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A SURVEY ON PREVALENCE OF *SALMONELLA ENTERITIDIS* ISOLATED FROM ROSS 308 BROILER CHICKENS ON THE BASIS OF *SEFA* AND *SPV* GENES DETECTION IN GUILAN PROVINCE, IRAN

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Abstract

Salmonella enteritidis is an important zoonotic pathogen with a risk for the public health due to capacity of dissemination between animals and human. Broiler chickens are the common source for *S. enteritidis*. The aim of this study was to investigate prevalence of *S. enteritidis* in commercial broiler chickens (Ross 308 lineage) in Guilan province during 2016-2018 by study of two important virulence genes and assessment of their antimicrobial resistance. In this cross-sectional study, a total of 700 specimens were collected from 7 different broiler chicken farms located in different regions, *S. enteritidis* was identified using culture and biochemical tests, and then processed for molecular detection of *S. enteritidis* by amplification of the *sefA* and *spv* genes. Two sets of primers specific for *spv* (250 bp) and *sefA* (310 bp) genes were used together in multiplex PCR a 250 bp fragment within the spv gene, and a 310 bp fragment within the sefA gene specific for *S. enteritidis*. The overall detection rate of *S. enteritidis* was 7.5% (n = 53), while the highest level of contamination with *S. enteritidis* was found in the broiler chicken liver tissue (n = 23; 13.1%). Antibiotic susceptibility performed and most of *S. enteritidis* isolates were resistant to penicillin group of antibiotics and the isolated *S. enteritidis* showed partial resistance to macrolide and tetracycline group of antibiotics. However, results showed no resistance against cephems (including ceftazidime, cefotaxime, and ceftriaxone). *SefA* gene and *spv* gene was detected in 100% and 38.9% of isolated *S. enteritidis* respectively. The present study showed prevalence of *S. enteritidis* in broiler chickens. The increased drug resistance in *S. enteritidis* is a concerning problem that could have negative impact on efforts to prevent dissemination and treat broiler-transmitted *S. enteritidis*.

Keywords: *Salmonella enteritidis*, broiler chicken, antibiotics, molecular detection, antimicrobial resistance.

Introduction

Salmonella is the primary cause of foodborne diseases globally (Gholam et al., 2019). A broad range of foodstuff has been associated with such diseases. However, food from animal sources, especially if poultry derived, has been implicated in periodic cases and outbreaks of human salmonellosis (Scaallan *et al.*, 2011). Moreover, *salmonella* infection in poultry industry can result in a huge economic loss (Lutticken *et al.*, 2007). *Salmonella* is the most commonly reported cause of foodborne disease among bacterial infections (Onyango *et al.*, 2009). It is estimated that about 94 million cases of gastroenteritis due to *Salmonella* species occur annually worldwide, leading to 155,000 deaths every year (Majowicz *et al.*, 2010; Jahani et al., 2019). Among *Salmonella* species, *S. enteritidis* is isolated mostly from poultry chicken and is the most frequent cause of human nontyphoidal salmonellosis (Akhtar *et al.*, 2010). In recent years, *S. enteritidis* has been reported as a major causative agent of foodborne gastroenteritis in humans (Clayton *et al.*, 2008). The current emergence of drug resistance in *S. enteritidis* is a major challenge due to the excessive use of antibiotics in the food and livestock sector (Gyles 2008; Sedaghat & Motamedifar, 2019). Poultry, especially broiler chickens, can be the carrier for the antimicrobial-resistant strains and can act as a vehicle for dissemination of these pathogens to humans (Rahmani *et al.*, 2013). Standard culture and serological methods for the detection of *S. enteritidis* are employed as disease control measures; however, polymerase chain reaction (PCR) is a preferred diagnostic method due to its reliable sensitivity, specificity, and detection speed (Vaneechoutte and Van Elare, 1997).

