Journal of Research in Medical and Dental Sciences
2018, Volume 6, Issue 2, Page No: 404-408

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Molecular Detection of Virulence Genes of *Salmonella gallinarum* from Iranian Poultry

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DOI: 10.5455/jrmds.20186262

ABSTRACT

The plasmid spvABCD genes are arranged in an operon that involved in intracellular survival and replication of Salmonella. This study aimed to evaluate the molecular detection of virulence genes of S. gallinarum recovered from food substance and feces samples of Iranian poultry. Diagnosis of Salmonella from 51 food substance and feces samples of poultry was performed using culture. Virulence gene of Hq703462 was used for identification of S. gallinarum serovars using Real-Time PCR. For differentiation between two biovars, ratA, tepB and rhs genes were used by Multi-Plex PCR. In determining the presence and absence of two virulence genes (sdiA and spvC), PCR was used. The data analyzed by SPSS software version 22 using Chi-square test. PCR detected the virulence genes sdiA-274 bp and spvC-571 bp. The prevalence of sdiA and spvC genes were reported 100% and 61%, respectively. Prevalence of both spvC and sdiA genes contributed in antimicrobial resistance in S. gallinarum strains recovered from Iranian poultry was high.

Key words: Virulence genes, *Salmonella gallinarum*, Iranian Poultry

HOW TO CITE THIS ARTICLE: Reza Khaltabadi Farahani, Pezhman Karami, Parastoo Ehsani, Azad Khaledi, Amir Hossien Khaltabadi Farahani, Molecular Detection of Virulence Genes of *Salmonella gallinarum* from Iranian Poultry, J Res Med Dent Sci, 2018, 6 (2):404-408, DOI: 10.5455/jrmds.20186262

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Received: 09/01/2018
Accepted: 02/03/2018

INTRODUCTION

Salmonella infection is one of the most significant causes of morbidity and mortality in the world and due to it is common between human and animals, the human food chain is known as the main source of infection [1]. At now, both *S. gallinarum* and *S. pullorum* are considered as biovars of Salmonella enterica subsp. enterica serovar[2] and because they belonged to serogroup D, having the common antigenic structure and similarity in genomic sequence [3], cannot differentiate them based on the serotyping method[4], rather the biochemical, and molecular tests used for differentiation[5]. Avian salmonellosis is divided into two forms; Fowl typhoid and pullorum disease which both have a main economic significance worldwide. Fowl typhoid is attributed to Salmonella enterica subsp. enterica serovar Gallinarum biovar Gallinarum (S. Gallinarum) can colonize and cause disease in various domestic and wild birds. And also, Pullorum disease is produced by Salmonella

enterica subsp. enterica serovar Gallinarum biovar Pullorum (S. Pullorum), which both can cause a significant morbidity and mortality in birds[6]. They can be transmitted via both horizontal and vertical ways[7]. Fowl typhoid (FT) there is as an endemic of poultry infection in various countries of Africa, Asia, South and Central America[6].These diseases lead to severe economic losses in many developing countries.

The extensive use of antibiotics in poultry and its products has resulted in producing the MRD species which may be transmitted to human through the food chain and it became concern globally[8].

Several virulence genes have been reported in *Salmonella* genus, the most of virulence genes of *Salmonella* are clustered in a region named *Salmonella* pathogenicity islands (SPI) that Scattered throughout the chromosome. In total, in most serovars, 19 SPI have been present [9].

In addition, *Salmonella* plasmid virulence (*spv*) locus which carries the *spv* genes were chiefly described in *Salmonella* Gallinarum-Pullorum[10]. Also, *sdiA* gene is related with biofilm formation in *Salmonella spp*, because, there are a few studies about the prevalence of *S. gallinarum* and mentioned virulence genes from Iran, this study aimed to evaluate the molecular detection of virulence genes of *S. gallinarum* strains recovered from food substance and feces samples of Iranian poultry.

