Original Article

Detecting Virulence Genes Among *Salmonella* Serovar Infantis Isolated From Poultry Sources

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ABSTRACT

Background: Salmonellosis is increasingly recognized as a worldwide public health concern. *Salmonella* Infantis can infect both humans and animals, including poultry. It has been one of the most reported isolated serovars from different parts of the world. Although some research has been carried out on the pathogenesis of *S.* Infantis, little scientific understanding of its pathogenesis is available.

Objectives: This study aimed to analyze the virulence genes of *S.* Infantis recovered from different sources of poultry in Iran.

Methods: Six virulence genes of 54 *S.* Infantis strains originated from broiler feces, poultry processing, and broiler carcasses were examined. Gene-specific polymerase chain reactions were designed and employed to detect the presence or absence of 6 important virulence genes (*sopB*, *sopE*, *sitC*, *pefA*, *sipA*, and *spvC*) in 54 *S.* Infantis isolates.

Results: In this study, *sopE*, *sitC*, *pefA*, *sipA*, and *sopB* virulence genes were detected in 51(94.4%), 49(90.7%), 26(48.1%), 15(27.7%), and 5(9.2%) isolates, respectively. The *spvC* gene was not detected in any of the isolates.

Conclusion: In the present study, a remarkably identical profile was found on virulence genes' presence in isolates recovered from broiler feces and poultry processing plant sources, that is a public health concern. However, more *S.* Infantis isolates from various poultry sources, and human origin should be examined and analyzed. The findings of this survey can help the health researchers better understand the pathogenesis and epidemiology of *S.* Infantis in Iran.

Keywords: Pathogenesis, Poultry, Public health, *Salmonella* Infantis, Virulence genes

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1. Introduction

Salmonella is one of the most important pathogens that cause different illnesses in humans and animals worldwide. This pathogen belongs to the family of *Enterobacteriaceae* (Lamas et al., 2018). *Salmonella* is highly prevalent in broilers, poultry feed, and the environment. Moreover, most *Salmonella* isolates are resistant to many antimicrobials and disinfectants used in medical and poultry practices (Sevilla-Navarro et al., 2019; Jovčić et al., 2020; Belachew et al., 2021; Li et al., 2021). So far, more than 2600 *Salmonella* serovars have been identified. Some serovars, such as Enteritidis, Infantis, and Typhimurium, have been the most reported isolated serovars throughout the world in recent years (Almeida et al., 2013; Shi et al., 2015; Mishra et al., 2020; Quino et al., 2020; Shome et al., 2020; Yu et al., 2021). *Salmonella enterica* subspecies *enterica* serovar Infantis (*S.* Infantis) can infect humans and animals, including poultry (Wajid et al., 2019). *Salmonella* Infantis has been one of the most reported serovars from different parts of the world, including Asian, African, European, and American countries (Hendriksen et al., 2011; Fuche et al., 2016; Mejía et al., 2020). The main source of salmonellosis in humans is food-producing animals such as cattle, pig, and poultry (Thorns, 2000; Wessels et al., 2021). *Salmonella* is transferred to eggs and poultry meat via fecal contamination, and humans are infected with *Salmonella* when they consume contaminated poultry products (Samiullah et al., 2013). *Salmonella* infection may cause enteritis and subclinical infections in humans (Antunes et al., 2016; Hindermann et al., 2017; Rincón-Gamboa et al., 2021).

According to the EFSA report (2017), about 50% of all *Salmonella* isolates reported by all European Union member states were *S.* Infantis. Moreover, the most common pathogenic serovars for humans were *S.* Typhimurium, *Salmonella* Enteritidis, *S.* Infantis, and *Salmonella* Derby (EFSA, 2017). More prevalence of *S.* Infantis among diverse environmental sources suggests that this pathogen can survive in various environmental situations and is still considered a public health concern (EFSA, 2017).

Salmonella serovars can encode and express virulence genes which help the microorganism interact with the host immune system. Various virulence properties play different roles in the pathogenesis of *Salmonella* in humans and animals. These virulence factors include capsule, flagella, adhesins, virulence plasmids, iron scavenging mechanisms, and the pathogenicity island (PAIs) (Wilharm & Heider, 2014; Elkenany et al., 2019; Lapierre et al., 2020).

