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Detection of Virulence Genes among *Salmonella* serovar Infantis Isolated from Poultry Sources

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Short title: Virulence genes of *Salmonella* Infantis

Abstract

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

OBJECTIVES: The purpose of this study was to analyzes the virulence genes of *S.* Infantis that had been recovered from different sources of poultry in Iran.

METHODS: Six virulence genes of 54 *Salmonella* 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 six 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.

CONCLUSIONS: In the present study, a remarkable identical profile was found on virulence genes' presence between isolates recovered from broiler feces and poultry processing plants 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 to better understand the process of pathogenesis and epidemiology of *Salmonella* Infantis in Iran.

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

Introduction

Salmonella is one of the most important pathogens that cause different illness in human and animals all over the world and belongs to the family of *Enterobacteriaceae* (Lamas *et al.*, 2018). *Salmonella* is highly prevalent in broilers, poultry feed and environment. Moreover, a majority of the *Salmonella* isolates are resistant to most 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, 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 both humans and animals, including poultry (Wajid *et al.*, 2019). *Salmonella* serovar Infantis, has been one of the most reported serovar 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 are food-producing animals such as cattle, pig and obviously 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 percent all *Salmonella* isolates reported by all European Union member states, were *S.* Infantis. Moreover, the most common pathogenic serovars for humans were *S.* Typhimurium, *S.* Entritidis, *S.* Infantis and *S.* Derby (EFSA, 2017). More prevalence of *Salmonella* Infantis among diverse environmental sources suggest that this pathogen has the ability to survive in various environmental situations and is still considered a public health concern (EFSA, 2017).

Salmonella serovars have the ability for coding and expression of virulence genes which help the microorganism to interacts with the host immune system. There are a variety of virulence properties that play various roles in the pathogenesis of *Salmonella* in human and

animals. These virulence factors include capsule, flagella, adhesins, virulence plasmids, iron scavenging mechanisms and the pathogenicity island (PAIs) (Wilharm and 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 plays 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 and 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 and Méresse, 2013). *Salmonella* virulence plasmid C (*spvC*) gene expressed from the operon of spv, which is composed of five 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 be also necessary to investigate the virulence properties of *Salmonella* serovars that are present in poultry intestinal tract during different phase of production.

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

Materials and Methods

Samples

A total of 54 *Salmonella* Infantis isolates that had been recovered from broiler feces, poultry processing, and broiler carcasses from different regions of Iran, were chosen from culture collection of the university (Table 1) (Peighambari *et al.*, 2015). Samples were cultured for regeneration under sterile conditions, next to the flame, by 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 of them showed turbidity of the environment which is a sign of bacterial growth, then 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 sterile loop and flame, incubated, and then refrigerated for the next step, DNA extraction.

Salmonella Isolation and Identification

The genomic DNA of each of 54 isolates was extracted by boiling method and the DNA concentration was determined. Five hundred µL of BHI broth culture was suspended in 350 µL sterile water and were placed in boiling water at 100 °C for 15 minutes. 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 employed to detect the presence or absence of six important virulence genes (*sopB*, *sopE*, *sitC*, *pefA*, *sipA* and *spvC*) in 54 *Salmonella* Infantis isolates. The primer sequences designed for the detection of six virulence gene are shown in Table 2. For each isolate, amplification reaction was prepared in a 25 µl reaction volume containing 2.5 µl 10 x PCR buffer, 3 µl of 50 mM $MgCl_2$, 0.5 µl of 10 mM dNTPs, one µl of each primer, 0.2 µl of *Taq* DNA polymerase and 14.8 µl of sterile deionized H_2O. Two µl of extracted DNA template was added to the mixture. In all reactions, positive (Peighambari *et al*., 2015; Taheri *et al*., 2018) and negative (deionized H_2O instead of template DNA) controls were included. Amplification was programed 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 one min, extension at 72 °C for one min and a final extension at 72 °C for 3 min. In order 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. Two markers including 100 bp DNA Ladder (Yekta Tajhiz Azma, Tehran, Iran) and 100 bp Plus DNA Ladder II (Dana Zist Asia, Mashhad, Iran) was employed as MW markers in each gel running, where appropriate. All materials used in PCR reactions were purchased from SinaClon (Tehran, Iran).

Results

All isolates showed the amplified band of 413 bp as a confirmation for the *Salmonella* Infantis (Figure. 1). The six virulence genes of *Salmonella* Infantis including *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 two genes, the highest detection percentage of this gene (52.38%) was in processing isolates (11/20) and 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 carcasses 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).

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.

Discussion

Salmonellosis is very important disease that affects human and poultry health. *Salmonella* Infantis has been one of the most reported *Salmonella* serovar throughout the world in recent years (Mejía *et al.*, 2020). Characterization and detection of virulence genes among *Salmonella* 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; Sever and Akan, 2019; Garcia-Soto *et al.*, 2020).

In Iran, due to lack of information on the status of virulence genes of *S.* Infantis isolates originating from poultry sources, the present study was conducted to determine the presence of six 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 on the presence of virulence genes among our isolates with those of previous investigations as is described below.

The high prevalence of *sopE* virulence gene among *S.* Infantis isolates was compatible with the previous findings (Hopkins and Threlfall, 2004; Karasova *et al.*, 2009; Sever and Akan, 2019). Earlier, Hopkins and Threlfall (2004) emphasized on the role of sopE protein in alteration of the actin structure that facilitates invasion to host cell. Also, it has been indicated that mutation in *sopE* gene leads to inability to attack nonpolarized epithelial cell lines (Raffatellu *et al.*, 2005). High frequency (90%) of *sopE* gene presence among *S.* Infantis isolates originated from poultry processing plants may be considered as 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 was comparable with the findings of previous investigations (Skyberg *et al.*, 2006; Gole *et al.*, 2013; Krawiec *et al.*, 2015; Sever and Akan, 2019; Dantas *et al.*, 2020). The high importance of the *sitC* virulence gene for the survival and multiplication of *Salmonella* in iron-deficient environments has been emphasized in previous works (Zhou *et al.*, 1999; Elemfareji and 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 entry of *Salmonella* into the host cell (Raffatellu *et al.*, 2005). Absence of sipA in the early stage of the pathogenesis of *Salmonella* usually reduces the invasion of this bacteria to host cell (Perrett and Jepson, 2009). Unlike the findings reported previously (Almeida *et al.*, 2013; Figueiredo *et al.*, 2015; Sever and 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; Sever and Akan, 2019; Dantas *et al.*, 2020), the presence of *sopB* gene, in the present study, was found in 5 (9.2%) *S.* Infantis isolates only. The role of fimbria, encoded by the *pefA* gene, in the adhesion phase of *Salmonella* and ability of this pathogen in adherence to different sites of the host cells and pathogenicity have been previously reported by other researchers (Elemfareji and thong, 2013; Figueiredo *et al.*, 2015). Our findings for the presence of *pefA* gene among *S.* Infantis isolates was compatible with those of Figueiredo *et al.* (2015) and Krawiec *et al.* (2015) but not with results reported by Sever and Akan (2019). In the present investigation in 26 (48.1%) isolates the *pefA* gene was detected but Sever and Akan (2019) reported 1 (0.44%) positive isolate 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 *spvC* gene unlike the findings of previous researchers (Huehn *et al.*, 2010; Krzyzanowski *et al.*, 2014; Figueiredo *et al.*, 2015;