MATERIALS AND METHODS

Isolation, diagnosis of *Salmonella* from fifty-one food substance and feces samples of poultry was performed during 2015-2017 using culture (ISO6579), in this process, 25 gr of feces was poured in a sterile bag and 225 gr of peptone water was added to it and mixed well and incubated overnight. Selenite cysteine (SC) broth and Tetrathionate broth base (TTB) were applied as the selective enrichment broth, and plating medium was xylose lysine desoxycholate agar (XLD) containing RVS broth (Rappaport-Vassiliadis Soy Peptone). Further identification of isolates was conducted by colony morphology, microbial tests and biochemical characterizations (Lysine decarboxylase, Voges-Proskauer, Indole reaction, Beta-galactosidase reaction, Urease and H2S production). After that, isolates were serotyped with specific O and H *Salmonella* antisera (Mast Company) and classified based on the Kauffman White scheme. The genomic DNAs of the *Salmonella* isolates were extracted using the kit (Roche Applied Science) based on the process defined by Turki et al in 2014[11].

Diagnosis of S. gallinarum serovars by Real Time PCR technique

Virulence gene of Hq703462 was used for identification of *S. gallinarum* serovars using Real-Time PCR with the primers of Hq703462: F--CGATATAGCTTACTGTGTCCCG, R-TCATGCACTACCACCATAACG,Prob-FAM-ACATCCCTCATATCGGCGCGAAC-TAMRA[12].

Differentiate Salmonella entrica biovar gallinarum of pollorum using Tri Plex PCR of ratA, tepB and rhs genes

For differentiation between two biovars, *ratA*, *tepB* and rhs genes were used by Multi-Plex PCR[13]. PCR was performed in final volume of 25 µl with a reaction mixture comprising 1µl of each primers using primer pairs listed in Table 1, the PCR program was as follows; a cycle for initial denaturation at 95 ºC for 5 min, 40 cycles with denaturation at 94 ºC for 40 sec, annealing step at 56 ºC for 30 sec, elongation stage at 72 ºC for 40 sec and final extension step in 72 ºC for 7 min.

Table1: The sequences of pair primers used in this study

Target gene	Primer name	Oligonucleotide Sequences (5to3)	Serovar	Annealing Temperature (ºC)	Ref.	PCR product size (bp)
steB	steB-F steB-R	TGTCGACTGGGACCCGCCCGCCCGC CCATCTTGTAGCGCACCAT	gallinarum(D1)	56	[14]	636
***rhs* locus**	rhs-F rhs-R	TCGTTTACGGCATTACACAAGTA CAAACCCAGAGCCAATCTTATCT	gallinarum +pullorum	56	[15]	402
ratA	ratA-f ratA-r	GACGTCGCTGCCGTCGTACC TACAGCGAACATGCGGGCGG	gallinarum +pullorum	56	[16]	SG:1047 SP:243

In determining the presence and absence of two virulence genes (*sdiA* and *spvC*), PCR reaction was performed in a 25 final volume with a reaction mixture comprising 1 µl of each primer with the PCR program; A cycle for initial denaturation at 95 ºC for 5 min, 30 cycles with denaturation at 94 ºC for 40 sec, annealing at 56 ºC for 30 sec, extension step at 72 ºC for 50 sec and final extension step in 72 ºC for 10 min. The oligonucleotides primers were as follows; *sdiA*-F: AATATCGCTTCGTACCAC, *sdiA*-R: GTAGGTAAACGAGGAGCAG; *spvC*-F:-ACTCCTTGCACAACCAAATGCGGA, *spvC*-R: TGTCTCTGCATTTCGCCACCATCA. Then data were analyzed by SPSS software (version 22.0; Chicago, Illinois, USA) using Chi-square test.

RESULTS

Real-Time PCR with the primers of Hq703462 virulence gene confirmed the *gallinarum* serovars. Also, it differentiated *Salmonella entrica* biovar *gallinarum* of *pollorum* using Tri-Plex PCR of *ratA*, *tepB* and *rhs* genes.

The bands achieved from PCR detection reflecting the virulence genes *sdiA*-274 bp and *spvC*-571 bp (Figure1). Multiple PCR technique presented that the prevalence of *sdiA* gene was reported 51(100%), while this rate for *spvC* was 31(61%).

