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Molecular Identification of *Salmonella* Strains Isolated from Livestock in Alborz Province and Their Serotyping

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ABSTRACT

Background and Aim: Salmonellosis is an important infectious zoonotic disease that makes it even more significant to identify and control the causative strains. Molecular methods, especially polymerase chain reaction (PCR), for virulence genes can help to quickly and accurately identify *Salmonella* strains. Accordingly, the purpose of this study was molecular identification based on *sivH*, *hilA* and *sefA* genes and serotyping of *Salmonella* strains isolated from livestock in Alborz province, Iran.

Materials and Methods: The present study was conducted on 30 *Salmonella* strains isolated from livestock in Alborz province. *Salmonella* strains were isolated using morphological identification and differential and selective culture media. DNA was then extracted by boiling, and PCR was performed to detect the virulence genes of *hilA*, *sivH,* and *sefA*. The sensitivity and specificity of the primers used were determined using PCR.

Results: The PCR findings showed that 27 (90%) isolates had the *hilA* gene, 10 (33.3%) isolates had the *sefA* gene, and 24 (80%) isolates had the *sivH* gene. Moreover, the highest frequency among serotypes was related to *Salmonella typhimurium* (10%). The sensitivity of ST11-ST15, *hilA*, *sefA,* and *sivH* primers were estimated at 0.0001, 1, 0.1, and 0.001 ng/mol, respectively. The specificity of primers for *Salmonella* strains was also confirmed.

Conclusion: Identifying livestock with salmonellosis and isolating pathogenic strains from other livestock are of the most important methods capable of reducing the prevalence of foodborne infection in consumers. This can be achieved by the PCR technique for virulence genes, especially *hilA,* which is more prevalent among *Salmonella* strains.

Keywords: Molecular identification, *Salmonella*, Serotyping, Virulence genes

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1 Introduction

Salmonella infection (salmonellosis) is a foodborne disease with a global spread in humans. It is one of the major public health problems that are of particular importance not only in developing countries but also in developed countries **(1)**. These diseases develop due to eating habits, food delivery methods, production time, storage and distribution of food, and cause great damage to public health and the food industry **(2, 3)**. Variety in hosts, serotypes, and natural carriers has made salmonellosis the most common foodborne microbial disease in humans **(4)**. Livestock and their products are the most important transmission sources of *Salmonella* serotypes and food poisoning in humans. Despite all the measures taken and health improvements, it is still a problem in the livestock industry **(5, 6)**.

Due to the importance of *Salmonella* strains in the occurrence of salmonellosis and its presence in various sources is a serious risk to humans, its rapid detection in various sources is of particular

importance **(7, 8)**. Since methods based on culture and isolation are time-consuming, using methods capable of quickly detecting the presence of Salmonella in suspected specimens can effectively prevent disease and outbreaks **(9, 10)**. Molecular methods are currently of the fastest and most sensitive diagnostic techniques **(11)**. Polymerase chain reaction (PCR) is one of the most common molecular detection techniques. This method utilizes different genes involved in the pathogenicity of *Salmonella* strains **(12, 13)**.

One of these genes is *hilA*, which encodes the central regulator of HilA, which is essential for the expression of components of the secretory system type III (TTSS). Salmonella species have pathogenicity islands (SPIs) that are critical to pathogenicity and encode TTSS pathogenic islands, which are able to inject bacterial effector proteins through bacterial and host membranes to interact with host cells **(14)**. In addition, the *hilA* gene is required to invade epithelial cells and induce apoptotic macrophages. It also encodes invasive proteins and facilitates the penetration of Salmonella into intestinal epithelial cells by encoding regulatory and transcription proteins **(15)**. The *sivH* is a specific gene *in Salmonella enterica subsp. Enterica serovar Typhi* is responsible for cell adhesion. In fact, this protein is related to the outer membrane of bacteria associated with intestinal colonization, which is also involved in invasion **(14, 16)**. Surface structures such as fimbriae are on the surface of Salmonella, which makes it possible to identify serotypes of a species **(17)**. Types of surface structures include SEF21, SEF14, and SEF17, which is the fimbriae encoding gene SEF14, called *sefA*, which is essential for survival and proliferation within the large vacuole **(17, 18)**. Other genes used in molecular detection of *Salmonella* include *sefA*, *spv*, *fliC*, *invA*, *fljB*, *rfbJ* **(15, 19)**.

