Molecular Identification and Antibiotic Resistance Profiling of *Salmonella* Species Isolated from Duck Eggs in IranMolecular identification and antibiotic resistance profiling of *Salmonella* species isolated from Duck eggs in Iran

Iradj Ashrafi Tamai[1], Zahra Ziafati Kafi[1], Golnar Sharafi[2], Naser Sadri[1], Shahrzad Babolmorad[3], Arian Abbassioun[1], Arash Ghalyanchi Langroudi[1*]

[1]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

[2]Department of Nutrition, Dietetics and food, Faculty of science, Science and Research Branch, Islamic Azad University, Tehran, Iran

[3]Department of Food Health and Hygiene, Veterinary Organization of Tehran Province, Iran Veterinary Organization, Tehran, Iran

Corresponding author's:

Arash Ghalyanchi Langroudi

Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

email: Arashghalyanchi@gmail.com Tel: +98-2161117154

Abstract

Background :Salmonellosis is a significant zoonotic disease that causes foodborne poisoning in humans through animal products and is considered a worldwide public health hazard. A significant hurdle in managing and preventing salmonellosis lies in accurately identifying individuals who may act as asymptomatic carriers, potentially spreading the pathogen without showing clinical signs. **Methods** :This study aimed to assess the prevalence, serotype distribution, and antimicrobial resistance profiles of *Salmonella* isolates recovered from duck eggs. Fifty-four local duck eggs were randomly obtained from villagers in northern Iran during a one-week sampling period in winter 2021 and subsequently transported to the Microbiology Laboratory. *Salmonella* was isolated according to standard culture and biochemical tests. The *Salmonella* positive samples were serotyped with O and H antisera based on slide and tube agglutination tests. Molecular test for

detection of genus-specific gene (*invA*) for *Salmonella* spp. Then, for serovars Infantis and Enteritidis was done by PCR reaction. Antibiotic resistance to 17 antimicrobial agents was evaluated. **Results**: indicated 11.11% (6 of 54) of duck eggs were positive for *Salmonella* in bacterial culture. Serotyping results showed one sample was serovar Infantis (1.85%) and five samples were serovar Enteritidis (9.25%), and PCR confirmed the serotyping results. *Salmonella* isolates in this study were sensitive to Ciprofloxacin, Kanamycin, Norfloxacin, Lincospectin, Sultrim, Chloramphenicol, Cephalothin, Flumequine, Florfenicol, Gentamicin, Nitrofurantoin, Co-amoxiclav, Enrofloxacin, and Cefotaxime, and resistant to Ampicillin, Oxytetracycline, and Tetracycline. . **Conclusion** The detection of *Salmonella* in duck eggs indicates that such eggs may act as potential reservoirs for *Salmonella*, representing a possible source of infection for humans. These findings highlight the necessity for proper hygiene practices and continuous monitoring of duck egg products to ensure food safety and public health protection.

Key words*:* *Salmonella* enteritidis, *Salmonella* infantis, Duck egg, Antibiotic resistance, *invA*

1. Introduction

Salmonella remains a leading cause of foodborne illness globally, with its burden disproportionately affecting populations in low- and middle-income countries (1). Public health concerns regarding food safety are increasing. *Salmonella* infection is caused by contaminated eggs or poultry meat, such as duck (2). *Salmonella* enteritidis infections in poultry are characterised by lesions, vascular damage at Specific site on the mucosal surface of the digestive tract, trauma in the lymphoid organs, and degenerative effects(3). Other groups include several serotypes that cause systemic typhoid disease in a limited range of host species, such as *Salmonella* gallinarum and *Salmonella* pullorum, which cause fowl typhoid and pullorum disease in poultry (4). Pullorum disease, paratyphoid, and fowl typhoid can result in high mortality rate in young birds; however, adults tend to develop a less lethal chronic or carrier state. *Salmonella* is present in the gastrointestinal tract and reproductive organs of carrier birds and can be transmitted to humans through contaminated meat and eggs. Humans who consume raw or partially cooked eggs or meat may contract salmonellosis(1).