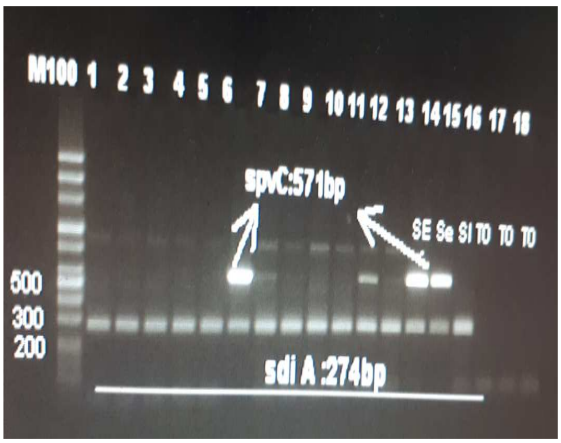

Figure1: Result detection of virulence genes; *spvA* and *sdiA*by Multiplex PCR. Left to the right; M100; Marker 100bp, wells 1-13; positive samples for *sdiA* gene with product size 274bp, wells 7 and 11; positive samples for spvC, wells 13,14 and 15 are positive control, wells 16,17 and 18 are the negative control.

DISCUSSION AND CONCLUSION

Poultry is usually infected by an extensive range of *Salmonella* serovars[17]. The transmission of salmonellae through poultry products, particularly egg to humans is well confirmed[18]. As shown in the results section, the prevalence of *sdiA* gene was reported 100%, while this rate for *spvC* was 61%. In contrast to our results, a study conducted by Ammar and et al. reported the prevalence 5.8% for spvC gene[19].

For our knowledge, the plasmid *spv*ABCD genes are arranged in an operon, which SpvB and SpvC have been recognized as necessary effector proteins for the SPV virulence phenotype [20], and based on evidence, in the absence of *spv*C and *spv*B genes does not present a noticeable virulence phenotype, also spvC locus is involved in intracellular survival(intra-macrophage survival) and replication of *Salmonella*, furthermore, in some cases, it carries five resistance genes [7]. For that existence of this virulence factor in *S. gallinarum* serovars is important to confer antibiotic resistance. Interestingly, a study conducted in Iran presented that the increasing prevalence of spvC gene in S. Enteritidis strains[21].

On the other hand, owing to the role of *sdiA* in biofilm formation and relationship between biofilm and antibiotic resistance, similar to the *spv*C, possibly *sdiA* gene has a role in antibiotic resistance. In confirmation this fact, several studies from different parts of the world revealed that the resistant *Salmonella* serovars are also able to exhibit several virulence genes[22, 23-25].

Regarding obtained results of the current study; prevalence both *spv*C and *sdiA* genes contributed in antimicrobial resistance in *S. gallinarum* strains recovered from Iranian poultry were high which should be considered because they are transmitted through the food chain to humans and consequently transfer the antibiotic resistance to humans that should be considered.

Conflict of interest

No conflict of interests is declared.

REFERENCES

1. Mkrtchyan M, Zakharyan M, Arakelova K. Molecular determinants of virulence genes of salmonella enteritidis prevailing in Armenia. Chemistry and Biology. 2016(1):55-60.
2. Pugliese N, Circella E, Pazzani C, Pupillo A, Camarda A. Validation of a seminested PCR approach for rapid detection of