Accordingly, this study aimed to investigate the presence of *sivH*, *hilA,* and *sefA* genes by PCR and serotyping of *Salmonella* strains isolated from livestock in Alborz province.

2.Materials and Methods

Salmonella Strains

In this study, 30 required livestock samples were obtained from the microbial collection of the microbiology department of the Razi Vaccine and Serum Research Institute, Iran, as lyophilized powders. To enrich the bacteria, 0.5 mL of TSB medium containing horse serum was added to the lyophilized vials, and the suspension was cultured on McConkey agar medium. The media were then incubated at 37°C for 24 hours.

Confirmation of *Salmonella* Strains

Morphological and biochemical tests were used to confirm the prepared strains. Morphological identification was performed by gram staining, and biochemical identification was performed by Simmon's Citrate, MR/VP, lysine, urease, indole, and lactose tests. Standard strains, including *Salmonella enteritidis* RTCC 1621 (ATCC: 13706) and *Salmonella typhimurium* RTCC 1735 (ATCC: 14028), were selected as positive controls.

Serotyping of Strains

Serotyping of isolated *Salmonella* strains was performed based on the standard rapid slide agglutination method, using O and H antibodies. A 24-hour suspension of bacteria in 9% normal saline was used on the slide, and the autoagglutination reaction was controlled by mixing one drop of antiserum. The occurrence of agglutination in less than two minutes was considered a positive reaction. First, a group set was determined using polyvalent antisera, and then multiple monovalent antisera were used. Serotypes were identified according to the Kauffman – White table.

DNA Extraction

The DNA extraction from bacteria was performed by boiling method. To this end, the strains were first cultured on a nutrient agar medium. After growth, some colonies were taken by sterile loop and dissolved in microtubes containing 500 µL of normal saline solution. The microtubes were centrifuged at 1000 rpm for 5 minutes. Then, the supernatant was discarded, and 600 µL of TE1X (Tris EDTA buffer) was added to the precipitate. After placing in a water bath at 90°C for 10 minutes, the microtubes were immediately placed in an ice container for 5 minutes and centrifuged for 4 minutes at 1000 rpm. In the last step, 6-7 µL of proteinase k was added to each microtubes, and the extracted DNA was stored at -20°C. The quantity and quality of the extracted DNA were measured by spectrophotometry and agarose gel electrophoresis, respectively **(20)**.

Polymerase Chain Reaction

A general PCR procedure was performed to evaluate the presence of *Salmonella* genera using ST11 and ST15 primers. Moreover, specific PCR procedures were performed to evaluate the presence of *hilA*, *sefA*, and *sivH* genes using the primers listed in Table 1. The PCR procedure was performed using Taq DNA l 2.0X Master Mix RED during 30 thermal cycles. The PCR products were observed using 3% agarose gel electrophoresis. The standard strains of *S. typhimuriom* ATCC 1735 (RTCC 14028) and *S. entritidis* ATCC 1621 (RTCC 13706) were used as positive controls **(21)**.

Table 1. Specifications of used primers

Gene	Sequence	Fragmet Length	CG%	Ref.
ST11	GCCAACCATTGCTAAATTGGCGCA	429bp	50. 0	(22)
ST15	GGTAGAAATTCCCAGCGGGTACTGG		56. 0	
***hil*A**	**F**-CGGAGGCTTATTTGCGCCATGCTGAGGTAG	854bp	53. 3	(23)
	R-GCATGGATCCCCGCCGGCGAGATTGTG		66. 7	
***sef*A**	**F**-GCAGCGGTTACTATTGCAGC	310bp	50. 0	(24)
	R-TGTGACAGGGACATTTAGCG		55. 0	
***siv*H**	**F**-CAGAATGCGAATCCTTCGCAC	763bp	52. 4	(14)
	R-GTATGCGAACAAGCGTAACAC		47. 6	

Determination of Gene Sensitivity

Dilutions of 0.100-0.000001 ng/mL were prepared from primers. After DNA purification from *S. typhimurium*, *S. aberdeen*, *S. enteritidis,* and *S. eastbuorne* strains for ST, hilA, sivH, and sefA primers, respectively, the sensitivity of the primers was measured by PCR with different dilutions of the primer.