Effector proteins, including *Salmonella* outer protein E (sopE), *Salmonella* outer protein B (sopB), and *Salmonella* inner protein A (sipA), are translocated from type III secretion system-1 (T3SS-1), which play a significant role in the attachment and invasion of *Salmonella* to the host cell. The plasmid-encoded fimbriae A (pefA) has also been reported as another important virulence element at this stage (Fabrega & Vila, 2013). *Salmonella* iron transporter C (*sitC*) is needed for the survival and spread of *Salmonella* in an iron-deficient environment, and its gene (*sitC*) is expressed from the sitABCD operon in SPI-I (Moest & Méresse, 2013). *Salmonella* virulence plasmid C (*spvC*) gene expressed from the operon of spv, which is composed of 5 genes (*spvRABCD*), is related to the spread of *Salmonella* serovars in the reticuloendothelial system and the expansion of systemic infection in different hosts (Foley et al., 2013). It may also be necessary to investigate the virulence properties of *Salmonella* serovars present in poultry intestinal tract during different production phases.

In this study, the presence or absence of 6 important virulence genes, including *sipA*, *sopB*, *sopE*, *spvC*, *pefA*, and *sitC*, were detected among *S.* Infantis isolates originating from poultry sources and analyzed.

2. Materials and Methods

Study samples

A total of 54 *S.* Infantis isolates recovered from broiler feces, poultry processing, and broiler carcasses from different regions of Iran were chosen from the culture collection of the university (Table 1) (Peighambari et al., 2015). Samples were cultured for regeneration under sterile conditions, next to the flame, by a sterile loop in a brain-heart broth (BHI), which had already been prepared and sterilized in a test tube with the number of each sample written on it and in a shaker incubator. The samples were incubated for 18 to 24 hours. After this time, the samples were taken out of the incubator, and most showed turbidity in the medium, which is a sign of bacterial growth. The samples were cultured again on McConkey's agar (MC) medium, and after 24 hours in the incubator, the samples were removed from the incubator, and single *Salmonella* colonies were observed. A single colony of these media was cultured again on a brain-heart broth medium with a sterile loop and flame, incubated, and then refrigerated for the next step, DNA extraction.

Table 1. Origins of *Salmonella* Infantis isolates of the study

Origin	Number of Isolates
Broiler feces	31
Poultry processing	20
Broiler carcasses	3
Total	54

Salmonella isolation and identification

The genomic DNA of each of the 54 isolates was extracted by boiling method, and the DNA concentration was determined. A total of 500 µL of BHI broth culture was suspended in 350 µL sterile water and placed in boiling water at 100°C for 15 min. Then, the suspension was spun for 5 min, and 70 µL of the supernatant containing chromosomal DNA was used as a template DNA in polymerase chain reaction (PCR) to reconfirm the *Salmonella* serovar Infantis strains (Kardos et al., 2007; Peighambari et al., 2015).

Molecular detection of genes

A conventional PCR was used to detect the presence or absence of 6 important virulence genes (*sopB*, *sopE*, *sitC*, *pefA*, *sipA*, and *spvC*) in 54 *S.* Infantis isolates. The primer sequences designed for detecting 6 virulence genes are shown in Table 2. For each isolate, an amplification reaction was prepared in a 25-µL reaction volume containing 2.5 µL 10x PCR buffer, 3 µL of 50 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs, 1 µL of each primer, 0.2 µL of *Taq* DNA polymerase and 14.8 µL of sterile deionized H_2O. Also, 2 µL of extracted DNA template was added to the mixture. Positive and negative (deionized H_2O instead of template DNA) controls were included in all reactions (Peighambari et al., 2015; Taheri et al., 2018). Amplification was programmed in a thermocycler (SensoQuest, Germany) as follows: 95˚C for 3 min followed by 30 cycles of denaturation at 94˚C for 1 min, annealing at 61˚C (*sitC*, *sipA*, and *sopB*), 51˚C (*spvC*), 65˚C (*sopE*) for 1 min, extension at 72˚C for 1 min and a final extension at 72˚C for 3 min. To detect the *pefA* gene, the PCR reaction was performed as previously described by Skyberg et al. (2006). All amplified products were detected by gel electrophoresis in 1.5% agarose gel in 1x TAE buffer with the addition of DNA Safe Stain® (SinaClon) and visualized under UV illumination. Where appropriate, two markers including 100 bp DNA ladder (Yekta Tajhiz Azma, Tehran, Iran) and 100 bp Plus DNA ladder II (Dana Zist Asia, Mashhad, Iran), were employed as MW markers in each gel running. All materials used in PCR reactions were purchased from SinaClon (Tehran, Iran).