Sever and Akan, 2019). The reason for this difference may be related to the fact that the number of our isolates that were tested was lower than those of in previous studies.

Conclusion

In current investigation, the presence of six virulence genes among 54 *Salmonella* serovar Infantis isolates originated from poultry sources in Iran were examined. A considerable 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, to reinforce these findings, more *Salmonella* serovar Infantis isolates obtained from various poultry sources and human should be examined and analyzed. The findings of this survey can help the health authorities to better understand the process of pathogenesis and epidemiology of *Salmonella* Infantis in Iran.

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Conflict of Interest

The authors declare they have no conflict of interest associated with this work.

References

Almeida, F., Pitondo-Silva, A., Oliveira, M. A. & Falcão, J. P. (2013). Molecular epidemiology and virulence markers of *Salmonella* Infantis isolated over 25 years in Sao Paulo state. *Infection, Genetics and Evolution*, *19*, 145-151. [DOI: 10.1016/j.meegid.2013.07.004] [PMID: 23860124]

Antunes, P., Mourão, J., Campos, J. & Peixe, L. (2016). Salmonellosis: the role of poultry meat. *Clinical Microbiology and Infection*, *22*(2), 110–121. [DOI.org/10.1016/j.cmi.2015.12.004] [PMID: **26708671**]

Belachew, T., Mulusew, E., Tolosa, Y., Asefa, Z., Negussie, H. & Sori, T. (2021). Prevalence and antimicrobial-susceptibility profiles of *Salmonella* in smallhold broiler supply chains in central Ethiopia. *Infection and Drug Resistance*, *14*, 4047–4055. [DOI: 10.2147/IDR.S331249] [PMID: 34616162]

Chiu, C., Su, L., Chu, C., Wang, M., Yeh, C., Weill, F. & Chu, C. (2006). Detection of multidrug-resistant *Salmonella* enterica serovar Typhimurium phage types DT102, DT104, and U302 by multiplex PCR. *Journal of Clinical Microbiology*, *44*(7), 2354–2358. [DOI: 10.1128/JCM.00171-06] [PMID: 16825349]

Elkenany, R., Elsayed, M. M., Zakaria, A. I., El-Sayed, S. A. & Rizk, M. A. (2019). Antimicrobial resistance profiles and virulence genotyping of *Salmonella* enterica serovars recovered from broiler chickens and chicken carcasses in Egypt. *BMC Veterinary Research*, *15*(1), 124. [DOI: 10.1186/s12917-019-1867-z] [PMID: 31029108]

Dantas, S., Camargo, C. H., Tiba-Casas, M. R., Vivian, R. C., Pinto, J., Pantoja, J., Hernandes, R. T., Fernandes Júnior, A. & Rall, V. (2020). Environmental persistence and virulence of *Salmonella* spp. isolated from a poultry slaughterhouse. *Food Research International (Ottawa, Ont.)*, *129*, 108835. [DOI: 10.1016/j.foodres.2019.108835] [PMID: **32036904**]

Elemfareji, O. I. & Thong, K. L. (2013). Comparative virulotyping of *Salmonella* Typhi and *Salmonella* Enteritidis. *Indian Journal of Microbiology*, *53*(4), 410–417. [DOI: 10.1007/s12088-013-0407-y] [PMID: **24426144**]

Fabrega, A. & Vila, J. (2013). *Salmonella* enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clinical Microbiology Reviews*, *26*(2), 308–341. [DOI: 10.1128/CMR.00066-12] [PMID: **23554419**]

Figueiredo, R., Card, R., Nunes, C., AbuOun, M., Bagnall, M. C., Nunez, J. & da Silva, G. J. (2015). Virulence characterization of *Salmonella* enterica by a new microarray: detection and evaluation of the cytolethal distending toxin gene activity in the unusual host *S.* Typhimurium. *PLoS one*, *10*(8), e0135010. [DOI: 10.1371/journal.pone.0135010] [PMID: **26244504**]

Foley, S. L., Johnson, T. J., Ricke, S. C., Nayak, R. & Danzeisen, J. (2013). *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiology and Molecular Biology Reviews*, *77*(4), 582–607. [DOI: 10.1128/MMBR.00015-13] [PMID: **24296573**]

Fuche, F. J., Sow, O., Simon, R. & Tennant, S. M. (2016). *Salmonella* serogroup C: Current status of vaccines and why they are needed. *Clinical and Vaccine Immunology*, *23*(9), 737–745. [DOI: 10.1128/CVI.00243-16] [PMID: **27413069**]

García-Soto, S., Abdel-Glil, M. Y., Tomaso, H., Linde, J. & Methner, U. (2020). Emergence of multidrug-resistant *Salmonella* enterica subspecies enterica serovar Infantis of multilocus sequence type 2283 in German broiler farms. *Frontiers in Microbiology*, *11*, 1741. [DOI: 10.3389/fmicb.2020.01741] [PMID: **32765483**]

Gole, V. C., Chousalkar, K. K. & Roberts, J. R. (2013). Survey of *Enterobacteriaceae* contamination of table eggs collected from layer flocks in Australia. *International Journal of Food Microbiology*, *164*(2-3), 161–165. [DOI: 10.1016/j.ijfoodmicro.2013.04.002] [PMID: **23680799**]