Determination of Primer Specificity

S. tsevie, *Citrobacter freundii*, *E. coli,* and Shigella strains were used to evaluate the specificity of primers. The standard strains of *S. enteritidis* RTCC 1621 and *S. typhimurium* RTCC 1735 were also positive controls.

3.Results

Biochemical Confirmation of Strains

Because *Salmonella* strains are lactose negative, the colonies of strains isolated in McConkey agar medium were the same color as the medium, confirming *Salmonella*. Gram-negative *Salmonella* bacilli were also observed in gram staining. Biochemical tests with the results of positive Simmon's Citrate, MR, and lysine and negative urease, VP, indole, and lactose confirmed the strains isolated as *Salmonella*.

Serotyping Strains

The standard and valid Salmonella serotyping method results are presented in Table 2. As seen, the highest frequency was related to *S. enteritidis* (10%), *S. abortusovis* (6.6%), and *S. tsevie* (6.6%).

Table 2. The results of serotyping *Salmonella* strains

Frequency	Serotype	Isolate number	Frequency	Serotype	Isolate number
3.3%	*S. cubana*	16	3.3%	*S. duesseldrof*	**1**
3.3%	*S. infantis*	17	3.3%	*S. Aberdeen*	**2**
3.3%	*S. dublin*	18	10%	*S. enteritidis*	**3**
10%	*S. entritidis*	19	3.3%	*S. ndolo*	**4**
3.3%	*S. dublin*	20	3.3%	*S. adelaide*	**5**
3.3%	*S. daytono*	21	3.3%	*S. typhimurium*	**6**
6.6%	*S. abortusovis*	22	3.3%	*S. sandow*	**7**
3.3%	*S. typhimurium*	23	3.3%	*S. derby*	**8**
6.6%	*S. tsevie*	24	3.3%	*S. eastbourne*	**9**
3.3%	*S. angusten borg*	25	3.3%	*S. typhimurium*	**10**
3.3%	*S. paratyphi B*	26	10%	*S. entritidis*	**11**
3.3%	*S. dublin*	27	3.3%	*S. newport*	**12**
3.3%	*S. typhimurium*	28	3.3%	*S. bovismorbifaciam*	**13**
6.6%	*S. tsevie*	29	3.3%	*S. anatum*	**14**
6.6%	*S. abortusbovis*	30	3.3%	*S. dublin*	**15**

Identification of Virulence Genes by PCR Procedure

The results of the PCR test showed that the control strains had all three genes studied. The two strains of *S. ndolo* and *S. adelaide* did not have any of the virulence genes studied. Overall, 27 (90%) isolates had *hilA* gene, 10 (33.3%) isolates had *sefA* gene and 24 (80%) isolates had *isoH* gene (Figure 1).