Table 2. Primer sequences used for the detecting virulence genes in *Salmonella* Infantis

Target Genes	Primer Sequences (5'-3')	Amplicon Size (bp)	References
sopE	F-ATTGTTGTGGCGTTGGCATCGT R-AATGCGAGTAAAGATCCGGCC	376	Zou et al., 2011
sitC	F-CAGTATATGCTCAACGCGATGTGGGTCTCC R-CGGGGCGAAAATAAAGGCTGTGATGAAC	768	Skyberg et al., 2006
pefA	F-GCGCCGCTCAGCCGAACCAG R-GCAGCAGAAGCCCAGGAAACAGTG	157	Skyberg et al., 2006
sipA	F-ATGGTTACAAGTGTAAGGACTCAG R-ACGCTGCATGTGCAAGCCATC	2055	Shah et al., 2011
sopB	F-GCTCTAGACCTCAAGACTCAAGATG R-GCGGCCGCTACGCAGGAGTAAATCGGTG	1987	Raffatellu et al., 2005
spvC	F-ACTCCTTGCACAACCAAATGCGGA R-TGTCTCTGCATTTCGCCACCATCA	571	Chiu et al., 2006

3. Results

All isolates showed the amplified band of 413 bp as a confirmation for the *S.* Infantis (Figure 1). The 6 virulence genes of *S.* Infantis, ie, *sopB*, *sopE*, *sitC*, *pefA*, *sipA*, and *spvC*, were detected by conventional PCR in 54 isolates and the results have been demonstrated in Table 3. The *sopE* gene was identified as the most prevalent virulence gene (Figure 2). The *sopE* gene was detected in 96.7% of fecal isolates (30/31 isolates), 90% of processing isolates (18/20 isolates), and all of the carcasses' isolates (3/3 isolates). The *sitC* virulence gene was the second-highest prevalent virulence gene (Figure 3). This virulence gene was positive in 93.5% of fecal isolates (29/31 isolates), 85% of processing isolates (17/20 isolates), and all other isolates. The *pefA* virulence gene was the third most prevalent virulence gene among isolates (Figure 4). Unlike the previous 2 genes, the highest detection percentage of this gene (52.38%) was in processing isolates (11/20). This gene was detected in 48.3% of fecal isolates (15 isolates out of 31 isolates). But this gene was negative in carcasses' isolates. The *sipA* gene was the fourth most prevalent virulence gene (Figure 5). This gene was detected in 33.3% of carcass isolates, 32.2% of fecal isolates, and 20% of slaughter isolates. It is noteworthy that the *sopB* gene was detected only in 16.12% of fecal isolates (10/31) (Figure 6). The *spvC* virulence gene was not detected in any of the isolates (Table 3).

Table 3. The results for detected virulence genes among 54 *Salmonella* Infantis isolates