In this study, *Salmonella* was detected in eggs purchased from several villages in northern Iran, and duck eggs from the region were found to be *Salmonella*-positive. *Salmonella* can be established into eggs by both direct and indirect pathways. It can persist directly in poultry and eggs, residing in both the reproductive organs and the spleen for extended periods. During sexual maturity of birds, *Salmonella* colonises both the oviduct and ovary oviduct of ducks, then infects eggs directly. Indirectly, *Salmonella* can attach to discharges and enter the egg during formation; it becomes concentrated on the inner surface of the eggshell, where antimicrobial factors are preserved in the egg white(1).

Additionally, the outer surface of the eggshell may be polluted by faeces. These routes of contamination can be affected by the egg production process, storage, handling, and food preparation(4). In recent years, demand for unprocessed and raw foods has increased. Many egg based products, such as ice cream, cold desserts, mayonnaise, or uncooked food, can support the growth of *salmonella* and increase the risk of salmonellosis. In these products, many bacteria present in the content or on the eggshell can survive and multiply, especially at warm room temperatures. Furthermore, light cooking, which may leave some albumen or yolk liquid, may not destroy all bacteria, for example, duck eggs boiled for less than 8 minutes (5). The main purpose of this study was to determine the prevalence, serotyping, and antimicrobial susceptibility of *Salmonella* species isolated from duck eggs.

2. Materials and Methods

2.1. Collection of Samples

A total of 54 local duck eggs were randomly collected from villagers in northern Iran over a one-week period during winter 2021 and transported to the Microbiology Laboratory for analysis.

2.2. Culture of Samples

The inner content of eggs were mixed with a sterile swab, and 1ml of the mixture was poured onto Rappaport enrichment culture medium, which was incubated for 18 hours at 37°C. Using a sterile needle, parts of the Rappaport medium were transferred to several selective culture medium, including MacConkey agar, SS agar, CHROM agar Salmonella, and XLD agar, and incubated for 24 hours. To identify the colonies, microbial tests were performed, including oxidase and catalase tests, as well as differential media including TSI, Urea, Indole, Methyl Red, Voges–Proskauer, Simmons Citrate, and Motility medium.

2.3. Serotyping

The serotyping of isolated *Salmonella* strains was conducted using commercial antisera (Difco, Detroit, USA), and the results were interpreted according to the Kaufmann-White scheme(6).

2.4. Antibiotic resistance test

Antibiotic sensitivity of the *Salmonella* isolates to 14 antibiotics was determined using the disk diffusion method, as explained by the Clinical and Laboratory Standards Institute (7). The antibiotics were selected based on their use in veterinary and human medicine to control *Salmonella* spp. The *Salmonella* isolates were cultured in tryptose soy broth (TSB) (Merck, USA), incubated at 37°C for 1-2 hours, and calibrated based on the 0.5 McFarland $BaSO_4$ turbidity standards. The resulting suspension was uniformly spread onto the surface of dry Mueller-Hinton (Merck) agar plates by swab. Antimicrobial disks were aseptically disposed of on the inoculated agar media, and the plates were incubated at 37°C for 24hours. The diameters of the inhibition zones were measured as millimeter. The isolates were classified as susceptible (S), intermediate (I), or resistant (R) according to guidelines published by CLSI.

2.5. Molecular diagnosis of Salmonella

The biochemically identified isolates were examined using Polymerase Chain Reaction (PCR) for *invA* gene, using S139 (5-GTGAAATTATCGCCACTGTCGGGCAA-3) and S141 (5 TCATCGCACCGTCAAAGGAACC-3) (Table-1)primers toampluify a 284bp verifing the isolates at the genus level (8). A single colony of each isolate from an agar plate was collected and suspended in 200 µl of distilled water. After whirlpooling, the suspension was boiled for 5 min, centrifuged for 10 min at 15,000 g, and 50 µl of the supernatant was gathered. PCR was done in a 25µl , for which 12.5 µl of prepared master mix (Denmark, Amplicon), 1 µl each of forward and reverse primers (10 pmol), 3 µl of DNA, and 7.5 µl of distilled water. Amplification was carried out in a thermocycler (BIORAD T100, UK) under the following conditions: the first denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec; and the last extension at 72°C for 7 min. PCR products were electrophoresed on a 1.25% agarose gel stained with ethidium bromide (2µg/ml) for 15 min. PCR bands were visualised with a UV transilluminator (BIORAD, UK).