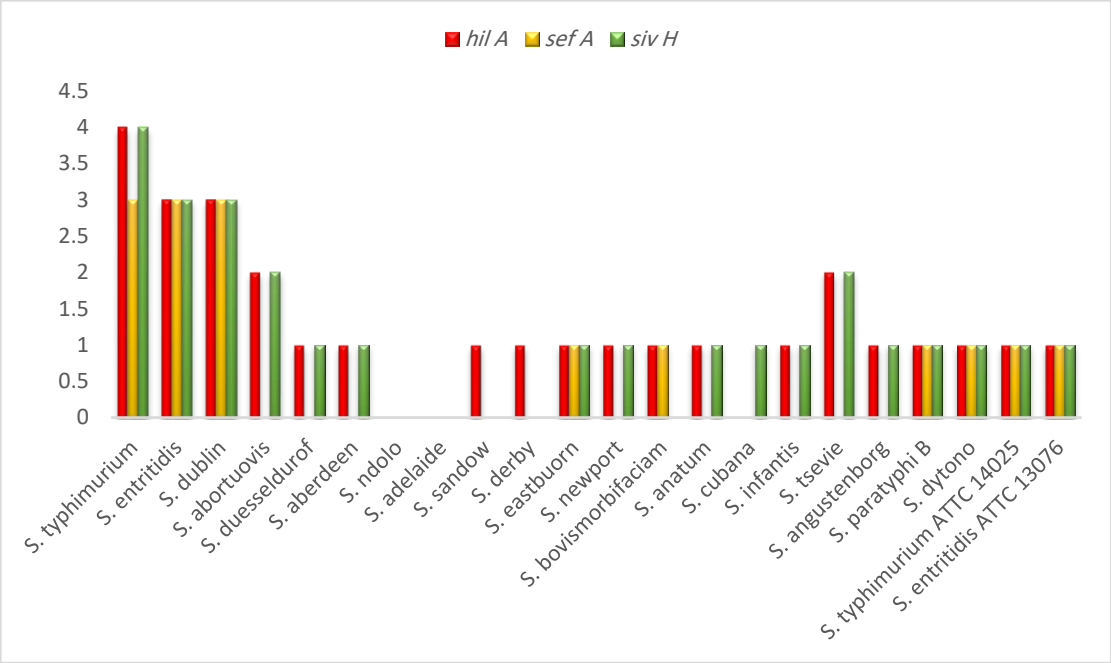

Figure 1. Frequency of *hilA*, *sefA*, *sivH* genes in different *Salmonella* serotypes

Sensitivity of primers: Primers used for ST11-ST15 gene had a sensitivity of 0.0001 ng/mL (Figure 2-A), while the *hilA* primer had a sensitivity of 1 ng/mL (Figure 2-B), the *SivH* primer had a sensitivity of 0.001 ng/mL (Figure 2-C) and the *sefA* primer had a sensitivity of 0.1 ng/mL (Figure 2-D).