Isolate #	Source	*sopE*	*sitC*	*pefA*	*sipA*	*sopB*	*spvC*
1	Poultry processing	-	-	-	-	-	-
2	Poultry processing	+	-	-	-	-	-
3	Poultry processing	+	+	+	+	-	-
4	Poultry processing	+	+	+	-	-	-
5	Poultry processing	+	+	+	+	-	-
6	Poultry processing	+	+	-	-	-	-
7	Poultry processing	+	-	-	-	-	-
8	Poultry processing	+	+	-	-	-	-
9	Poultry processing	+	+	-	-	-	-
10	Poultry processing	+	+	+	-	-	-
11	Poultry processing	+	+	-	-	-	-
12	Poultry processing	+	+	-	+	-	-
13	Broiler feces	+	+	-	+	-	-
14	Broiler feces	+	-	-	-	-	-
15	Broiler feces	+	+	-	-	-	-
16	Broiler feces	+	+	-	-	-	-
17	Broiler feces	+	+	+	+	+	-
18	Broiler feces	+	+	-	+	+	-
19	Broiler feces	-	+	-	-	-	-
20	Broiler feces	+	-	-	+	-	-
21	Broiler feces	+	+	+	-	-	-
22	Broiler carcasses	+	+	-	+	-	-

Isolate #	Source	*sopE*	*sitC*	*pefA*	*sipA*	*sopB*	*spvC*
23	Broiler feces	+	+	-	-	-	-
24	Poultry processing	+	+	+	-	-	-
25	Poultry processing	+	+	+	-	-	-
26	Poultry processing	+	+	+	-	-	-
27	Poultry processing	+	+	-	-	-	-
28	Poultry processing	+	+	-	+	-	-
29	Poultry processing	+	+	+	-	-	-
30	Poultry processing	-	+	+	-	-	-
31	Poultry processing	+	+	+	-	-	-
32	Broiler carcasses	+	+	-	-	-	-
33	Broiler carcasses	+	+	-	-	-	-
34	Broiler feces	+	+	+	-	-	-
35	Broiler feces	+	+	-	-	-	-
36	Broiler feces	+	+	+	-	-	-
37	Broiler feces	+	+	+	+	-	-
38	Broiler feces	+	+	+	-	-	-
39	Broiler feces	+	+	-	+	-	-
40	Broiler feces	+	+	+	-	-	-
41	Broiler feces	+	+	+	-	-	-
42	Broiler feces	+	+	-	-	+	-
43	Broiler feces	+	+	+	-	-	-
44	Broiler feces	+	+	-	+	-	-
45	Broiler feces	+	+	+	-	-	-
46	Broiler feces	+	+	-	-	-	-
47	Broiler feces	+	+	+	+	-	-
48	Broiler feces	+	+	+	+	+	-
49	Broiler feces	+	+	+	+	-	-
50	Broiler feces	+	+	+	-	-	-
51	Broiler feces	+	+	-	-	+	-
52	Broiler feces	+	+	-	-	-	-
53	Broiler feces	+	+	+	-	-	-
54	Broiler feces	+	+	-	-	-	-

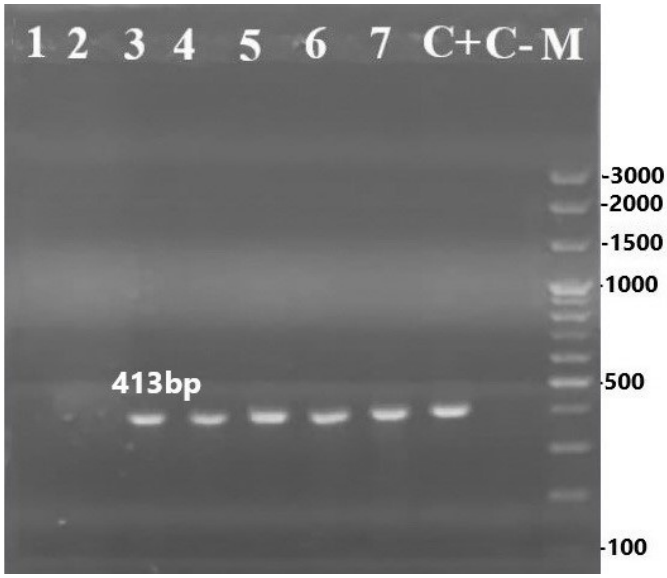

Figure 1. Electrophoresis of PCR products on 1% agarose gel to confirm *Salmonella* Infantis

Amplified 413 bp bands of 5 isolates are shown in lanes 3 to 7. Lanes M, C+, and C- indicate commercial 100 bp plus DNA ladder II, positive control, and negative control (dH_2O instead of DNA), respectively. Other lanes demonstrate negative results for tested *Salmonella* isolates.