Hendriksen, R. S., Vieira, A. R., Karlsmose, S., Wong, D., Jensen, A. B., Wegener, H. C. & Aarestrup, F. M. (2011). Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country data bank: results of quality assured laboratories from 2001 to 2007. *Foodborne Pathogens and Disease*, *8*(8), 887–900. [DOI: 10.1089/fpd.2010.0787] [PMID: **21492021**]

Hindermann, D., Gopinath, G., Chase, H., Negrete, F., Althaus, D., Zurfluh, K., Tall, B.D., Stephan, R. & Nüesch-Inderbinen, M. (2017). *Salmonella* enterica serovar Infantis from food and human infections, Switzerland, 2010-2015: poultry-related multidrug resistant clones and an emerging ESBL producing clonal lineage. *Frontiers in Microbiology*, *8*, 1322. [DOI: 10.3389/fmicb.2017.01322] [PMID: **28751886**]

Hopkins, K. L. & Threlfall, E. J. (2004). Frequency and polymorphism of sopE in isolates of *Salmonella* enterica belonging to the ten most prevalent serotypes in England and Wales. *Journal of Medical Microbiology*, *53*(6), 539–543. [DOI: 10.1099/jmm.0.05510-0] [PMID: **15150335**]

Huehn, S., La Ragione, R. M., Anjum, M., Saunders, M., Woodward, M. J., Bunge, C. & Malorny, B. (2010). Virulotyping and antimicrobial resistance typing of *Salmonella* enterica serovars relevant to human health in Europe. *Foodborne Pathogens and Disease*, *7*(5), 523–535. [DOI: 10.1089/fpd.2009.0447] [PMID: **20039795**]

Jovčić, B., Novović, K., Filipić, B., Velhner, M., Todorović, D., Matović, K., Rašić, Z., Nikolić, S., Kiškarolj, F. & Kojić, M. (2020). Genomic characteristics of colistin-resistant *Salmonella* enterica subsp. enterica serovar Infantis from poultry farms in the Republic of Serbia. *Antibiotics (Basel, Switzerland)*, *9*(12), 886. [DOI: 10.3390/antibiotics9120886] [PMID: **33321688**]

Karasova, D., Havlickova, H., Sisak, F. & Rychlik, I. (2009). Deletion of *sodCI* and *spvBC* in *Salmonella* enterica serovar Enteritidis reduced its virulence to the natural virulence of serovars Agona, Hadar and Infantis for mice but not for chickens early after infection. *Veterinary Microbiology*, *139*(3-4), 304–309. [DOI: 10.1016/j.vetmic.2009.06.023] [PMID: **19595520**]

Kardos, G., Farkas, T., Antal, M., Nógrády, N. & Kiss, I. (2007). Novel PCR assay for identification of *Salmonella* enterica serovar Infantis. *Letters in Applied Microbiology*, *45*(4), 421–425. [DOI: 10.1111/j.1472-765X.2007.02220.x] [PMID: **17897386**]

Krawiec, M., Kuczkowski, M., Kruszewicz, A. G. & Wieliczko, A. (2015). Prevalence and genetic characteristics of *Salmonella* in free-living birds in Poland. *BMC Veterinary Research*, *11*, 15. [DOI: 10.1186/s12917-015-0332-x] [PMID: **25636375**]

Krzyzanowski, F. Jr, Zappelini, L., Martone-Rocha, S., Dropa, M., Matté, M. H., Nacache, F. & Razzolini, M. T. (2014). Quantification and characterization of *Salmonella* spp. isolates in sewage sludge with potential usage in agriculture. *BMC Microbiology*, *14*, 263. [DOI: 10.1186/s12866-014-0263-x] [PMID: **25927729**]

Lamas, A., Miranda, J. M., Regal, P., Vázquez, B., Franco, C. M. & Cepeda, A. (2018). A comprehensive review of non-enterica subspecies of *Salmonella* enterica. *Microbiological Research*, *206*, 60–73. [DOI: 10.1016/j.micres.2017.09.010] [PMID: **29146261**]

Lapierre, L., Cornejo, J., Zavala, S., Galarce, N., Sánchez, F., Benavides, M. B., Guzmán, M. & Sáenz, L. (2020). Phenotypic and genotypic characterization of virulence factors and susceptibility to antibiotics in *Salmonella* Infantis strains isolated from chicken meat: first findings in Chile. *Animals*, *10*(6), 1049. [DOI: 10.3390/ani10061049] [PMID: **32570768**]

Li, C., Wang, Y., Gao, Y., Li, C., Ma, B. & Wang, H. (2021). Antimicrobial resistance and CRISPR typing among *Salmonella* isolates from poultry farms in China. *Frontiers in Microbiology*, *12*, 730046. [DOI: 10.3389/fmicb.2021.730046] [PMID: **34603259**]

Mejía, L., Medina, J. L., Bayas, R., Salazar, C. S., Villavicencio, F., Zapata, S., Matheu, J., Wagenaar, J. A., González-Candelas, F. & Vinueza-Burgos, C. (2020). Genomic epidemiology

of *Salmonella* Infantis in Ecuador: from poultry farms to human infections. *Frontiers in Veterinary Science*, *7*, 547891. [DOI: 10.3389/fvets.2020.547891] [PMID: **33134346**]

Mishra, P., Gattani, A. & Mahawar, M. (2020). Isolation and identification of protein l-Isoaspartate-O-Methyltransferase (PIMT) interacting proteins in *Salmonella* Typhimurium. *Current Microbiology*, *77*(5), 695–701. [DOI: 10.1007/s00284-019-01724-6] [PMID: **31263924**]

Moest, T. P. & Méresse, S. (2013). *Salmonella* T3SSs: successful mission of the secret(ion) agents. *Current Opinion in Microbiology*, *16*(1), 38–44. [DOI: 10.1016/j.mib.2012.11.006] [PMID: **23295139**]

Peighambari, S. M., Sorahi Nobar, M., & Morshed, R. (2015). Detection of *Salmonella entrica* serovar Infantis among serogroup C *Salmonella* isolates from poultry using PCR and determination of drug resistance patterns. *Iranian Veterinary Journal, 11*, 54-60. [DOI: 10.22055/ivj.2015.10112]

Perrett, C. A. & Jepson, M. A. (2009). Regulation of *Salmonella*-induced membrane ruffling by SipA differs in strains lacking other effectors. *Cellular Microbiology*, *11*(3), 475–487. [DOI: 10.1111/j.1462-5822.2008.01268.x] [PMID: **19046340**]