The virulence of *Salmonella* spp. is associated with a combination of chromosomal and plasmid factors, and many studies have identified genes that encode these factors. Some virulence factors are associated with the cellular structure of the bacteria, such as fimbriae (Pan and Liu, 2002). The salmonella virulent plasmid (*spv*) gene, commonly involved in salmonella virulence and routinely used for detection of *Salmonella spp* (Madadi *et al.*, 2013), and *sefA* (*Salmonella enteritidis* fimbrial antigen) gene is routinely used for the detection of *S. enteritidis* (Edwards and Puente, 1998). To date, few data exist on the prevalence of *S. enteritidis* in broiler chicken from broiler flocks in Guilan province by application of both molecular methods and antimicrobial susceptibility. The present study has documented prevalance of *S. enteritidis by molecular detection of sefA* and *spv* genes and invastigated associated drug resistance pattern of *S. enteritidis* against commonly used antibiotics. The findings of this study may be useful in better controlling antibiotic resistance among *S. enteritidis* isolates from broiler chicken samples.

Materials and Methods

Sample collection

The present study was conducted at the Department of Microbiology, Lahijan, Guilan, during the period from May 2016 to November 2018. A total of 700 different samples from liver, wing, drumstick and breast tissue of broiler chicken (Ross 308 lineage) were collected from different broiler chicken farms in Guilan povince. Random samples were collected from individual chickens (one sample from one chicken). All the samples were carried separately to avoid any cross contamination between two samples. The samples were collected in peptone water-filled sterile plastic bags and immediately transported on ice to the laboratory for inoculation on enriched medium.

Bacteriological culture procedure

The culturing method performed by using one gram of boiler tissue sample that was added to 9 mL of tetrathionate broth and was incubated at 37°C for 24 hours. A loop full of broth culture from tetrathionate was streaked onto a plate of bismuth sulfite agar. The plates were incubated at 37°C for 48 hours and checked for the growth of typical black *Salmonella* species colonies. The presumptive colonies of *Salmonella* species were taken for further confirmation by biochemical testing, including oxidase, catalase, triple sugar iron slant reaction, and motility, indole, urease, and citrate utilization tests (Barrow *et al.*, 2003).

DNA preparation

A loopful of colonies of each isolate on agar plate were picked and suspended in 200 µL of distilled water. After vortexing, the suspension was boiled for 5 min, and 50 µL of the supernatant was collected after spinning at 14,000 rpm for 10 min. The DNA concentration of the boiled extracts was determined with spectrophotometer (Lin *et al.*, 1996).

Molecular detection of *S. enteritidis*

Molecular detection performed by using of Multiplex Polymerase Chain Reaction (PCR) method (Corbett Rotor-Gene 6000) performed for detection of *SefA* (*Salmonella enteritidis* fimbrial gene) and *spv* genes using two set of specific primers including SefA2- SefA4, S1-S4 (Table 1) (Pan and Liu, 2002). Briefly for PCR, 3 µL of DNA was added to 25 µL of the reaction mixture containing 4 µL prepared master mix (Deoxynucleotide Triphosphate (dNTPs), 10× PCR buffer, Taq polymerase, and $MgCl_2$), and 1 µL of each primers (including forward and reverse primers), while the remaining volume was equalized by nuclease-free water. The prepared PCR tubes with the master mixture were placed in a gradient thermal cycler. Amplification was carried out with initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation (94°C for 30 seconds), annealing (56°C for 90 seconds), and primer extension (72°C for 30 second). A final extension step was carried out at 72°C for 10 minutes. The amplified DNA products from *S. enteritidis*-specific PCR, *Escherichia coli* ATCC 25922 and *S. enteritidis* ATCC 13076 were used as negative and positive controls, respectively. The amplification products were electrophoresed on 1.2% agarose gels and 100-bp ladder was used as a molecular weight marker. The gel was stained with ethidium bromide (2 µg mL–1) to visualize fluorescent bands while using UV light in the gel document system (BIORAD, UK).

Table 1: Primer sequences that used for the detection of *Salmonella enteritidis* isolates.