In summary, in the present study, *sopE*, *sitC*, *pefA*, *sipA*, and *sopB* virulence genes were detected in 51(94.4%), 49(90.7%), 26(48.1%), 15(27.7%), and 5(9.2%) isolates, respectively. The *spvC* gene was not found in any of the isolates.

4. Discussion

Salmonellosis is an important disease that affects human and poultry health. *Salmonella* Infantis has recently been among the most reported *Salmonella* serovars worldwide (Mejía et al., 2020). Characterization and detection of virulence genes among *S.* Infantis isolates obtained from various sources have been the subject of investigations by many researchers worldwide, leading to different findings (Skyberg et al., 2006; Gole et al., 2013; Krawiec et al., 2015; Karacan Sever & Akan, 2019; Garcia-Soto et al., 2020).

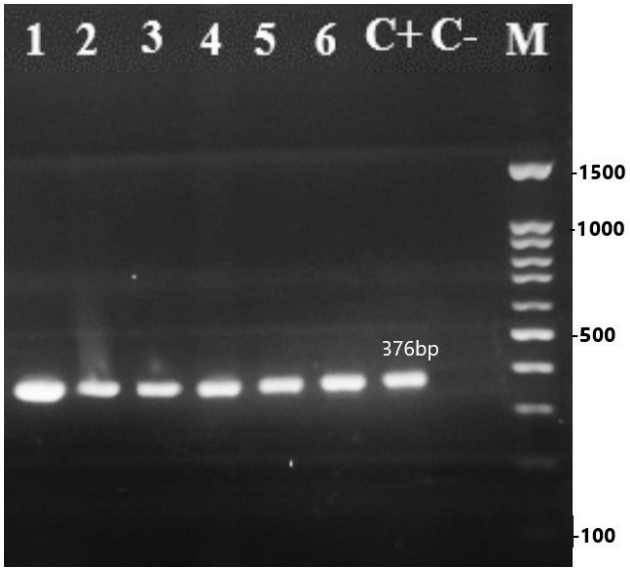

Figure 2. Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *sopE* gene

Amplified 376 bp bands of isolates are shown in lanes 1 to 6. Lanes M, C+, and C- indicate commercial 100 bp DNA ladder, positive control, and negative control (dH_2O instead of DNA), respectively.

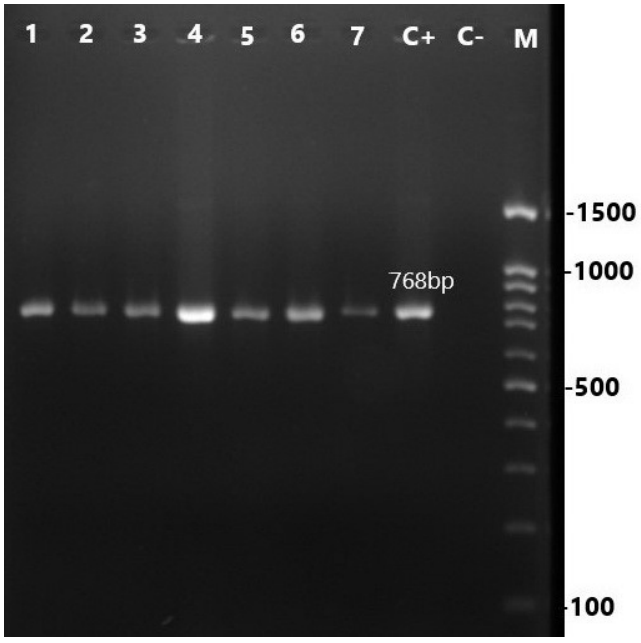

Figure 3. Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *sitC* gene

Amplified 768 bp bands of isolates are shown in lanes 1 to 7. Lanes M, C+, and C- indicate commercial 100 bp DNA ladder, positive control, and negative control (dH_2O instead of DNA), respectively.