Quino, W., Caro-Castro, J., Mestanza, O., Hurtado, C.V., Zamudio, M.L. & Gavilan, R.G. (2020). Phylogenetic structure of *Salmonella* Enteritidis provides context for a foodborne outbreak in Peru. *Scientific Reports*, *10*(1), 22080. [DOI: 10.1038/s41598-020-78808-y] [PMID: **33328486**]

Raffatellu, M., Wilson, R. P., Chessa, D., Andrews-Polymenis, H., Tran, Q. T., Lawhon, S., Khare, S., Adams, L. G. & Bäumler, A. J. (2005). SipA, SopA, SopB, SopD, and SopE2 contribute to *Salmonella* enterica serotype Typhimurium invasion of epithelial cells. *Infection and Immunity*, *73*(1), 146–154. [DOI: 10.1128/IAI.73.1.146-154.2005] [PMID: **15618149**]

Rincón-Gamboa, S. M., Poutou-Piñales, R. A. & Carrascal-Camacho, A. K. (2021). Antimicrobial resistance of non-typhoid *Salmonella* in meat and meat products. *Foods (Basel, Switzerland)*, *10*(8), 1731. [DOI: 10.3390/foods10081731] [PMID: **34441509**]

Samiullah, S. (2013). *Salmonella* Infantis, a potential human pathogen has an association with table eggs. *International Journal of Poultry Science, 12*, 185-191. [DOI: 10.3923/ijps.2013.185.191]

Sever, N. K. & Akan, M. (2019). Molecular analysis of virulence genes of *Salmonella* Infantis isolated from chickens and turkeys. *Microbial Pathogenesis*, *126*, 199–204. [DOI: 10.1016/j.micpath.2018.11.006] [PMID: **30403968**]

Sevilla-Navarro, S., Catalá-Gregori, P., García, C., Cortés, V. & Marin, C. (2020). *Salmonella* Infantis and *Salmonella* Enteritidis specific bacteriophages isolated form poultry feces as a complementary tool for cleaning and disinfection against *Salmonella*. *Comparative Immunology, Microbiology and Infectious Diseases*, *68*, 101405. [DOI: 10.1016/j.cimid.2019.101405] [PMID: **31887484**]

Shah, D. H., Zhou, X., Addwebi, T., Davis, M. A., Orfe, L., Call, D. R., Guard, J. & Besser, T. E. (2011). Cell invasion of poultry-associated *Salmonella* enterica serovar Enteritidis isolates is associated with pathogenicity, motility and proteins secreted by the type III secretion system. *Microbiology (Reading, England)*, *157*(5), 1428–1445. [DOI: 10.1099/mic.0.044461-0] [PMID: **21292746**]

Shi, C., Singh, P., Ranieri, M. L., Wiedmann, M. & Moreno Switt, A. I. (2015). Molecular methods for serovar determination of *Salmonella*. *Critical Reviews in Microbiology*, *41*(3), 309–325. [DOI: 10.3109/1040841X.2013.837862] [PMID: **24228625**]

Shome, A., Kumawat, M., Pesingi, P. K., Bhure, S. K. & Mahawar, M. (2020). Isolation and identification of periplasmic proteins in *Salmonella* Typhimurium. *International Journal of Current Microbiology and Applied Sciences*, *9*, 1923-193. [DOI: 10.20546/ijcmas.2020.906.238]

Skyberg, J. A., Logue, C. M. & Nolan, L. K. (2006). Virulence genotyping of *Salmonella* spp. with multiplex PCR. *Avian Diseases*, *50*(1), 77–81. [DOI: 10.1637/7417.1] [PMID: **16617986**]

Taheri, H., Peighambari, S. M., Shahcheraghi, F. & Solgi, H. (2018). Pulse-field gel electrophoresis (PFGE) of *Salmonella* serovar Infantis isolates from poultry. *Iranian Journal of Veterinary Medicine*, *12*(3), 187-197. [DOI: 10.22059/ijvm.2018.236580.1004821]

Tarabees, R., Elsayed, M., Shawish, R., Basiouni, S. & Shehata, A. A. (2017). Isolation and characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium from chicken meat in Egypt. *Journal of Infection in Developing Countries*, *11*(4), 314–319. [DOI: 10.3855/jidc.8043] [PMID: **28459222**]

The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016 (2017). *EFSA Journal*, 15(12): e05077. [DOI: 10.2903/j.efsa.2017.5077] [PMID: **32625371**]

Thorns, C. J. (2000). Bacterial food-borne zoonoses. *Revue Scientifique et technique (International Office of Epizootics)*, *19*(1), 226–239. [DOI: 10.20506/rst.19.1.1219] [PMID: **11189717**]

Wajid, M., Saleemi, M. K., Sarwar, Y. & Ali, A. (2019). Detection and characterization of multidrug-resistant *Salmonella* enterica serovar Infantis as an emerging threat in poultry farms of

Faisalabad, Pakistan. *Journal of Applied Microbiology*, *127*(1), 248–261. [DOI: 10.1111/jam.14282] [PMID: **30990250**]

Wessels, K., Rip, D. & Gouws, P. (2021). *Salmonella* in chicken meat: consumption, outbreaks, characteristics, current control methods and the potential of bacteriophage use. *Foods (Basel, Switzerland)*, *10*(8), 1742. [DOI: 10.3390/foods10081742] [PMID: **34441520**]

Wilharm, G. & Heider, C. (2014). Interrelationship between type three secretion system and metabolism in pathogenic bacteria. *Frontiers in Cellular and Infection Microbiology*, *4*, 150. [DOI: 10.3389/fcimb.2014.00150] [PMID: **25386411**]

Yu, X., Zhu, H., Bo, Y., Li, Y., Zhang, Y., Liu, Y. & Zhang, X. (2021). Prevalence and antimicrobial resistance of *Salmonella* enterica subspecies enterica serovar Enteritidis isolated from broiler chickens in Shandong province, China, 2013–2018. *Poultry Science*, *100*(2), 1016–1023. [DOI: 10.1016/j.psj.2020.09.079] [PMID: **33518060**]

Zhou, D., Hardt, W. D. & Galán, J. E. (1999). *Salmonella* Typhimurium encodes a putative iron transport system within the centisome 63 pathogenicity island. *Infection and Immunity*, *67*(4), 1974–1981. [DOI: 10.1128/IAI.67.4.1974-1981.1999] [PMID: **10085045**]