Primer	Target gene	Length	Sequence (5'- 3')	Amplification product
S1	Spv*	20	GCCGTACACGAGCTTATAGA	250
S4	Spv	20	ACCTACAGGGGCACAATAAC	250
SefA2	SefA**	20	GCAGCGGTTACTATTGCAGC	310
SefA4	SefA	19	TGTACAGGGACATTTAGCG	310

* *Salmonella* plasmide virulent gene ** *Salmonella enteritidis* fimbrial antigen gene

Antimicrobial susceptibility testing of *S. enteritidis*

All the isolated bacteria that were identified as *S. enteritidis* on M-PCR were tested for antimicrobial susceptibility according to the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2015). The following disks (Oxoid, Basingstoke, UK) were included in the test: ampicillin (10 µg), piperacillin (10µg), ceftriaxone(30µg), ceftazidime (30µg), cefotaxime (30µg), florfenicol (30µg), colistin (10µg), neomycin (30µg), gentamicin (10µg), amikacin (30µg), doxycycline (30µg), fosfomycincefotaxime (30 µg), florfenicol (30 µg), amikacin (30 µg), gentamicin (10 µg), Trimethoprim (5 µg), doxycycline (5 µg), tetracycline (30 µg), ciprofloxacin (5 µg), fosfomycin (30 µg), erythromycin (15 µg), and azithromycin (15 µg). *Escherichia coli* ATCC 25922 were used for quality control. Results were interpreted as recommended by CLSI (Clinical and Laboratory Standards Institute, 2019).

Results and Discussion

A total of 700 broiler chicken samples were obtained from different broiler chicken flocks in Guilan province and processed for molecular detection, *S. enteritidis* was detected in 53 (7.5%) samples among the biochemically identified *Salmonella* species. The size of the amplified sequences of *sefA* and *spv* genes was 250bp and 310bp respectively (Fig. 1).

In specimen-wise distribution of *S. enteritidis* that included different parts (breast tissue, drumstick, liver and wing) of broiler chicken, a higher isolation rate of *S. enteritidis* was noticed in the liver (n = 23, 13.1%), while the lowest detection rate was observed in drumstick tissue samples (n = 6; 3.42%; Table 2).

