content was observed in a subset of outbreaks. This variation, especially in poultry-associated outbreaks, highlights the potential of plasmid profiling as a useful tool for understanding the epidemiology of *Salmonella* beyond core genome similarities (Trees et al., 2024). In a comparative genomic study of 197 SE isolates from China, the U.S., Europe, and Africa, researchers identified 14 plasmid types with notable differences in their distribution across

Table 3. ERIC-PCR profiles of 100 *S.* Enteritidis isolates using the ERIC2 primer

Profile	Molecular Weight (bp)	Number of Isolates
A	1100, 800, 490, 450, 400, 370, 320, 230	5
B	1300, 600, 500, 400, 230	5
C	400, 230	3
D	1300, 800, 490, 400, 370, 320, 230	21
E	1300, 850, 800, 490, 400, 370, 320, 230	2
F	1300, 1000, 800, 490, 400, 370, 320, 230	29
G	2500, 1300, 800, 490, 400, 370, 320, 230	35

Table 4. Results of plasmid profiling and ERIC-PCR fingerprinting of *S.* Enteritidis isolates using ERIC1R and ERIC2 primers

ERIC Profile		Plasmid Profile	% of Isolates
ERIC1R	ERIC2		
A	G	A	11
C	F	A	9
C	G	A	Each pattern included 7% of isolates
F	F	A	
F	G	A	
A	D	A	
A	F	A	6
E	D	A	4
F	D	A	3
F	G	B	Each pattern included 2% of isolates
C	D	A	
F	B	A	
F	B	B	
E	G	A	
F	F	C	Each pattern included only 1% of isolates
D	F	A	
B	D	A	
F	G	F	
B	G	C	
F	F	E	
F	D	E	
C	D	E	
F	F	B	
D	D	A	
E	C	C	
G	A	B	
C	E	B	
B	F	C	
A	F	B	

ERIC Profile		Plasmid Profile	% of Isolates
ERIC1R	ERIC2		
A	D	B	
F	A	A	
D	E	D	
B	F	A	
D	G	A	
C	A	A	
C	C	A	
B	C	A	
B	A	A	
B	B	A	
C	A	B	
C	G	D	
C	G	F	
G	G	A	

geographic regions. Most isolates carried plasmids associated with virulence. Certain plasmid types were exclusively found in isolates from China, while others were regionally associated with Africa. Several hybrid plasmids were also identified, carrying both resistance and virulence genes (Cao et al., 2023). These findings demonstrate the role of plasmid diversity in the evolutionary dynamics of SE and its adaptation to different ecological and geographic contexts. In another study, plasmid profiling of *S. enterica* isolates from food products in Russia identified large plasmids ranging from 92 kb to 280 kb, associated with antimicrobial resistance (AMR) and virulence factors. The presence of these plasmids highlighted their role in the spread of AMR and pathogenicity in *Salmonella* populations. This study underscores the importance of monitoring plasmid-mediated resistance to prevent the dissemination of both AMR and virulence factors in foodborne pathogens (Egorova et al., 2023). A study analyzed four multidrug-resistant *Salmonella* isolates from poultry in Brazil, revealing complex plasmid profiles with resistance to antibiotics and heavy metals. Whole-genome sequencing identified circular chromosomes and megaplasmids carrying resistance genes. The presence of heavy metal tolerance operons suggests an adaptive advantage in challenging environments, like poultry farms. The highly mobile megaplasmids were found to spread across different farms in Brazil, highlighting their role in the dissemination of antimicrobial resistance (Galetti et al., 2021). In our study, six plasmid profiles were identified among 100 isolates. Sixty-seven percent of the samples had only one plasmid, which belonged to profile A. All samples contained one plasmid with a molecular weight greater than 10 kb. In a previous study (Morshed & Peighambari 2010), six plasmid profiles were found among 49 SE isolates from human and animal sources and 98% of the isolates had one to six plasmids with molecular weights between 1.5 and 68 kb. All isolates containing plasmids carried the 68 kb plasmid (Morshed & Peighambari, 2010). In a study (Fardsanei et al. 2016), 30 SE isolates from food and clinical samples in Tehran were analyzed for their plasmid content. All isolates carried a 68 kb plasmid, which may represent a serotype-specific virulence plasmid, and seven distinct plasmid profiles were identified. Despite some variation in plasmid content, a high degree of similarity was observed between food and clinical isolates, suggesting a potential link between food sources and human infections. In another study, 27 *Salmonella* isolates recovered from clinical, food, water, and hand swab samples were investigated for their antimicrobial resistance and plasmid profiles. A total of 12 distinct plasmid profiles were identified, with plasmid sizes