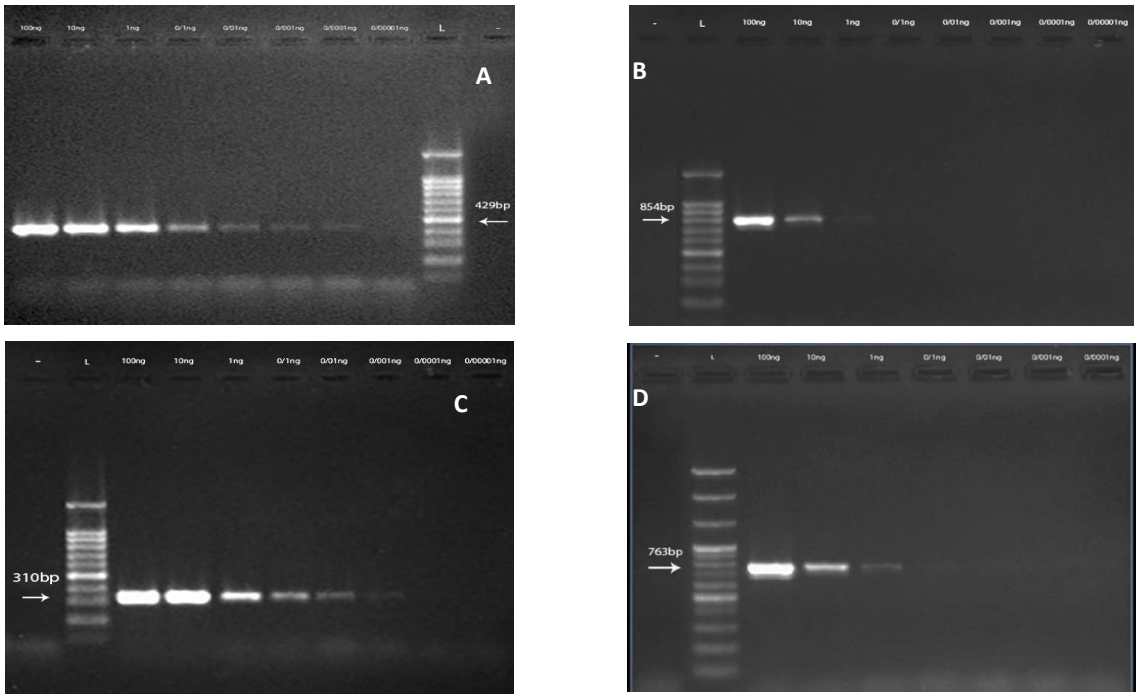

Figure 2. PCR results to determine the sensitivity of ST11-ST15 (A), *hilA* (B), *SivH* (C) and *sefA* (D) primers (L: molecular ladder, other wells: dilutions of 100 ng to 0.00001 primer)

Specificity of Primers

Primers used for ST11-ST15, *hilA*, *sefA,* and *sivH* genes were able to form bands in *S. typhimurium*, *S. enteritidis,* and *S. tsevie*. Still, no band was observed for *C. freundii*, *E. coli* and Shigella strains, indicating the specificity of the primers (Figure 3).

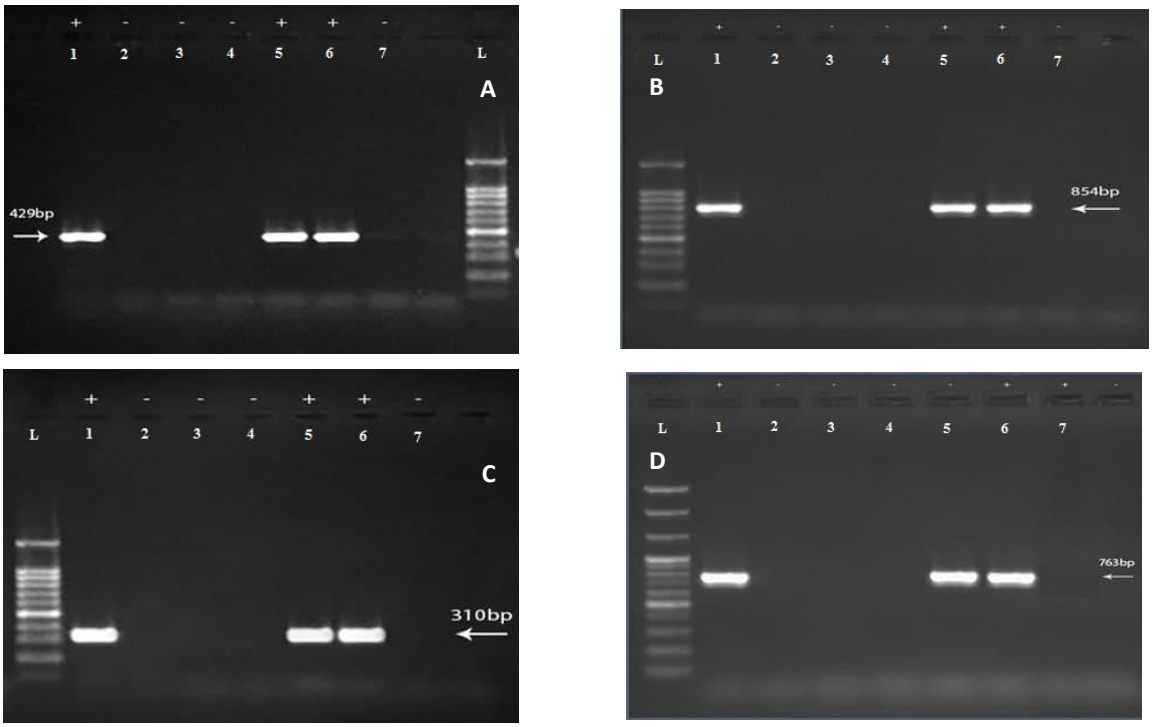

Figure 3. PCR results to determine the specificity of ST11-ST15 (A), *hilA* (B), *SivH* (C), and *sefA* (D) primers (L: molecular ladder 1: *S. tsevie*, 2: *Citrobacter freundii*, 3: *E. coli*, 4: Shigella, 5: positive control of *S. typhimurium* ATCC 14028, 6: positive control of S.