2.6. Multiplex PCR (mPCR) for detection of S. enteritidis and *S. infantis*

All *Salmonella* strains were identified by multiplex PCR to verify the *S.* enteritidis serovar as described by Soumet et al. and Pan and Liu (9, 10), using ST11 and ST14 specific primers for the genus of *Salmonella* (Random), S1 and S4 primers which is related to virulence (*Spv* gene) and particular for *S.* enteritidis and SEFA2 and SEFA4 primers for specificity within *S.* enteritidis (*SefA* gene) (Table 1)(11, 12). The mPCR reaction was done in 25µl, containing 3 µl of extracted DNA, 12.5 µl of Master mix (Amplicon, Danmark), 0.5 µl of each primer, and 6.5 µl of distilled water in total 25 µl volume. The first denaturation was at 94°C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 56°C for 90 sec, extension at 72°C at 30 sec and a final extension at 72°C for 10 min. Also, another PCR reaction was ran to verify *S. infantis* serovar (13) using SI-F and SI-R primers which amplifies a 413 bp of *fljB* gene. The reaction was fulfilled in 25 µl containing 3µl of template DNA, 12.5 µl of Master mix (Amplicon, Danmark) and 0.5 µl of each primer and 8.5 µl of distilled water. The first denaturation was at 95°C for 60 sec, followed by 35 cycles, of denaturation at 95°C for 60 sec, annealing at 56°C for 15 sec and extension at 72°C for 60 sec, with a final extension at 72°C for 240 sec. The PCR products were electrophoresed on 1% agarose gel for 1 hour at 90V and stained with ethidium bromide.

Table 1. Primers used for the detection of *S.* enteritidis and *S.* infantis by mPCR

Target sequence	Sequence (5´-3´)	Size (bp)	Reference
*Random**	ST11: GCCAACCATTGCTAAATTGGCGCA ST14: GGTAGAAATTCCCAGCGGGTACTGG	429	(17)
*Spv***	S1: GCCGTACACGAGCTTATAGA S4: ACCTACAGGGGCACAATAAC	250	(17)
*SefA****	SEFA2: GCAGCGGTTACTATTGCAGC	310	(15)

	SEFA4: TGTGACAGGGACATTTAGCG		
fljB	Si-F: TTGCTTCAGCAGATGCTAAG Si-R: CCACCTGCGCCAACGCT	413	(16)

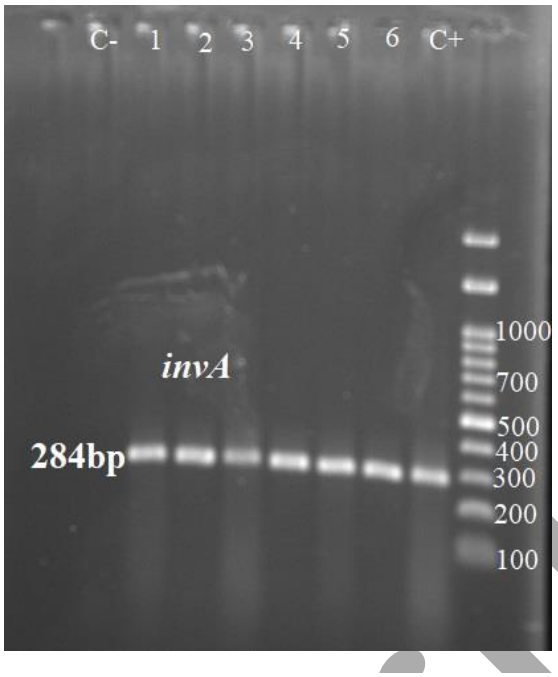
* Randomly cloned sequence specific for the genus *Salmonella*, ** *Salmonella* plasmid virulent gene, *** *S*. Enteritidis fimbrial antigen gene,