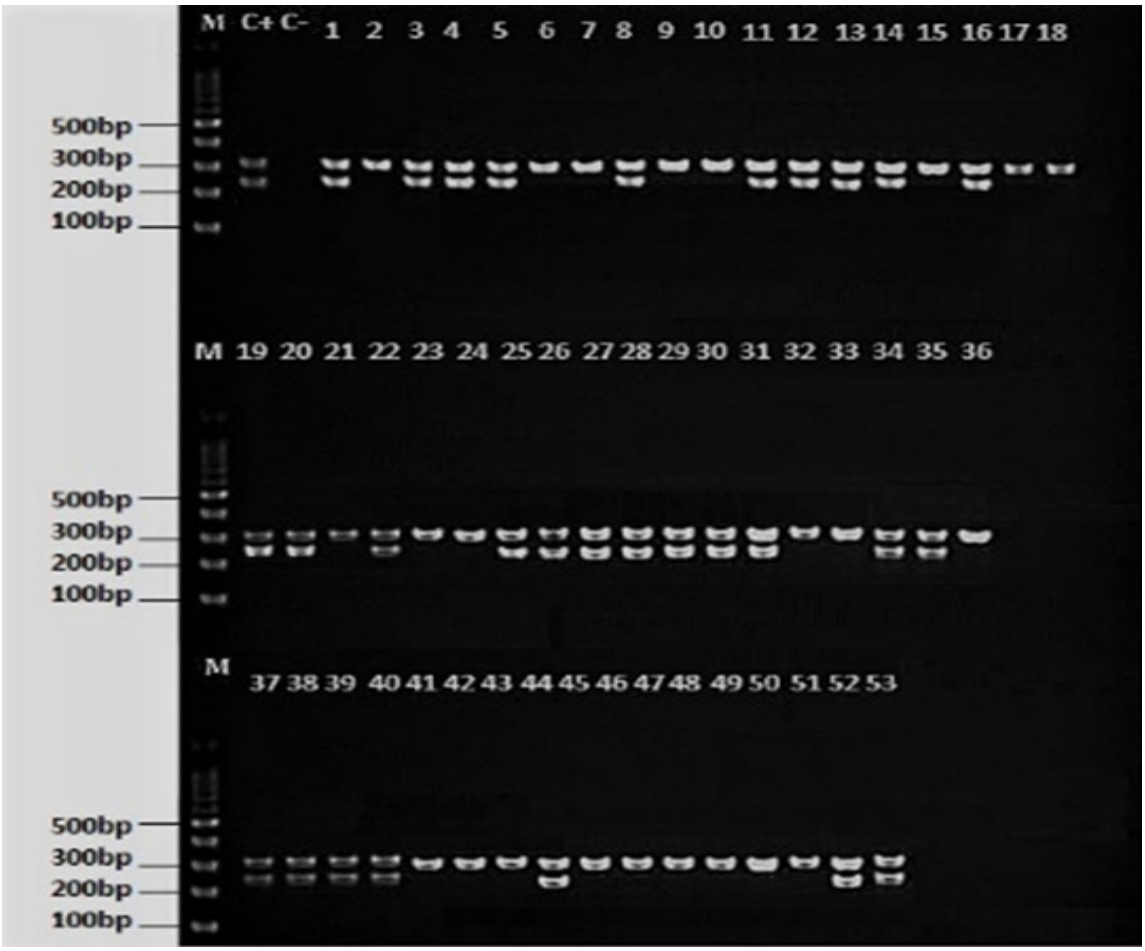

Fig. 1: Multiplex PCR result of *S. enteritidis*. Amplified sequences in *sefA and spv gene* of *S. enteritidis*, product size = 250 bp and 310 bp respectivly. L = ladder (100bp); C+ = positive control (*S. enteritidis ATCC13076*); C– = negative control (*Escherichia coli* ATCC 25922); *sefA* gene were present in all isolates but only 29 out of 53 (38.9%) isolates were positive for the presence of *spv* gene.

Table 2: Specimen wise distribution of *S. enteritidis*.

Source	Sample No*.*	*S. enteritidis* positive No (%)	*S. enteritidis* negative N (%)
Breast	175	17 (9.7%)	158 (90.2%)
Drumstick	175	6 (3.42%)	169 (99.4%)
Liver	175	23 (13.1%)	152 (86.8%)
Wing	175	7 (4%)	168 (98.8%)

When antibiotic susceptibility test was verified, most *S. enteritidis* isolates were resistant to ampicillin ($n = 38$; 71.6%), piperacillin (n=35; 66%), the isolate *S. enteritidis* showed relative high resistance to macrolide group of antibiotics including azithromycin (n=27; 50.9%) and erythromycin (n=28; 52.8%) and resistance to tetracycline and doxycycline were moderate. however, results showed no resistance against cephems (including ceftazidime, cefotaxime, and ceftriaxone); (Table 2).

Multi drug resistant and extensively drug resistant patterns were also reported among 53 *S. enteritidis* isolates, in which 58.4% ($n = 31$) were non-MDR. Additionally, 35.8% ($n = 19$) were MDR, while 5.6% ($n = 3$) were the XDR isolates (Table 4).

Table 3: General number and percentage drug resistance pattern of *Salmonella enteritidis.* (Clinical and Laboratory Standard Institute 2014).

Antibiotics	Sensitive [n (%)]	Intermediate [n (%)]	Resistant [n (%)]
PENICILLINS			
Ampicillin(10 µg)	6(11.3%)	9(16.9%)	38(71.6%)
piperacillin(100 µg)	7(13.2%)	11(20.7%)	35(66%)
CEPHEMS			
Ceftriaxone(30 µg)	53(100%)	0 (0%)	0 (0%)
Ceftazidime(30 µg)	53 (100%)	0 (0%)	0 (0%)
Cefotaxime(30 µg)	53 (100%)	0 (0%)	0 (0%)
FENICOLS			
Florfenicol(30 µg)	53 (100%)	0 (0%)	0 (0%)
LIPOPEPTIDES			
Colistin(10 µg)	51(96.2%)	2 (3.7%)	0 (0%)