ranging from 3.2 to 30.2 kb. Isolates of the same species but from different sources exhibited variation in plasmid content, highlighting the discriminatory power of plasmid profiling in source tracking (Akinyemi et al., 2018). In a long-term molecular epidemiological study, Russian researchers analyzed over 22,000 SE isolates, mainly from human patients, along with samples from food, the environment, and rodents. Using plasmid profile analysis, they identified around 500 distinct plasmid types. However, three dominant profiles accounted for the majority of human infections throughout the surveillance period. These dominant types were also consistently found in food isolates, suggesting a foodborne route of transmission (Rakov et al., 2020). Liebana et al. (2004) reported a 57 kb-plasmid in 56% of the studied isolates. They also demonstrated that plasmid profiling had greater discriminatory power than PFGE and was more effective than ribotyping. Extragenomic DNA, such as plasmids, is known as an unstable and mutable genetic marker. As a result, isolates with identical chromosomal characteristics may have different plasmid patterns (David et al., 2020). Additionally, identical plasmid patterns can be observed in isolates with different chromosomal characteristics (Chu & Chiu, 2006).

ERIC-PCR is a powerful DNA-based typing and fingerprinting method used for classification of bacterial isolates. It is particularly useful for studying the epidemiology of SE (Suh et al., 2006). *Salmonella* isolates with different genotypes can exhibit varying pathogenicity. ERIC-PCR can serve as an appropriate tool for linking genotype and bacterial virulence (Saravanan et al., 2015). In a study (Fardsanei et al. 2016), 30 SE isolates of food and clinical origin showed five distinct ERIC patterns using ERIC1R and ERIC2 primers, with two patterns representing 76% of all samples. Additionally, the patterns exhibited 60% similarity with each other. This genotypic homogeneity supports the hypothesis that foodborne transmission is a likely route for human infection. In another study, using ERIC-PCR, 22 isolates produced 1 to 8 bands on an electrophoresis gel, with molecular weights ranging from 163 to 3074 bp (Elsayed et al., 2024). By comparing the profiles of the isolates, 11 different ERIC profiles were identified, which indicates considerable heterogeneity within the population. The study concluded that in broiler chickens, two profiles were most frequent in cloacal and feed swabs, and another profile was found in cloacal and drinking water swabs, indicating that the main source of contamination was contaminated water and feed (Elsayed et al., 2024).