Salmonella enterica subsp. enterica serovar Gallinarum. Journal of Microbiological Methods. 2011; 85(1):22-27.
3. Grimont PA, Weill F-X. Antigenic formulae of the Salmonella serovars. WHO collaborating centre for reference and research on Salmonella. 2007; 9:1-66.
4. Shah DH, Park J-H, Cho M-R, Kim M-C, Chae J-S. Allele-specific PCR method based on rfbS sequence for distinguishing Salmonella gallinarum from Salmonella pullorum: serotype-specific rfbS sequence polymorphism. Journal of Microbiological Methods. 2005; 60(2):169-77.
5. Kang MS, Kwon YK, Jung BY, Kim A, Lee KM, An BK, Song EA, Kwon JH, Chung GS. Differential identification of Salmonella enterica subsp. enterica serovar Gallinarum biovars Gallinarum and Pullorum based on polymorphic regions of glgC and speC genes. Veterinary Microbiology. 2011; 147(1-2):181-85.
6. Barrow P, Neto OF. Pullorum disease and fowl typhoid—new thoughts on old diseases: a review. Avian Pathology. 2011;40(1):1-13.
7. Guiney DG, Fierer J. The role of the spv genes in Salmonella pathogenesis. Frontiers in Microbiology. 2011;2:129.
8. Tollefson L, Miller MA. Antibiotic use in food animals: controlling the human health impact. Journal of AOAC International. 2000; 83(2):245-54.
9. Foley SL, Lynne AM, Nayak R. Salmonella challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates 1 2. Journal of Animal Science. 2008;86(14_suppl):E149-62.
10. Chiu CH, Ou JT. Rapid identification of Salmonella serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. Journal of Clinical Microbiology. 1996; 34(10):2619-22.
11. Turki Y, Mehr I, Ouzari H, Khessairi A, Hassen A. Molecular typing, antibiotic resistance, virulence gene and biofilm formation of different Salmonella enterica serotypes. The Journal of General and Applied Microbiology. 2014; 60(4):123-30.
12. Bianco P, Cao X, Frenette PS, Mao JJ, Robey PG, Simmons PJ, Wang CY. The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. Nature Medicine. 2013; 19(1):35-42.
13. Batista DF, de Freitas Neto OC, Lopes PD, de Almeida AM, Barrow PA, Berchieri Jr A. Polymerase chain reaction assay based on ratA gene allows differentiation between Salmonella enterica subsp. enterica serovar Gallinarum biovars Gallinarum and Pullorum. Journal of veterinary Diagnostic Investigation. 2013; 25(2):259-62.
14. Zhu Y, Peng L, Chen D, Yu G. Intercalation pseudocapacitance in ultrathin VOPO4 nanosheets: toward high-rate alkali-ion-based electrochemical energy storage. Nano Letters. 2015;16(1):742-47.
15. Zhu C, Yue M, Rankin S, Weill F-X, Frey J, Schifferli DM. One-step identification of five prominent chicken Salmonella serovars and biotypes. Journal of Clinical Microbiology. 2015;53(12):3881-83.
16. Batista DFA. Análise comparativa dos genomas de Salmonella enterica subsp. enterica sorovar Gallinarum biovares Gallinarum 287/91 e Pullorum 449/87 para identificação de regiões de diferenças (RODs), 2013.
17. Salm-Surv WG, Organization WH. Progress report (2000-2005): building capacity for laboratory-based foodborne disease surveillance and outbreak detection and response, 2006.
18. Sanchez S, Hofacre CL, Lee MD, Maurer JJ, Doyle MP. Animal sources of salmonellosis in humans. Journal of the American Veterinary Medical Association. 2002; 221(4):492-97.
19. Ammar AM, Mohamed AA, El-Hamid MI, El-Azzouny MM. Virulence genotypes of clinical SalmonellaSerovars from broilers in Egypt. The Journal of Infection in Developing Countries. 2016; 10(04):337-46.
20. Li H, Xu H, Zhou Y, Zhang J, Long C, Li S, Chen S, Zhou JM, Shao F. The phosphothreonine lyase activity of a bacterial type III effector family. Science. 2007; 315(5814):1000-03.
21. Amini K, Salehi TZ, Nikbakht G, Ranjbar R, Amini J, Ashrafganjooei SB. Molecular detection of invA and spv virulence genes in Salmonella enteritidis isolated from human and animals in Iran. African

Journal of Microbiology Research. 2010; 4(21):2202-10.
22. Parvathi A, Vijayan J, Murali G, Chandran P. Comparative Virulence Genotyping and Antimicrobial Susceptibility Profiling of Environmental and Clinical Salmonellaenterica from Cochin, India. Current Microbiology. 2011; 62(1):21-26.
23. Osman KM, Elhariri M, Amin ZM, AlAtfeehy N. Consequences to international trade of chicken hatchlings: Salmonella enterica and its. International Journal. 2014; 2(5):45-63.
24. Song, W. (2017). Effects of a Training Program on Lifestyle Modification for Adolescents Identified with Overweight. NeuroQuantology, 15(4), 174-179. doi:10.14704/nq.2017.15.4.1147
25. Xu, L., Liu, H. (2017). Effects of Rational Emotive Behavior Therapy (REBT) Intervention Program on Mental Health in Female College Students. NeuroQuantology, 15(4), 156-161 doi:10.14704/nq.2017.15.4.1122