AMINOGLYCOSIDES			
Neomycin(30 µg)	44(83%)	7 (13.2%)	2 (3.7%)
Gentamicin(10 µg)	53 (100%)	0 (0%)	0 (0%)
Amikacin(30 µg)	53 (100%)	0 (0%)	0 (0%)
FOLAT PATHWAY INHIBITORS			
Trimethoprim (5 µg)	25 (47.1%)	0 (0%)	10 (18.8%)
TETRACYCLINES			
Doxycycline(30 µg)	53 (100%)	0 (0%)	0 (0%)
Tetracyclin(30 µg)	5 (9.4%)	33 (62.2%)	15 (28.3%)
FOSFOMYCINS			
Fosfomycin(30 µg)	53 (100%)	0 (0%)	0 (0%)
FLUOROQUINOLONES			
Ciprofloxacin(5 µg)	18(33.9%)	11(39.6%)	24(45.2%)
MACROLIDES			
Erythromycin (15 µg)	7(13.2%)	18(33.9%)	28 (52.8%)
Azithromycin (15 µg)	11(20.7%)	15(28.3%)	27(50.9%)

Table 4: Multidrug resistant and extensively drug resistant patterns of 53 isolate of *Salmonella enteritidis*.

Drug resistance pattern	*S. enteritidis*, No.
Nonmultidrug resistant	31 (58.4%)
MDR	19 (35.8%)
XDR	3 (5.6%)

MDR = multidrug resistant (no susceptible to ≥1 agent in ≥3 antimicrobial categories); XDR = extensively drug resistant (no susceptible to ≥1 agent in all but sensitive to ≥2 categories).

Salmonella is one of the most important cause of foodborn llness globaly. there is a wide variety of foods that has been associated with such diseases. It has been shown that the foods from animal sources, especially broiler chicken derived, has been implicated in periodic cases and outbreaks of human salmonellosis (Magiorakos *et al.*, 2012). The use of antibiotics in poultry and livestock production is favorable to farmers and the economy as well because it has generally improved poultry performance effectively and economically but at the same time, the likely dissemination of antibiotic resistant strains of pathogenic and non-pathogenic organisms into the environment and their further transmission to humans via the food chain could also lead to serious consequences on public health (Apata, 2008). Currently, increasing bacterial resistance to antimicrobial agents including penicillins, macrolides and fluoroquinolones poses a serious problem throughout the world and for many years antibiotics have been used for treating or preventing disease in raising food animals. The animal feed often contains antibiotics in amounts that range from below therapeutic levels to full therapeutic levels, and the used antibiotics come from most of the antimicrobial classes used in humans. There is evidence to support the idea that feeding antibiotics to animals may result in development of antimicrobial resistant organisms, and that those resistant organisms may be transferred to the humans who consume those animals (Eley, 1996; Landers *et al*., 2012). To date, several reports from Iran have described the presence of resistance to antimicrobial agents such as fluoroquinolones and third-generation cephalosporin's that are critically important for treatment of infections in human and increased number of Multi drug resistant bacteria has been reported in *Salmonella* isolates in many countries including Iran (Rad *et al.*, 2012; Tajbakhsh *et al.*, 2012). The widespread overuse and misuse of antimicrobial agents are associated with the development of resistance to these drugs that has emerged as a major problem worldwide (Medeiros *et al.*, 2011). In Iran, patients referred to hospitals with *Salmonella* infections are usually treated with ciprofloxacin, co-amoxiclave (amoxicillin + clavulanic acid) or cephalosporins (Tajbakhsh *et al.*, 2012). Quinolones family of antibiotics, especially Fluoroquinolones are widely used in poultry farms in Iran (Rad *et al.*, 2012). In the present study, *S. enteritidis* was detected in broiler chicken samples. Moreover, their antimicrobial susceptibility pattern was also reported. In a total of 700 broiler chicken samples, the prevalence of *S. enteritidis* was 7.5%, while an increased detection rate (13.1%) was observed in liver tissue. The results of our investigation are compatible with those of a study conducted by Azizpoor in Ardabil province, Iran (Azizpoor, 2018). Another study in Iran also reported 25% prevalence of *S. enteritidis* from broiler chicken farms (Rahmani *et al.*, 2013). According to a study that was published in 2014 on the presence of Salmonella in Guilan province. Liver samples had the highest rate of infection (50%) which is also consistent with our study, and the skin and cecum were in the next positions (Asadpour *et al.*, 2014). Resistance of *Salmonella* to antibiotics is an emerging problem worldwide (Lu *et al.*, 2014). In current study, *S. enteritidis* isolates were resistant to some of commonly used antibiotics, i.e., ampicillin (82.85%), piperacillin (66%), erythromycin (52.8%), azithromycin (50.9%) and ciprofloxacin (45.2%), but cephem class of antibiotics, aminoglycosides and fosfomycin were found to be most effective. Penicillin class of antibiotics, fluoroquinolones and macrolides resistance among *S. enteritidis* isolates might designate the common use of these antibiotics. Our findings indicate that isolated *S. enteritidis* was least resistant to third-generation cephalosporin's. Lower rates of cephalosporin resistance in this study are consistent with the results of Abdel-Maksoud et al's study in 2015, who reported a low prevalence of cephalosporin resistance among *S. enteritidis* isolates from poultry sources. Lower resistance of *S. enteritidis* to cephalosporin is valuable to the community as cephalosporin resistance is a noteworthy public health concern (Abdel-Maksoud *et al.*, 2015). Overall, MDR was observed among 54.2% *S. enteritidis* isolates. Our findings are in line with the results of a study by Hur et al in 2011, in which 65.2% of *Salmonella* isolates were multiple drug resistant (Hur *et al.*, 2011). Another study from Brazil also reported 63.9% multidrug-resistant *S. enteritidis* isolates from chicken carcass samples (Medeiros *et al.*, 2011). However, another