In Iran, due to a lack of information on the virulence genes of *S.* Infantis isolates originating from poultry sources, the present study was conducted to determine the presence of the 6 most important virulence genes among *S.* Infantis isolates recovered from poultry flocks. The investigated virulence genes included *sopB, sopE, sitC*, *pefA*, *sipA*, and *spvC* among 54 *S.* Infantis isolates. There were similarities and differences in the presence of virulence genes among our isolates with those of previous investigations, as is described below.

The high prevalence of the *sopE* virulence gene among *S.* Infantis isolates was compatible with the previous findings (Hopkins & Threlfall, 2004; Karasova et al., 2009; Karacan Sever & Akan, 2019). Earlier, Hopkins and Threlfall (2004) emphasized the role of the sopE

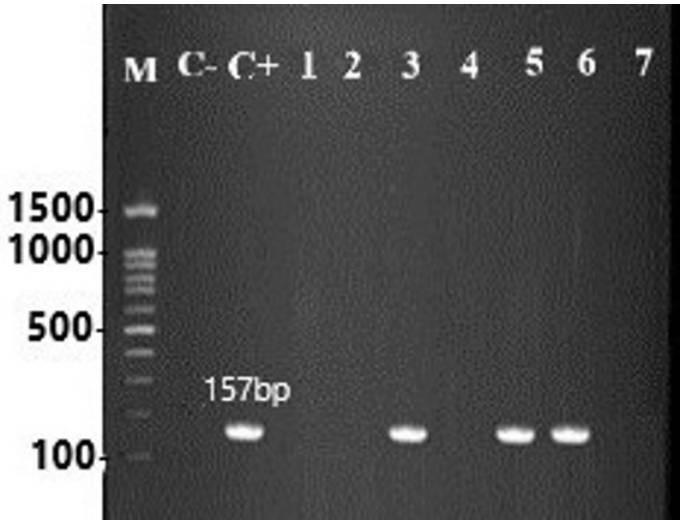

Figure 4. Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *pefA* gene

Amplified 157 bp bands of isolates are shown in lanes 3, 5, and 6. Lanes M, C+, and C- indicate commercial 100 bp DNA ladder, positive control, and negative control (dH_2O instead of DNA), respectively. Other lanes demonstrate negative results for the tested *pefA* gene.

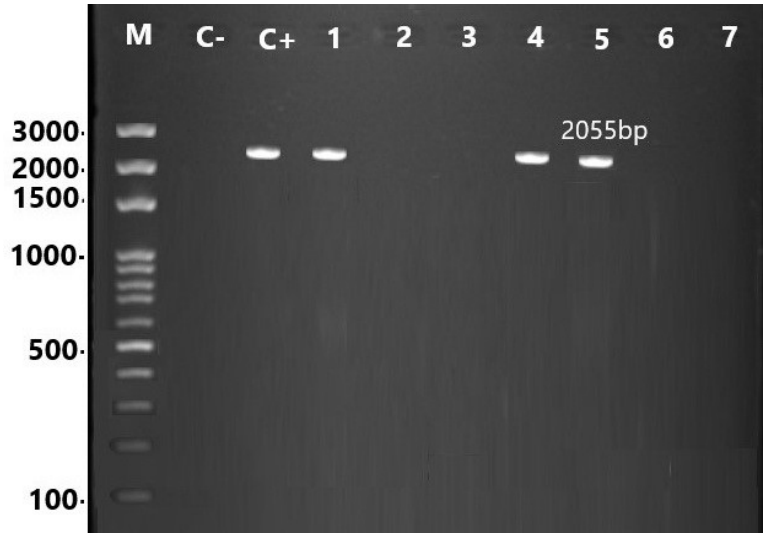

Figure 5. Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *sipA* gene. Amplified 2055 bp bands of isolates are shown in lanes 1, 4, and 5. Lanes M, C+, and C- indicate commercial 100 bp Plus DNA ladder II, positive control, and negative control (dH_2O instead of DNA), respectively. Other lanes demonstrate negative results for the tested *sipA* gene.

protein in altering the actin structure that facilitates invasion to the host cell. Also, it has been indicated that mutation in the *sopE* gene leads to an inability to attack nonpolarized epithelial cell lines (Raffatellu et al., 2005). High frequency (90%) of sope gene presence among *S.* Infantis isolates originating from poultry processing plants may be considered an intimidating factor because there is a chance of human infection with these isolates due to the consumption of infected poultry products.