3. Results

While examining the culture media for the growth of various bacteria, it was observed that *Salmonella*, *Escherichia coli*, and *Proteus* spp. had the highest bacterial counts. *Salmonella* spp. were isolated from six out of 54 (11.11%) duck eggs by culture and biochemical tests(Table-2). A PCR test targeting the *invA* gene was performed, and in all positive samples, the characteristic 284 bp product was observed in the agarose gel (Figure 1). At multiplex PCR. Five isolates were identified as *S*. enteritidis (group D), and one isolate was determined as *S*. infantis (group C1) by serotyping, presenting antigenic formulas of (O:1, 9, 12, H1: g, m) and (O:6, 7, H1: b, H2: 1, 2), respectively. PCR results for detection of *S*. enteritidis and *S*. infantis serovars were consistent with the serotyping results (Figures 2 and 3). Antibiogram results showed that all *Salmonella* strains were highly susceptible to Ciprofloxacin, Kanamycin, Norfloxacin, Lincospectin, Sultrim, Chloramphenicol, Cephalothin, Flumequine, Florfenicol, Gentamicin, Nitrofurantoin, Co-amoxiclav, Enrofloxacin, and Cefotaxime, and resistant to Ampicillin, Oxytetracycline, and Tetracycline.

Table 2. The frequency of microorganisms isolated from the eggs of Duck

Microorganisms	**No. (%)**
Salmonella entritidis	5 (9.26%)
Salmonella infantis	1 (1.85%)
E. coli	5 (9.26%)
Proteus mirabilis	5 (9.26%)
Klebsiella sp.	2 (3.7%)
Pseudomonas aeruginosa	4 (7.4%)
Citrobacter sp.	3 (5.55%)



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160 **Figure 1.** Results of PCR assays for identification of *invA* gene

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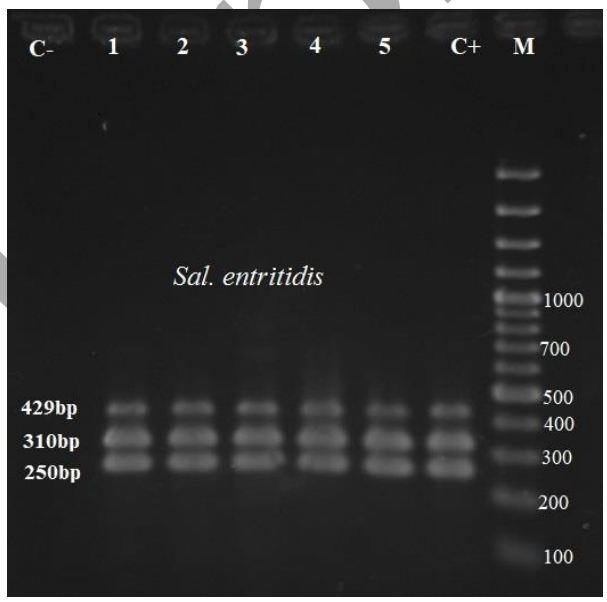

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165 **Figure 2.** Multiplex polymerase chain reaction for detection of *S.* enteritidis

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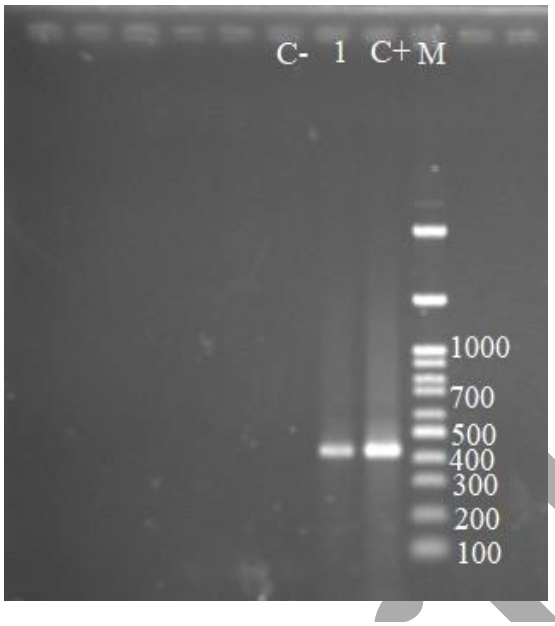

Figure 3. Results of PCR assays used to identify *S.* Infantis.