study reported a high prevalence of MDR of 90.9% to *Salmonella enterica* serovars *Indiana* and *enteritidis* (Lu *et al.,* 2014). In one recent study, 35.8% of MDR *Salmonella* species isolates were reported. The increased MDR isolates can be due to the use of antimicrobial drugs in poultry food at a sub therapeutic level, which can promote antimicrobial-resistant strains (Rizi *et al.*, 2015).

Set primers of S1-S4 is specific for detection of *Salmonella* spp. Also set primers SEFA2-SEFA4 are specific for detection of *S. enteritidis* serovar (Amini *et al.*, 2010; Craciunas *et al.*, 2012). SefA gene is specific for detection of *S. enteritidis*. and it can be considered as a target gene to identify the serovar *S. enteritidis* by polymerase chain reaction method (Oliveira et al., 2003). In our study, *spv* gene was present in 38.9% of samples and also was similar to those found by other studies (Amini *et al.*, 2010; Craciunas *et al.*, 2012; Derakhshandeh *et al.*, 2013). There are also other studies that have found lower frequencies for this gene in strains of avian origin (Okamoto et al., 2009; Refai and Moussa, 2013). It is possible that the presence of this gene is related to the host from which the sample was isolated (Oliveira *et al.*, 2003).

In the current study, elevated levels of *S. enteritidis* were detected in broiler chickens. Increased drug resistance was observed to some of commonly used antibiotics, which indicates an emerging problem and could have negative impact on the effort to prevent and treat broiler-transmitted zoonotic *S. enteritidis*. besides Our study demonstrated that the broiler chicken meat could be a source of multiple antimicrobial-resistance *S. enteritidis* and may constitute a public health concern in Guilan province, Iran. Moreover, due to variety of virulence plasmid gene (*spv* gene) that observed in current study, further studies suggested for determination of virulence and genetic profile of salmonella enteritidis in the region.

References

Abdel-Maksoud, M.; Abdel-Khalek, R. and El-Gendy, A. (2015). Genetic characterization of multidrug-resistant *Salmonella enterica* serotypes isolated from poultry in Cairo. Egypt. Afr. J. Lab. Med.; 4: 158-165.

Akhtar, F.; Hussain, I. and Khan, A. (2010). Prevalence and antibiogram studies of *Salmonella enteritidis* isolated from human and poultry sources Pakistan. *Vet. J.;* 30: 25-28.