The presence of the *sitC* virulence gene was very high among isolates which were comparable with the findings of previous investigations (Skyberg et al., 2006; Gole et al., 2013; Krawiec et al., 2015; Karacan Sever & Akan, 2019; Dantas et al., 2020). Previous works have emphasized the importance of the *sitC* virulence gene for the survival and multiplication of *Salmonella* in iron-deficient environments (Zhou et al., 1999; Elemfareji & Thong, 2013).

sipA protein is encoded by genes located on *Salmonella* pathogenicity island I, and also, this protein is a main virulence property of *Salmonella* that accelerates the entry of *Salmonella* into the host cell (Raffatellu et

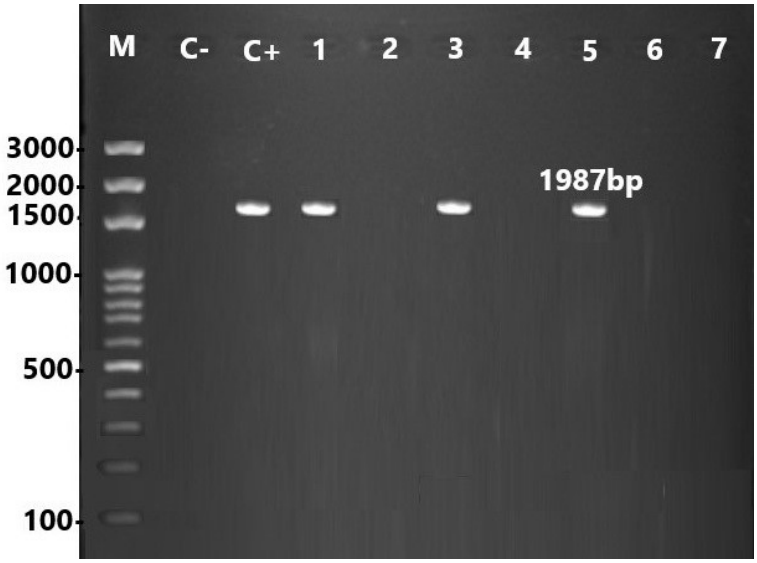

Figure 6. Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *sopB* gene. Amplified 1987 bp bands of isolates are shown in lanes 1, 3, and 5. Lanes M, C+, and C- indicate commercial 100 bp plus DNA ladder II, positive control, and negative control (dH_2O instead of DNA), respectively. Other lanes demonstrate negative results for the tested *sopB* gene.

al., 2005). The absence of sipA in the early stage of the pathogenesis of *Salmonella* usually reduces the invasion of this bacteria to host cells (Perrett & Jepson, 2009). Unlike the findings reported previously (Almeida et al., 2013; Figueiredo et al., 2015; Karacan Sever & Akan, 2019; Dantas et al., 2020; Lapierre et al., 2020), a somewhat low positive rate of *sipA* gene presence (27.7%) was observed among the isolates of this study.

Both sopB and pefA proteins have important roles in host recognition and invasion (Tarabees et al., 2017). Contrary to the previously reported investigations (Karasova et al., 2009; Huehn et al., 2010; Almeida et al., 2013; Gole et al., 2013; Karacan Sever & Akan, 2019; Dantas et al., 2020), the presence of *sopB* gene, in the present study, was found in 5 *S.* Infantis isolates (9.2%) only. The role of fimbria, encoded by the *pefA* gene, in the adhesion phase of *Salmonella* and the ability of this pathogen to adhere to different sites of the host cells and pathogenicity have been previously reported by other researchers (Elemfareji & Thong, 2013; Figueiredo et al., 2015). Our findings for the presence of the *pefA* gene among *S.* Infantis isolates were compatible with those of Figueiredo et al. (2015) and Krawiec et al. (2015) but not with results reported by Karacan Sever & Akan (2019). In the present investigation, in 26 isolates (48.1%), the *pefA* gene was detected, but Karacan Sever and Akan (2019) reported 1 positive isolate (0.44%) only.