Zou, W., Al-Khaldi, S. F., Branham, W. S., Han, T., Fuscoe, J. C., Han, J., Foley, S. L., Xu, J., Fang, H., Cerniglia, C. E. & Nayak, R. (2011). Microarray analysis of virulence gene profiles in *Salmonella* serovars from food/food animal environment. *Journal of Infection in Developing Countries*, *5*(2), 94–105. [DOI: 10.3855/jidc.1396] [PMID: **21389588**]

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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

Table 2: Primer sequences used for the detection of 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

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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	+	+	-	+	-	-
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	+	+	-	-	-	-

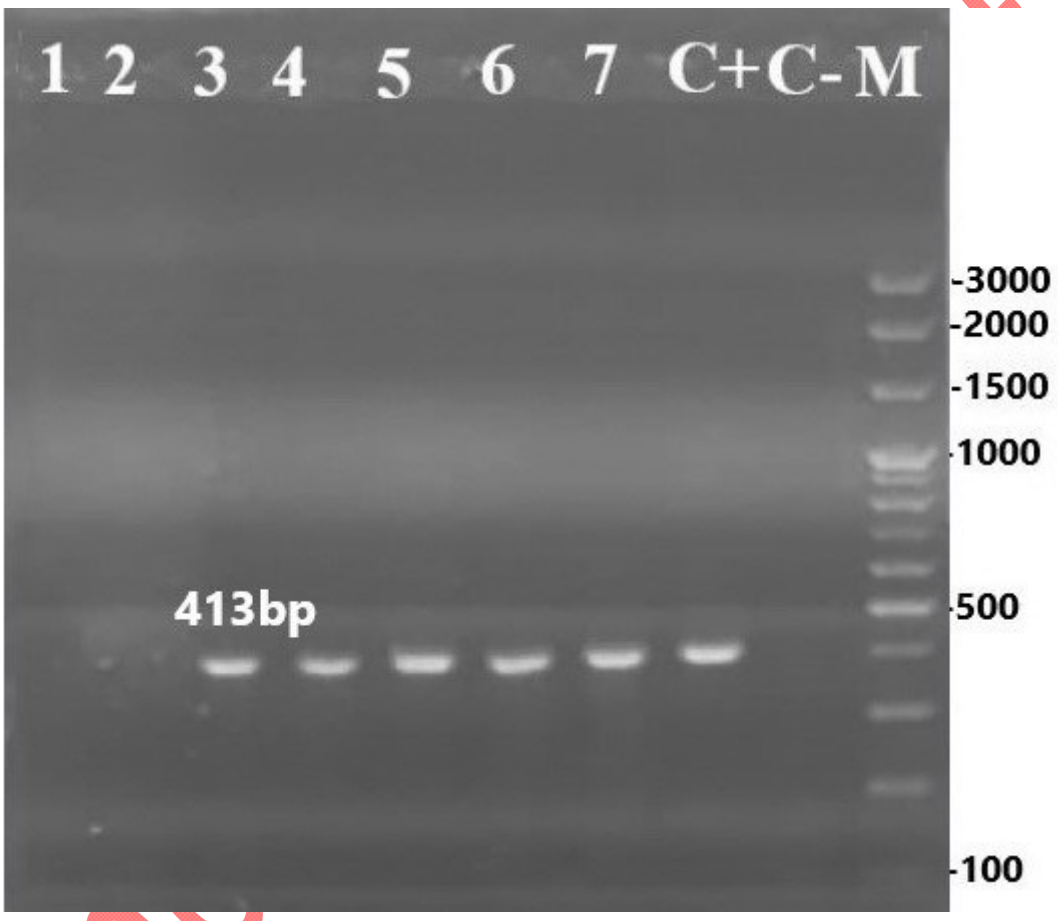

Figure 1. Electrophoresis of PCR products on 1% agarose gel to confirm *Salmonella* Infantis. Amplified 413 bp bands of 5 isolates are shown in lanes 3 to 7. Lanes M, C+, and C- indicate commercial 100 bp Plus DNA Ladder II, positive control, and negative control (dH2O instead of DNA), respectively. Other lanes demonstrate the negative results for tested *Salmonella* isolates.