A study aimed to detect *Salmonella* spp. in retail chicken samples and investigate their phylogenetic relationships using ERIC-PCR (Telli et al., 2022). *Salmonella* spp. was detected in 21.3% of samples, but no pathogenic serotypes, such as *S.* Enteritidis or *S.* Typhimurium were found. ERIC-PCR analysis revealed high genetic diversity among the *Salmonella* isolates, with no identical profiles observed. The findings indicated multiple contamination sources, and ERIC-PCR was suggested as a rapid, effective, and low-cost tool for genotyping and assessing genetic diversity in *Salmonella* spp. (Telli et al., 2022). In a study (Saravanan et al. 2015), ERIC-PCR was used to investigate diversity among *Salmonella* isolates. Eight distinct profiles were identified, with profiles containing 2 to 6 bands, and the molecular weights of the bands ranged from 150 to 2000 bp. Isolates from the same flock exhibited similar profiles. In another study, 38 *Salmonella* isolates were divided into 25 different genotypes, suggesting that the sources of the *Salmonella* isolates in these flocks were diverse (Zhao et al., 2016). Another study employed ERIC-PCR to examine the genetic diversity and clonal relationships of *Salmonella* isolates from poultry and calves (Tawfik et al., 2022). The results showed that two *S.* Enteritidis isolates from poultry and calves had 100% similarity, indicating possible transmission between these animals. However, the two *S.* kentucky isolates showed only 33% similarity, suggesting genetic diversity among the isolates from different sources (Tawfik et al., 2022). In a study, ERIC-PCR analysis revealed a high degree of genetic diversity among *S. enterica* isolates collected throughout the broiler production chain (Ramtahal et al., 2022). The banding patterns ranged from 3 to 13 bands, with fragment sizes between 150 bp and 3 kb, indicating the presence of genetically diverse clonal populations. The clustering of ERIC profiles showed a tendency for isolates to group according to their sources, suggesting that environmental factors and specific sampling points significantly influenced their genetic patterns. This observed diversity highlights the complex epidemiological dynamics of *Salmonella* transmission and underscores the importance of ongoing molecular surveillance in food production systems (Ramtahal et al., 2022).

Hassena et al. (2022) utilized ERIC-PCR to investigate the genetic relatedness of 54 foodborne *Salmonella* isolates, primarily of the Enteritidis, London, and Kentucky serotypes. The ERIC-PCR technique yielded 10 distinct profiles, clustering 37 isolates, while 17 isolates remained unclustered. ERIC-PCR revealed substantial genetic diversity among the isolates, showing that strains of the same serotype were distributed across different clusters, reflecting the complexity of contamination

sources (Hassena et al. 2022). In a study by Campioni et al. (2014), 60 *Salmonella* isolates were analyzed by ERIC-PCR. The band sizes ranged from 100 to 5000 bp, and the 60 isolates produced 24 different ERIC profiles. This study demonstrated the strong discriminatory power of ERIC-PCR for distinguishing isolates from different sources. In the present study, ERIC-PCR method was successfully used to create distinct profiles for SE isolates originated from broiler chicks.

The key benefits of ERIC-PCR genotyping include its accessibility, speed, ease of use, and stability. However, it does not always provide a full representation of genetic relatedness. Identical bands are determined by their size, rather than their genetic composition, meaning that two genetically distinct segments could be mistaken for identical if the ERIC targets are the same distance apart on the DNA (Ranjbar et al., 2013).

The findings of this study showed that the discriminatory power of the two techniques used in this research—plasmid profiling and ERIC-PCR—were quite similar, in that both methods differentiated isolates into a nearly equal number of subgroups: six subgroups for plasmid profiling and seven subgroups for ERIC-PCR. A single method may not have sufficient power to discriminate subgroups among SE isolates (Ling et al., 1998). To enhance the discriminatory power of isolates, researchers have combined the results of several molecular methods to achieve this goal (Ling et al., 1998; Liebana et al., 2004). In the present study, by combining the results of plasmid profiling and ERIC-PCR with two different primers, 100 SE isolates were divided into 43 groups, with seven groups comprising 54% of the isolates.

Conclusion

Given the increasing importance of paratyphoid *Salmonella*, especially *S.* Enteritidis, in poultry and public health worldwide, there is a need for extensive and advanced research to reduce and control *Salmonella* infections in poultry and humans. This study provided genetic data related to the isolates recovered from day-old broiler chicks and can be used for a broader epidemiological study at the national level.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this work according to the principles outlined by the ethical committee of the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Funding

This research was funded by the Research Council of the University of Tehran, Tehran, Iran (Grant No.: 7508007.6.51).

Authors' contributions

Study design, data analysis, and writing: Farzaneh Jeldi, Seyed Mostafa Peighambari, and Jamshid Razmyar; Experiments: Farzaneh Jeldi, Aazam Yazdani, and Fattaneh Naderinezhad; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to sincerely thank Office of Research Affairs of the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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