The contribution of *Salmonella* virulence plasmid C to the spread of *Salmonella* serovars in the reticuloendothelial system and the expansion of systemic infection in different hosts have been documented by some researchers (Foley et al., 2013; Krzyzanowski et al., 2014). In this study, no *S.* Infantis isolate was positive for the presence of the *spvC* gene, unlike the findings of previous researchers (Huehn et al., 2010; Krzyzanowski et al., 2014; Figueiredo et al., 2015; Karacan Sever & Akan, 2019). The reason for this difference may be related to the fact that the number of our tested isolates was lower than those of previous studies.

5. Conclusion

The current investigation examined the presence of 6 virulence genes among 54 *Salmonella* serovar Infantis isolates originating from poultry sources in Iran. A considerably identical profile was found on virulence genes' presence between isolates recovered from broiler feces and poultry processing plants sources that may be a cause of concern for health authorities. However, more *Salmonella* serovar Infantis isolates obtained from various poultry sources and human should be examined and analyzed to reinforce these findings. This survey can help the health authorities better understand the pathogenesis and epidemiology of *S.* Infantis in Iran.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Investigation, writing–original draft preparation: Hossein Haghighatnezhad; Study design, data analysis, review, editing and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

جست‌وجوی ژن‌های حدت سالمونلا سروار اینفنتیس جداشده از منابع طیوری

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چکیده

زمینه مطالعه: سالمونلوز به‌صورت گسترده، به‌عنوان یک بیماری همه‌گیر و دارای اهمیت بهداشت عمومی شناخته می‌شود. سالمونلا اینفنتیس توانایی ایجاد عفونت در انسان و حیوانات مختلف شامل طیور را دارد. این باکتری یکی از مهم‌ترین سروارهای جداسازی‌شده از مناطق مختلف جهان محسوب می‌شود. با وجود اینکه تحقیقات مختلفی در مورد روند بیماری‌زایی سالمونلا اینفنتیس صورت گرفته است، اما درک علمی چندانی در این زمینه وجود ندارد.

هدف: هدف این مطالعه بررسی ژن‌های حدت سالمونلا اینفنتیس جداشده از منابع مختلف طیور در کشور ایران است.

روش کار: در این مطالعه ۵۴ جدایه سالمونلا اینفنتیس که از لاشه طیور، مدفوع طیور و کشتارگاه جداسازی شده بودند، مورد بررسی قرار گرفتند. تکنیک ملکولی PCR اختصاصی هر ژن، به منظور بررسی ۶ ژن حدت مهم سالمونلا اینفنتیس (sopB, sopE, sitC, pefA, sipA, spvC) طراحی و مورد استفاده قرار گرفت.

نتایج: تعداد ۵۱ جدایه (۹۴/۴ درصد) دارای ژن حدت sopE، ۴۹ جدایه (۹۰/۷ درصد) دارای ژن حدت sitC، ۲۶ جدایه (۴۸/۱ درصد) واجد ژن حدت pefA، ۵ جدایه (۹/۲ درصد) واجد ژن حدت sopB و ۱۵ جدایه (۷/درصد) واجد ژن حدت sipA بودند. همچنین ژن حدت spvC در هیچ‌کدام از جدایه‌ها مشاهده نشد.

نتیجه‌گیری نهایی: در مطالعه حاضر، ویژگی‌های مشابه و قابل توجهی در ژن‌های حدت جدایه‌های به‌دست‌آمده از مدفوع طیور و کشتارگاه طیور مشاهده شد که ازنظر بهداشت عمومی حائز اهمیت و باعث نگرانی است. نیاز است جدایه‌های سالمونلا اینفنتیس بیشتری از منابع مختلف طیور و انسان مورد بررسی و تحلیل قرار بگیرند، اما یافته‌های این بررسی می‌تواند به محققان بهداشتی به منظور درک روند بیماری‌زایی و همه‌گیرشناسی سالمونلا اینفنتیس در ایران کمک‌کننده باشد.

کلیدواژه‌ها: بیماری‌زایی، بهداشت عمومی، سالمونلا اینفنتیس، ژن‌های حدت، طیور

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