4. Discussion

Salmonella infection (salmonellosis) is a common bacterial disease that causes morbidity and mortality worldwide through the consumption of contaminated eggs or poultry meat (14). *Salmonella* spp. usually live in animal and human intestines and are transmitted via the fecal-oral route. Humans most frequently become infected through contaminated food or water (15). In the present study, two different Salmonella serotypes were identified, with the more prevalent serotype *S.* entritidis and *S.* infantis in duck eggs. The predominant serotypes vary in different countries. In South America, such as Argentina, *S.* typhimurium was the predominant cause of gastroenteritis until 1987 (16). Although *S.* enteritidis is the most frequently isolated serovar from eggs and eggshell contents in many countries (17), it appears that the most common isolates from eggs in Iran were of groups D and B, with the main serovars being Enteritidis and Typhimurium(18). In our study, the isolates from the eggs were identified as *S.* enteritidis and *S.* infantis. Our results are consistent with other studies (19); however, these finding are based on a pilot study. In European countries, the prevalence of *S.* enteritidis and *S.* typhimurium has decreased due to vaccination programmes, but the serovar infantis is still spreading and remains a significant problem in some countries, such as Hungary.Furthermore, human cases of serovar infantis have been increasingly reported worldwide in countries such as Australia, New Zealand, Brazil, Finland, Canada, Japan, and Russia (20). A survey of *S.* infantis in hospitals in Iran shows a rise in the prevalence of this serovar (21). *Salmonella* infantis was the second most prevalent in human cases of salmonellosis in Iran between the years 2007 and 2010 (22). In recent years, the rate of *Salmonella* infections with different serovars among poultry has been increasing in some countries, such as Japan (13). Therefore, this foodborne pathogen can be transmited to humans through poultry products. To identify *S.* enteritidis and *S.* infantis strains, we developed an m-PCR assay. In the present study, we used S139 and S141 primers for *Salmonella* species detection, which target the *invA* gene. The

InvA gene encodes a protein in the inner membrane of bacteria, responsible for the invasion of host epithelial cells. The presence of this gene in *Salmonella* isolates demonstrates the pathogenicity of the organisms and the ability of the bacteria to penetrate host epithelial cells and cause infections (23). Thus, detection of the *invA* gene has been suggested as an international standard for *Salmonella* identification (24).

In this study, specific detection of *S.* entritidis in the multiplex PCR assay was performed using S1, S4, SEFA2, and SEFA4 primers which target the *Spv* and *SefA* genes, respectively (10, 12). SEFA is a pair of primers required for the identification of strains of S. enteritidis (10); thus,the *sef* gene is a strong marker for the detection of *Salmonella* serovar enteritidis (25). For specific detection of *S.* infantis, we used SI-F and SI-R primers which target the *fljB* gene (13). Salmonella isolates showed varynig degrees of sensitivity to 14 antibiotics in the present study, using the disk diffusion method and antibiogram. All *Salmonella* strains were sensitive to ciprofloxacin, kanamycin, norfloxacin, lincospectin, sultrim, chloramphenicol, cephalothin, flumequine, florfenicol, gentamicin, nitrofurantoin, coamoxiclav, enrofloxacin, and cefotaxime, and they were resistant to ampicillin, oxytetracycline, and tetracycline. The widespread use of antimicrobial agents in food animal production has increased the incidence of resistant zoonotic pathogens such as *Salmonella* in animals. Antimicrobial resistance is moderate in the predominant poultry-related serovar *S.* enteritidis(26).