Amini, K.; Salehi, T.Z. and Nikbakht, G. (2010) Molecular detection of *invA* and *spv* virulence genes in *Salmonella Enteritidis* isolated from human and animals in Iran. *Afr. J. Microbiol. Res.;* 4(21): 2202-2210.

Apata, D.F. (2008) Antibiotic resistance in poultry. *Int. J. Poult. Sci.;* 8: 404-408.

Asadpour, Y.M. and Pourbakhsh, S.A. (2014). Isolation, serotyping and antibiotic resistance of Salmonella isolated from chicken carcasses in Guilan province. *Iran. Vet. J.;* 9(4): 5-14.

Azizpoor, A. (2018). A Survey on Prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* Serotypes in Broiler Flocks of Ardabil Province and Determination of Their Antibiotics Resistance to Five Antibacterial Agents Widely Used in the Iranian Medical Field. *J. Health,* 9: 143-151.

Barrow, G.I. and Feltham, R.K.A. (2003). Cowan and Steel's manual for the identification of medical bacteria (3rd ed.). Cambridge University Press, Cambridge.

Clayton, D.J.; Bowen, A.J. and Hulme, A.W. (2008). Analysis of the role of 13 major fimbrial subunits in colonization of the chicken intestines by *Salmonella enteric*a serovar*enteritidis* reveals a role for a novel locus. J. BMC Microbiol.; 8: 228-242.

Clinical and Laboratory Standards Institute (2015) Performance standards for antimicrobial disk susceptibility tests: approved standard (11th ed.). Wayne CLSI Publication M2-A100.

Clinical and Laboratory Standards Institute (2019) Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. Wayne CLSI Publication.

Craciunas, C.; A.L. Keul, M. Flonta, et al. (2012) DNA-based diagnostic tests for *Salmonella* strains targeting *hilA, agfA. spvC* and *sefC* genes. *J. Env. Man.;* 95: 512-518.

Derakhshandeh, A.; Firouzi, R. and Khoshbakht, R. (2013). Association of three plasmid-encoded spv genes among different *Salmonella* serotypes isolated from different origins. Indian J. Microbiol.; 53(1): 106-110.

Edwards, R.A. and Puente, J.L. (1998). Fimbrial expression in enteric bacteria: a critical step in intestinal pathogenesis. Trends Microbiol.; 6(7): 282-287.

Eley, R.A. (1996). Infective bacterial food poisoning. Eley R.A. (Ed.), Microbial food poisoning. London, UK. Chapman & Hall.

Gholam, A.I.; Alosaimi, E.A.; Aldhafeeri, M.D.; Alahmari, A.S.; Alharbi, A.S.; Bohassan, R.H.A. and Aldosari, F.M.A. (2019). Gastroenteritis Diagnosis and Management in Children: A simple Literature Review. Arch. Pharm. Pract.; 1: 43-46.

Gyles, C.L. (2008). Antimicrobial resistance in selected bacteria from poultry. Anim Health Res. Rev.; 9: 149-158.

Hur, J.; Kim, J.H.; Park, J.H. (2011). Molecular and virulence characteristics of multi-drug resistant *Salmonella enteritidis* strains isolated from poultry. Vet. J.; 189: 306-311.

Jahani, S.; Baniadam, A.; Imani, H.; Khosravi, M. and Gooraninejad, S. (2019). Comparison of the Effect of Ovariectomy and Ovariohysterctomy on Some Immunity Responses in Dogs. Int. J. Pharm. Res. Allied Sci.; 8(2): 58-70.

Landers, T.F.; Cohen, B. and Wittum, T.E. (2012). A review of antibiotic use in food animal perspective, policy, and potential. Public Health Rep.; 127: 4–22.

Lin, A.W.; Usera, M.A.; Barrett, T.J. (1996). Application of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella enteritidis*. J. Clin. Microbiol.; 34: 870–876.

Lu, Y.; Zhao, H.; Sun, J. (2014). Characterization of multidrug-resistant *Salmonella enterica* Serovars *Indiana* and *enteritidis* from chickens in eastern China. PLoS One, 9(5): e96050.