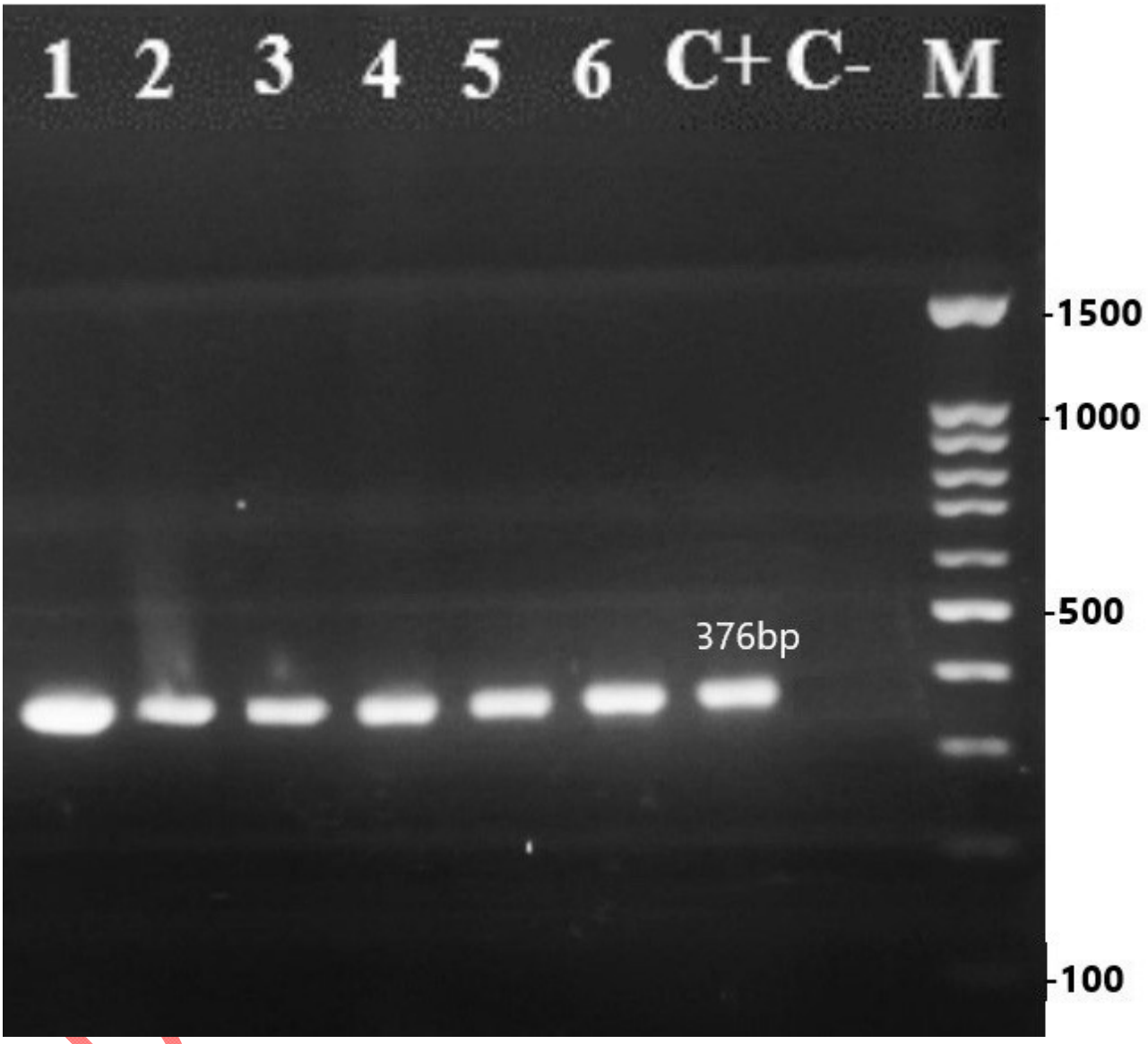

Figure 2. Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *sopE* gene. Amplified 376 bp bands of isolates are shown in lanes 1 to 6. Lanes M, C+, and C- indicate commercial 100 bp DNA Ladder, positive control, and negative control (dH2O instead of DNA), respectively.

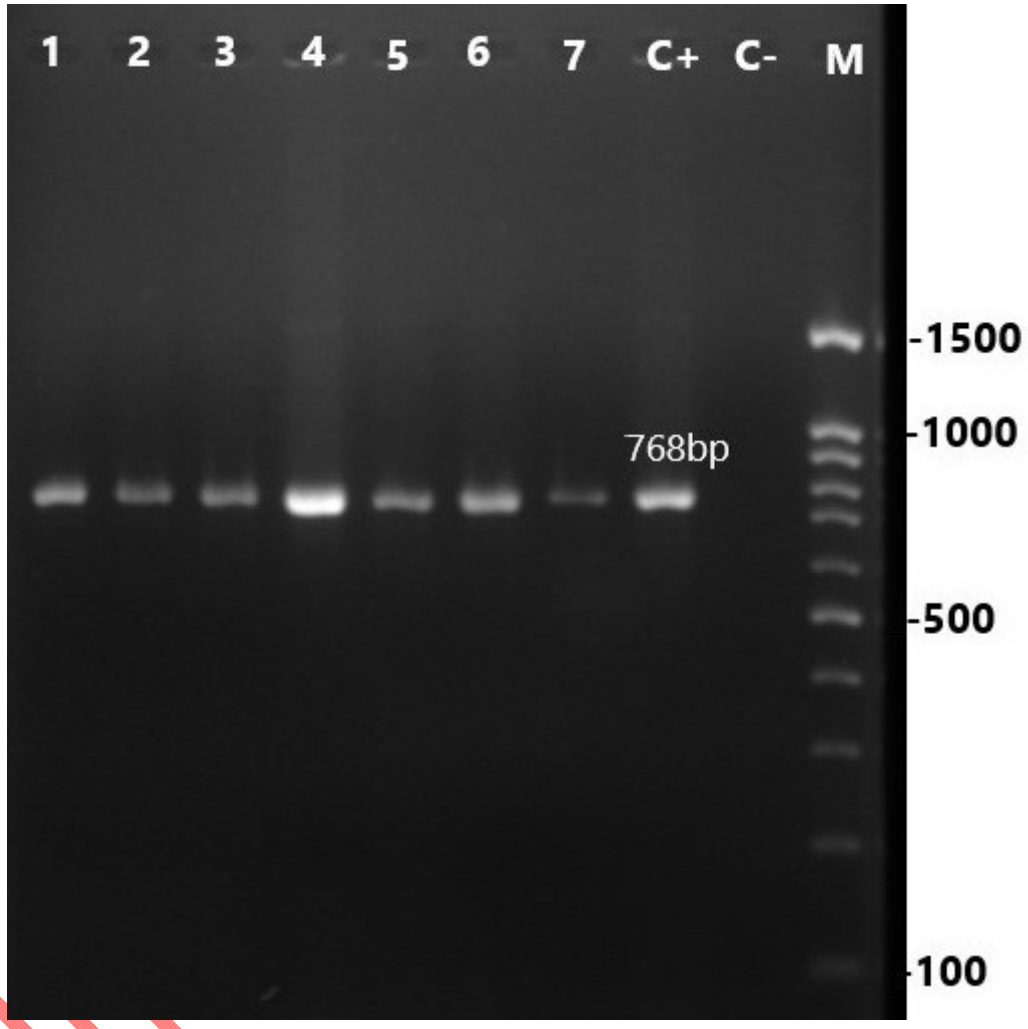

Figure 3: Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *sitC* gene. Amplified 768 bp bands of isolates are shown in lanes 1 to 7. Lanes M, C+, and C- indicate commercial 100 bp DNA Ladder, positive control, and negative control (dH2O instead of DNA), respectively.