As a consequence, poultry can contain antimicrobial-resistant strains and serve as a vehicle for their transmission to humans. The major common resistance observed in *S.* enteritidis, and *S.* infantis was to tetracycline and ampicillin. In addition,tetracycline was the second most frequently observed type of antimicrobial resistance among the different studies. In Iran, poultry is mostly treated with tetracycline and ciprofloxacin. Tetracycline is used for growth promotion and disease prevention in animals and humans. The antimicrobial resistance profile of pathogenic isolates can indicate the antimicrobial substances used for animal treatment. As a result, the widespread incidence of resistance among all bacterial species is probably a consequence of the extensive use of antibiotics in poultry . Recently, tetracycline resistance in *Salmonella* spp. isolated from poultry in Iran has been reported (27). Another antibiotic resistance observed in our study was to ampicillin, which is combined with chloramphenicol to treat bacterial infections such as meningitis, salmonellosis, and endocarditis. The combined use of chloramphenicol and ampicillin has demonstrated therapeutic efficacy in the management of salmonellosis, particularly in cases involving invasive or multidrug-resistant strains (28). Thus, it is not surprising that the ampicillin resistance pattern is observed. These results indicate that it is tremendously important to control the overtaking of antibiotics in the poultry industry to reduce the multi-drug resistance of *Salmonella* spp.

In conclusion, studies have shown eggs can be a source of human infection. The consumption of contaminated, undercooked, or raw eggs and poultry meat, such as duck poses a high risk to humans. In duck eggs, both *S.* enteritidis and *S.* infantis have been shown contaminate the reproductive organs and developing eggs. *S.* enteritidis can also persist after the eggs are laid. In addition to contamination via the reproductive organs, eggs can become polluted through contact

with *Salmonella* contaminated equipment, staff, the environment, and through the spread of disease by rodents and wild birds

Authors' contributions

These authors have contributed equally to this work.

Ethics

We verify that the current study has been carried out under ethical regulations. All animal experiments including the sampling procedure were done considering animal welfare and relief. Furthermore, no harmful techniques or procedures to animal health were used in this study.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Chen Z, Bai J, Wang S, Zhang X, Zhan Z, Shen H, et al. Prevalence, Antimicrobial Resistance, Virulence Genes and Genetic Diversity of *Salmonella* Isolated from Retail Duck Meat in Southern China. Microorganisms. 2020;8(3):444.
2. Youn SY, Kwon YK, Song CS, Lee HJ, Jeong OM, Choi BK, et al. Increased efficacy of inactivated vaccine candidates prepared with *Salmonella enterica* serovar Typhimurium strains of predominant genotypes in ducks. Poult Sci. 2016;95(8):1764-73.
3. Deng SX, Cheng AC, Wang MS, Yan B, Yin NC, Cao SY, et al. The pathogenesis of *Salmonella* enteritidis in experimentally infected ducks: a quantitative time-course study using taqman polymerase chain reaction. Poult Sci. 2008;87(9):1768-72.
4. Whiley H, Ross K. *Salmonella* and eggs: from production to plate. Int J Environ Res Public Health. 2015;12(3):2543-56.
5. Duguid JP, North RA. Eggs and *Salmonella* food-poisoning: an evaluation. J Med Microbiol. 1991;34(2):65-72.
6. Popoff MY, Bockemühl J, McWhorter-Murlin A. Supplement 1991 (no. 35) to the Kauffmann-White scheme. Res Microbiol. 1992;143(8):807-11.
7. Bauer A. Antibiotic susceptibility testing by a standardized single disc method. Am J clin pathol. 1966;45:149-58.
8. Zahraei Salehi T, Mahzounieh M, Khaksar E. Detection of *Salmonella* serovars in zoo and pet reptiles, rabbits, and rodents in Iran by culture and PCR methods. Comp Clin Pathol. 2010;19(2):199-202.
9. Soumet C, Ermel G, Rose V, Rose N, Drouin P, Salvat G, et al. Identification by a multiplex PCR-based assay of *Salmonella* typhimurium and *Salmonella* enteritidis strains from environmental swabs of poultry houses. Lett Appl Microbiol. 1999;29(1):1-6.