4.Discussion

Isolation, identification, control, and prevention of Salmonella strains in animal source foods are essential in public health. Among diagnostic methods, the PCR technique is a suitable tool for identifying food contaminated with *Salmonella* strains due to its sensitivity and high rapidity. Accordingly, the present study employed the PCR technique to detect three genes of *hilA*, *sefA,* and *sivH* among *Salmonella* strains isolated from livestock. The results showed the presence of the *hilA* gene in 90% of samples, the *SefA* gene in 33.3% of samples, and the *sivH* gene in 80% of samples.

Many studies detected the virulence genes in *Salmonella* strains of different origins, which have reported various genes. Consistent with the present study results, in a study on the identification of *Salmonella* strains in clinical samples from Saudi Arabia using PCR based on *hilA* and *invA* genes, 23 *Salmonella* strains were isolated from stool samples **(25)**. Also, in line with the present study results, the prevalence, virulence genes, and antimicrobial resistance profiles of *Salmonella* serovars from retail beef in Selangor, Malaysia, by Thung *et al.*, out of 240 retail beef meat samples, 23 *Salmonella* strains were isolated. In their study, PCR screening via *Salmonella*-specific *hilA* primers showed that the frequency of this virulence gene in strains without considering their serovars was 82.61% **(23)**. In a study by Pathmanathan *et al.* on the simple and rapid detection of Salmonella strains using the *hilA* primer, 33 Salmonella strains from 27 serovars and 15 non-Salmonella species from 8 different genera from Medical Research Institutes and the Medical University of Malaya were selected. All of the studied Salmonella samples had the *hilA* gene, while this gene was not observed in any non-salmonella strains **(26)**.

In the present study, 90% of the samples had the *hilA* gene, which is close to the results of the mentioned studies. More studies should be done on animal samples about this gene. The type of host should also be considered. There may be differences in identifying this gene from all samples compared to the Malay study for the reasons mentioned above.

In another part of the results of the present study, the *SefA* gene was observed in 33.3% of the samples. Since this gene encodes surface structures such as fimbriae on the surface of Salmonella, it is possible to identify serotypes of a species through these structures **(17)**. In a study inconsistent with the present results by Borges *et al.,* which is aimed to

identify virulence genes in *S. enteritidis* strains isolated from chickens in southern Brazil, the PCR results showed that 100% of the strains had *hilA* and *SefA* genes **(14)**. In the study of Ranjbar *et al.* In 2020, out of 110 samples, 101 samples (91.81%) with 16S rRNA gene were positive for Salmonella, and 86 samples (85.14%) carried the *sefA* gene **(27)**. Also, in line with the results of this study, in a study by Zahedi *et al.* that investigated the prevalence of *S. enteritidis* and *S. typhimurium* in marketed meat in Shahrekord, Iran, 360 meat samples of cow, camel, sheep, goat, and chicken were collected from sale centers and tested for *Salmonella* contamination by culture, biochemical and PCR tests, followed by testing *S. typhimurium* and *S. entertidis* isolated on the bases of virulence genes *invA*, *rfbj*, fliC, *fljB*, *spV,* and *sefA*. Out of 54 *Salmonella* isolates, 24 isolates were *S. typhimurium*, 20 isolates were *S. enteritidis,* and 10 isolates belonged to other *Salmonella* serotypes. Based on PCR results, all strains had virulence genes **(28)**.

The results showed the presence of the *SefA* gene in 80% of the samples. Also, in line with the present study, Webber *et al.,* in a study in 2019, stated that all the studied species have the mentioned gene, and all the samples were *Salmonella enterica* subspp. *Enterica* **(29)**.

Generally, in the present study, 90% of the *hilA* gene, 33.3% of the *SefA* gene, and 80% of the *sivH* gene were reported in the studied strains, which indicates the suitability of these genes, especially the *hilA* gene, to identify *Salmonella* strains.

5.Conclusion

The results of this study and its comparison with other studies show that the genes *hilA*, *sefA,* and *sivH* are present in some Salmonella serotypes and cannot be used with certainty as diagnostic genes but can be used their presence in selecting serotypes for the production of biological products.