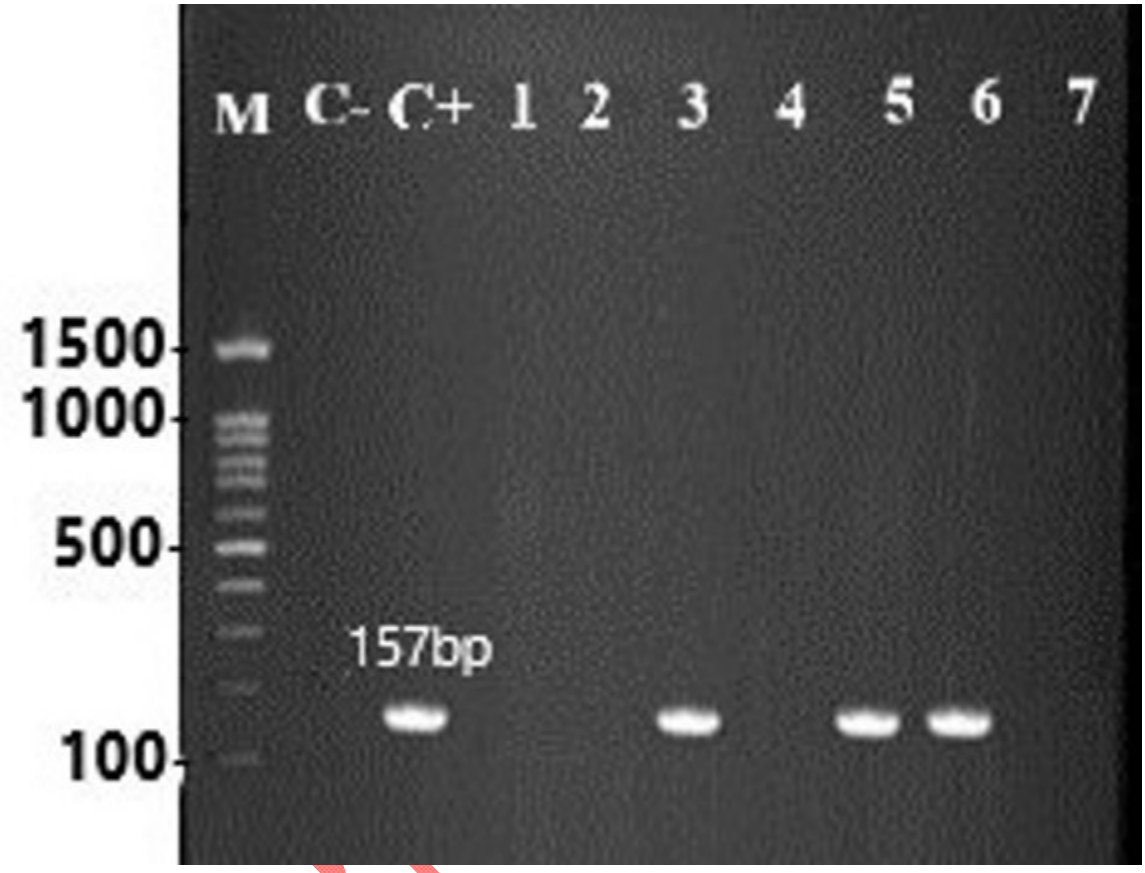

Figure 4: Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *pefA* gene. Amplified 157 bp bands of isolates are shown in lanes 3, 5 and 6. Lanes M, C+, and C- indicate commercial 100 bp DNA Ladder, positive control, and negative control (dH2O instead of DNA), respectively. Other lanes demonstrate the negative results for tested *pefA* gene.

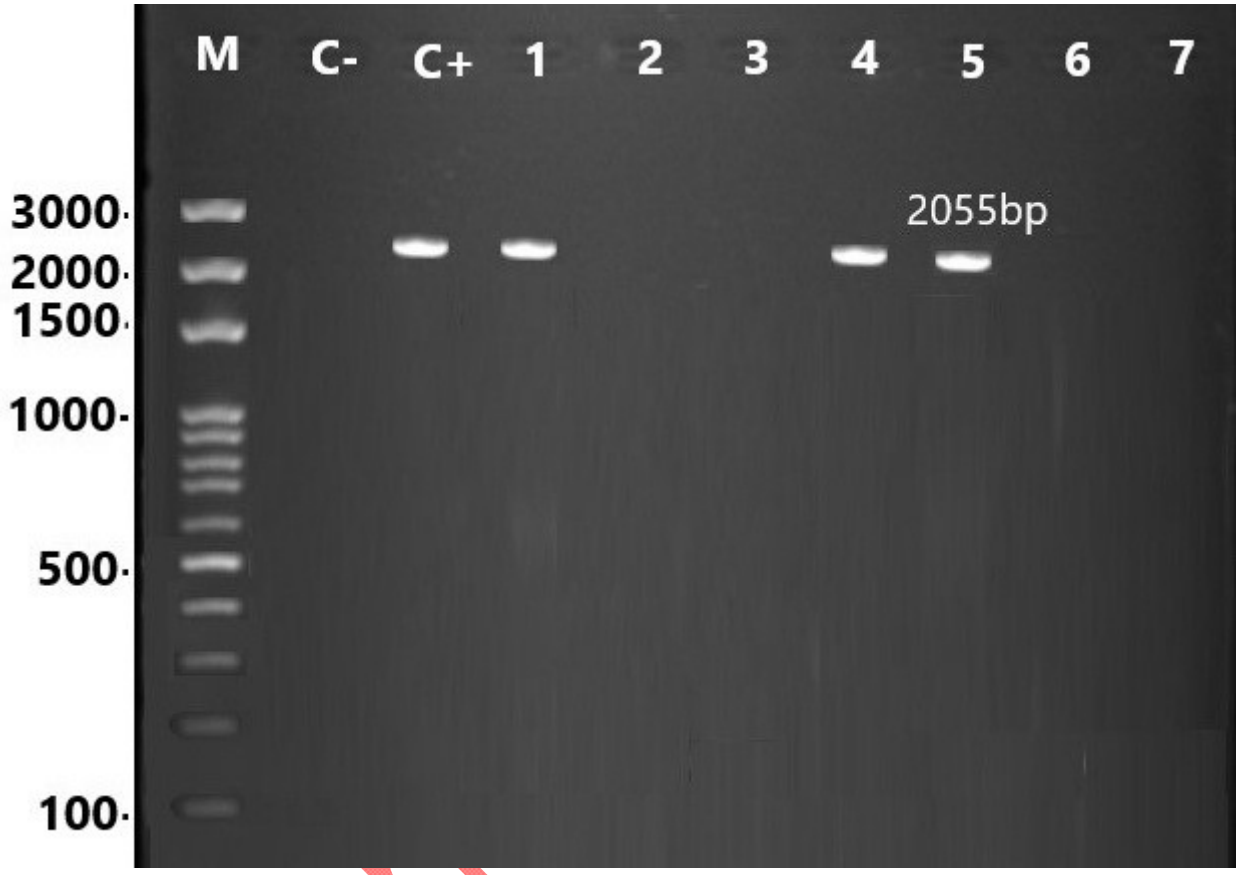

Figure 5: Electrophoresis of PCR products on 1% agarose gel to confirm the presence of *sipA* gene. Amplified 2055 bp bands of isolates are shown in lanes 1, 4 and 5. Lanes M, C+, and C- indicate commercial 100 bp Plus DNA Ladder II, positive control, and negative control (dH2O instead of DNA), respectively. Other lanes demonstrate the negative results for tested *sipA* gene.