Lutticken, D.; Segers, R.P. and Visser, N. (2007). Veterinary vaccines for public health and prevention of viral and bacterial zoonotic diseases. Rev. Sci. Tech.; 26: 165-177.

Madadi, M.S.; Hassanzadeh, M. and Nikbakht, G.H.R. (2013). A comparative study on the colonization of

Salmonella enteritidis hilA mutant and its parent strains in laying hens. Int. J. Vet. Med.; 6: 227-233.

Magiorakos, A.P.; Srinivasan, A.; Carey, R.B. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance Clin. Microbiol. Infect.; 18: 268-281.

Majowicz, S.E.; Musto, J.; Scallan, E. (2010) The global burden of non-typhoidal *Salmonella* gastroenteritis. Clin. Infect. Dis.; 50: 882-889.

Medeiros, M.A.N.; Oliveira, D.C.N.; Rodrigues, D.P. (2011). Prevalence and antimicrobial resistance of *Salmonella* in chicken carcasses at retail in 15 Brazilian cities. Rev. Panam. Salud. Publica, 30: 555-560.

Okamoto, A.S.; Filho, R.L.A.; Rocha, T.S. (2009). Relation between the *spvC* and *invA* genes and resistance of *Salmonella* Enteritidis isolated from avian material. Int. J. Poult. Sci.; 8(6): 579-582.

Oliveira, S.D.; Rodenbusch, C.R. and Michael, G.B. (2003). Detection of virulence genes in *Salmonella* Enteritidis isolates from different sources. Braz. J. Microbiol.; 34(1): 123-124.

Onyango, M.D.; Ghebremedhin, B. and Waindi, E.N. (2009). Phenotypic and genotypic analysis of clinical isolates *Salmonella* serovar *Typhimurium* in western Kenya. J. Infect. Dev. Ctries.; 3: 685-694.

Pan, T.M. and Liu, Y.J. (2002). Identification of *Salmonella enteritidis* isolates by polymerase chain reaction and multiplex polymerase chain reaction. J. Microbiol. Immunol. Infect.; 35: 147-151.

Rad, M.; Kooshan, M. and Mesgarani, H. (2012). Quinolone resistance among *Salmonella enterica* and *Escherichia coli* of animal origin. Comp. Clin. Pathol.; 21:161–165.

Rahmani, M.; Peighambari, S.M. and Svendsen, C.A. (2013). Molecular clonality and antimicrobial resistance in *Salmonella enterica* serovars *Enteritidis* and *Infantis* from broilers in three Northern regions of Iran. BMC Vet. Res.; 9: 57-66.

Refai, M. and Moussa, I. (2013). Molecular characterization of *Salmonella* virulence genes isolated from different sources relevant to human health. J. Food Agric. Environ.; 11(2): 197-201.

Rizi, K.S.; Peerayeh, S.N.; Bakhshi, B. (2015). Prevalence of ESBLs and integrons in clinical isolates of *Salmonella spp*. from four hospitals of Tehran. Int. J. Enteric. Pathog.; 3: e21820.

Scaallan, E.; Hoekstra, R.M. and Angulo, F.J. (2011) Food-borne illness acquired in the United States. Jmajor. pathogen Emerg Infect Dis.; 17: 7-15.

Sedaghat, F. and Motamedifar, M. (2019). Investigating the prevalence of vascular catheter related bloodstream infections in ICU units of Shiraz Namazi Hospital. J. Adv. Pharm. Educ. Res.; 9(S2): 127-135.

Tajbakhsh, M.; Hendriksen, R.S. and Nochi, Z. (2012). Antimicrobial resistance in *Salmonella* spp. recoveredfrom patients admitted to six different hospitals in Tehran. Iran from 2007 to 2008. Folia Microbiol.; 57: 91–97.

Vaneechoutte, M. and Van Elare, J. (1997). The possibilities and limitations of nucleic acid amplification technology in diagnostic microbiology. J. Med. Microbiol.; 46: 188-194.