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Conflict of Interest

There is no conflict of interest between the authors.

Reference

1. Azizpour A. Prevalence and Antibiotic Resistance of Salmonella Serotypes in Chicken Meat of Ardabil, Northwestern Iran. Iran J Med Microbiol. 2021;15(2):232-46. [DOI:10.30699/ijmm.15.2.232]
2. Ferrari RG, Rosario DK, Cunha-Neto A, Mano SB, Figueiredo EE, Conte-Junior CA. Worldwide epidemiology of Salmonella serovars in animal-based foods: a meta-analysis. Applied and environmental microbiology. 2019;85(14):e00591-19. [DOI:10.1128/AEM.00591-19] [PMID] [PMCID]
3. Jabin T, Siam EA-d, Dipu MR, Asaduzzaman M, Uddin A. Phenotypic characterization of Salmonella typhi from clinical specimens and determination of the drug susceptibility pattern of the isolates. Iran J Med Microbiol. 2021;15(2):189-94. [DOI:10.30699/ijmm.15.2.189]
4. Nozohour Y, Jalilzadeh G. Antibacterial Activities of Ethanolic Extract of Malva sylvestris L. Against Salmonella enterica and Escherichia coli Isolated from Diarrheic Lambs. Iran J Med Microbiol. 2021;15(1):121-9. [DOI:10.30699/ijmm.15.1.121]
5. Gast RK, Porter Jr RE. Salmonella infections. Dis Poult. 2020:717-53. [DOI:10.1002/9781119371199.ch16]
6. Bahadur DL, Prasad DI, Kumar YS, Md A, Zohorul IM. Prevalence and antibiotic resistance profile of Salmonella from livestock and poultry raw meat, Nepal. Int J Mol Vet Res. 2016;6.
7. Lee K-M, Runyon M, Herrman TJ, Phillips R, Hsieh J. Review of Salmonella detection and identification methods: Aspects of rapid emergency response and food safety. Food Control. 2015;47:264-76. [DOI:10.1016/j.foodcont.2014.07.011]
8. Odumeru JA, León-Velarde CG. Salmonella detection methods for food and food ingredients. Salmonella-A Dangerous Foodborne Pathogen Rijeka, Croatia: InTec. 2012:373-92.
9. Eriksson E, Aspan A. Comparison of culture, ELISA and PCR techniques for Salmonella detection in faecal samples for cattle, pig and poultry. BMC veterinary research. 2007;3(1):1-19. https://doi.org/10.1186/1746-6148-3-21 [DOI:10.1186/1746-6148-3-1] [PMID] [PMCID]
10. Oliveira S, Rodenbusch C, Ce M, Rocha S, Canal C. Evaluation of selective and non-selective enrichment PCR procedures for Salmonella detection. Letters in Applied Microbiology. 2003;36(4):217-21. [DOI:10.1046/j.1472-765X.2003.01294.x] [PMID]

11. Mthembu TP, Zishiri OT, El Zowalaty ME. Molecular detection of multidrug-resistant Salmonella isolated from livestock production systems in South Africa. Infection and drug resistance. 2019;12:3537. [DOI:10.2147/IDR.S211618] [PMID] [PMCID]

12. Moradi Bidhendi S. A Review of studies on isolation, diagnosis and antimicrobial resistance of Salmonella in Iran. Vet Res Biol Prod. 2015;109(4):21-30.

13. Alam SB, Mahmud M, Akter R, Hasan M, Sobur A, Nazir K, et al. Molecular detection of multidrug resistant Salmonella species isolated from broiler farm in Bangladesh. Pathogens. 2020;9(3):201. [DOI:10.3390/pathogens9030201] [PMID] [PMCID]

14. Borges KA, Furian TQ, Borsoi A, Moraes HL, Salle CT, Nascimento VP. Detection of virulence-associated genes in Salmonella Enteritidis isolates from chicken in South of Brazil. Pesqui Vet Bras. 2013;33:1416-22. [DOI:10.1590/S0100-736X2013001200004]