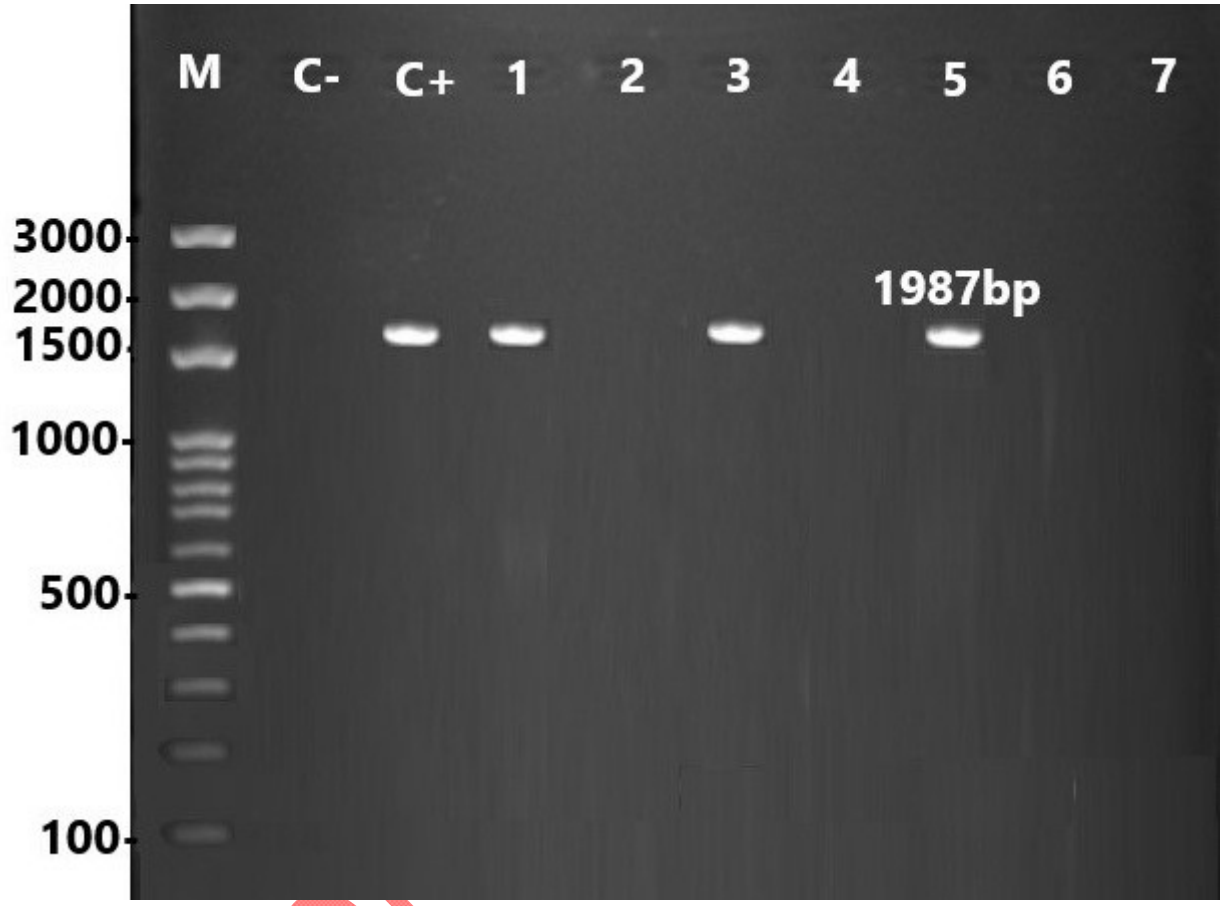

Figure 6: Electrophoresis of PCR products on 1% agarose gel to confirm the presence of s*opB* gene. Amplified 1987 bp bands of isolates are shown in lanes 1, 3 and 5. Lanes M, C+, and C- indicate commercial 100 bp Plus DNA Ladder II, positive control, and negative control (dH2O instead of DNA), respectively. Other lanes demonstrate the negative results for tested s*opB* gene.

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جستجوی ژن‌های حدت سالمونلا سروار اینفنتیس جداشده از منابع طیوری

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عنوان کوتاه: ژن های حدت سالمونلا اینفنتیس

چکیده

زمینه مطالعه: سالمونلوز به صورت گسترده، به عنوان 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 جداسازی شده 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 جدا شده از منابع مختلف طیور در کشور ایران است.

روش کار: در این مطالعه 54 جدایه سالمونلا اینفنتیس که از لاشه طیور، مدفوع طیور و کشتارگاه جداسازی شده بودند، مورد بررسی PCR (اختصاصی هر ژن، به منظور بررسی 6 ژن حدت مهم سالمونلا اینفنتیس *sopB*, *sopE*, *sitC*, *pefA*, *sipA*, *spvC*) طراحی و مورد استفاده قرار گرفت.

نتایج: تعداد 51 جدایه (94/4٪) دارای ژن حدت *sopE*، 49 جدایه (90/7٪) دارای ژن حدت *sitC* واجد (48/1٪) ، 26 جدایه بودند. همچنین ژن حدت *sipA* و 15 جدایه (27/7٪) واجد ژن حدت *sopB*، 5 جدایه (9/2٪) واجد ژن حدت *pefA* ژن حدت *spvC* در هیچکدام از جدایه ها مشاهده نشد.

نتیجه گیری نهائی: در مطالعه حاضر، ویژگی های مشابه و قابل توجهی در ژن های حدت جدایه های بدست آمده از مدفوع طیور و کشتارگاه طیور مشاهده شد که به لحاظ بهداشت عمومی حائز اهمیت و باعث نگرانی است. اگرچه نیاز است که جدایه های سالمونلا اینفنتیس بیشتری از منابع مختلف طیور و انسان مورد بررسی و آنالیز قرار بگیرند. یافته های این بررسی می تواند به محققان بهداشتی به منظور درک روند بیماری زایی و همه گیر شناسی سالمونلا اینفنتیس در ایران کمک کننده باشد.

واژه های کلیدی: بیماری‌زایی، بهداشت عمومی، سالمونلا اینفنتیس، ژن‌های حدت، طیور