10. Pan TM, Liu YJ. Identification of *Salmonella* enteritidis isolates by polymerase chain reaction and multiplex polymerase chain reaction. J Microbiol Immunol Infect. 2002;35(3):147-51.
11. Woodward MJ, Kirwan SE. Detection of *Salmonella* enteritidis in eggs by the polymerase chain reaction. Vet Rec. 1996;138(17):411-3.
12. Soumet C, Ermel G, Rose N, Rose V, Drouin P, Salvat G, et al. Evaluation of a multiplex PCR assay for simultaneous identification of *Salmonella* sp., *Salmonella* enteritidis and Salmonella typhimurium from environmental swabs of poultry houses. Lett Appl Microbiol. 1999;28(2):113-7.
13. Kardos G, Farkas T, Antal M, Nógrády N, Kiss I. Novel PCR assay for identification of *Salmonella enterica* serovar Infantis. Lett Appl Microbiol. 2007;45(4):421-5.
14. Kurtz JR, Goggins JA, McLachlan JB. *Salmonella* infection: Interplay between the bacteria and host immune system. Immunol Lett. 2017;190:42-50.
15. Angelo KM, Chu A, Anand M, Nguyen TA, Bottichio L, Wise M, et al. Outbreak of *Salmonella* Newport infections linked to cucumbers--United States, 2014. MMWR Morb Mortal Wkly Rep. 2015;64(6):144-7.
16. Pucciarelli AB, Benassi FO. Inactivation of *Salmonella* enteritidis on raw poultry using microwave heating. Braz Arch Biol Technol. 2005;48(6).
17. Musgrove MT, Jones DR, Northcutt JK, Harrison MA, Cox NA, Ingram KD, et al. Recovery of *Salmonella* from commercial shell eggs by shell rinse and shell crush methodologies. Poult Sci. 2005;84(12):1955-8.
18. Salehi TZ, Mahzounieh M, Saeedzadeh A. Detection of invA gene in isolated *Salmonella* from broilers by PCR method. Int J Poult Sci. 2005;4(8):557-9.
19. Indar L, Baccus-Taylor G, Commissiong E, Prabhakar P, Reid H. Salmonellosis in Trinidad: evidence for transovarian transmission of *Salmonella* in farm eggs. West Indian Med J. 1998;47(2):50-3.
20. Miller T, Prager R, Rabsch W, Fehlhaber K, Voss M. Epidemiological relationship between *Salmonella* Infantis isolates of human and broiler origin. Lohmann Inf. 2010;45(2):27.
21. Naghoni A, Ranjbar R, Tabaraie B, Farshad S, Owlia P, Safiri Z, et al. High prevalence of integron-mediated resistance in clinical isolates of *Salmonella enterica*. Jpn J Infect. 2010;63(6):417-21.
22. Ranjbar R, Sarshar M, Sadeghifard N. Characterization of genetic diversity among clinical strains of *Salmonella enterica* serovar infantis by ribotyping method. J Adv Med Biomed Res2012;20(81):75-84.
23. Zahran SI, Bkheet AA, Torky HA. *Salmonella* Species Isolated from Different Sources with Special Reference to Biofilm Formation. Alex J Vet Sci. 2020;64(2).
24. Malorny B, Hoorfar J, Bunge C, Helmuth R. Multicenter validation of the analytical accuracy of *Salmonella* PCR: towards an international standard. App Environ Microbiol. 2003;69(1):290-6.
25. Malkawi HI, Gharaibeh R. Rapid and simultaneous identification of two *Salmonella enterica* Serotypes, enteritidis and typhimurium from chickpea and meatproducts by multiplex PCR. Biotechnology (Pakistan). 2004;3(1):44-8.
26. Foley S, Lynne A. Food animal-associated *Salmonella* challenges: pathogenicity and antimicrobial resistance. J Anim Sci. 2008;86(suppl_14):E173-E87.
27. Morshed R, Peighambari SM. Drug resistance, plasmid profile and random amplified polymorphic DNA analysis of Iranian isolates of *Salmonella* enteritidis. New Microbiol. 2010;33(1):47-56.
28. Hur J, Choi YY, Park JH, Jeon BW, Lee HS, Kim AR, et al. Antimicrobial resistance, virulence-associated genes, and pulsed-field gel electrophoresis profiles of *Salmonella* enterica subsp. enterica serovar Typhimurium isolated from piglets with diarrhea in Korea. Can J Vet Res. 2011;75(1):49-56.