15. Allam SA, Mostafa NY, Kirrella GA, Eleiwa NZ, El-Magd MA. Molecular detection of invA and hilA virulent genes in Salmonella serovars isolated from fresh water fish. Slov Vet Res. 2019;56(Suppl 22):693-8. [DOI:10.26873/SVR-809-2019]

16. Bugarel M, Tudor A, Loneragan G, Nightingale K. Molecular detection assay of five Salmonella serotypes of public interest: Typhimurium, Enteritidis, Newport, Heidelberg, and Hadar. Journal of microbiological methods. 2017;134:14-20. [DOI:10.1016/j.mimet.2016.12.011] [PMID]

17. Morshed R, PEYGHAMBARI S. Distribution of sefA gene among Salmonella Enteritidis isolates from poultry sources and potential as diagnostic and epidemiological tools. 2008.

18. Newell D, Elvers K, Dopfer D, Hansson I, Jones P, James S, et al. Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms. Applied and environmental microbiology. 2011;77(24):8605-14. [DOI:10.1128/AEM.01090-10] [PMID] [PMCID]

19. Sever NK, Akan M. Molecular analysis of virulence genes of Salmonella Infantis isolated from chickens and turkeys. Microb Pathog. 2019;126:199-204. [DOI:10.1016/j.micpath.2018.11.006] [PMID]

20. Dashti AA, Jadaon MM, Abdulsamad AM, Dashti HM. Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques. Kuwait Med J. 2009;41(2):117-22.

21. Pritha ST, Rahman S, Punom SA, Rahman MM, Nazir KNH, Islam MS. Isolation, Molecular Detection and Antibiogram of Multi-drug Resistant Salmonella Typhimurium DT104 from Selected Dairy Farms in Mymensingh, Bangladesh. Am J Microbioll Res. 2020;8(4):136-40.

22. Pui C, Wong W, Chai L, Tunung R, Jeyaletchumi P, Hidayah N, et al. Salmonella: A foodborne pathogen. Intl Food Res J. 2011;18(2).

23. Thung TY, Radu S, Mahyudin NA, Rukayadi Y, Zakaria Z, Mazlan N, et al. Prevalence, virulence genes and antimicrobial resistance profiles of Salmonella serovars from retail beef in Selangor, Malaysia. Frontiers in microbiology. 2018;8:2697. [DOI:10.3389/fmicb.2017.02697] [PMID] [PMCID]

24. Pan T-M, Liu Y-J. Identification of Salmonella enteritidis isolates by polymerase chain reaction and multiplex polymerase chain reaction. Journal of microbiology, immunology, and infection. 2002;35(3):147-51.

25. Ohud M, Eman MH, Hayam SA. Detection of Salmonella strains in clinical samples from Saudi Arabia by invA and hilA polymerase chain reaction (PCR)-based assays. Afr J Microbiol Res. 2012;6(25):5410-6. [DOI:10.5897/AJMR12.771]

26. Pathmanathan S, Cardona-Castro N, Sanchez-Jimenez M, Correa-Ochoa M, Puthucheary S, Thong KL. Simple and rapid detection of Salmonella strains by direct PCR amplification of the hilA gene. Journal of Medical Microbiology. 2003;52(9):773-6. [DOI:10.1099/jmm.0.05188-0] [PMID]

27. Ranjbar R, Dehkordi FS, Heiat M. The frequency of resistance genes in Salmonella enteritidis strains isolated from cattle. Iranian Journal of Public Health. 2020;49(5):968. [DOI:10.18502/ijph.v49i5.3215] [PMID] [PMCID]

28. Zahedi M, Rahimi E, Zahedi M, Momtaz H. Prevalence of Salmonella enteritidis and S. typhimurium in marketed meat in Shahrekord in 2014. J Shahrekord Univ Med Sci. 2017;19.

29. Webber B, Borges KA, Furian TQ, Rizzo NN, Tondo EC, Santos LRd, et al. Detection of virulence genes in Salmonella Heidelberg isolated from chicken carcasses. Revista do Instituto de Medicina Tropical de São Paulo. 2019;61. [DOI:10.1590/s1678-9946201961036] [PMID] [PMCID]