Characterization of Salmonellae isolated from different animal and human sources by PCR and resistance trends

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Summary

Salmonella is one of the most important zoonotic pathogens with many virulence factors playing a major role in its pathogenesis. The aims of this study were to detect *spvA*, *int2* and *invC* virulence genes of different *Salmonella* serotypes isolated from various clinical animal and human sources, and to investigate antibiotic resistance patterns among these serotypes. Using a PCR assay, a total of 64 *Salmonella* isolates were evaluated for the presence of virulence genes. Results revealed that *spvA*, *int2* and *invC* genes were found in 65.6%, 39.1%, and 76.6% of the *Salmonella* isolates, respectively. Seven different serotypes were differentiated according to the specific antisera. Antibiotic susceptibility results showed that isolates were susceptible to all tested antibiotics (31.25%), Amikacin (84.4%), Co-Amoxiclav (81.2%), Cefepime (73.4%), Ceftizoxime (76.6%), Ceftriaxone (60.9%), Meropenem (50%), Norfloxacin (82.8%), and Piperacillin (75%). S*pvA* is a plasmid gene and the *int2* gene has been identified on mobile elements. In addition, the chromosomal *invC* gene is associated with type III secretion systems (TTSS; not present in all Salmonellae). Hence, the detection of these genes could be used to identify the *Salmonella* genus. High prevalence of *int2* and *spvA* genes was also observed in multidrug resistance *Salmonella* isolates which might play an important role in the dissemination of antimicrobial resistance in multi-drug resistance (MDR) *Salmonella* isolates.

Key words: *Salmonella*, Virulence genes, Serotyping, Antibiotic resistance, PCR

Introduction

A large number of *Salmonella* serotypes with a wide range of animal and human reservoirs show different disease syndromes according to their antigenic profiles. To discriminate *Salmonella* serotypes from each other, it is important to ensure that each pathogen and epidemiology are correctly recognized (Lim *et al*., 2003; Ranjbar *et al*., 2010).

The availability of reliable, rapid, and internationally accepted test systems used to determine the presence or absence of food-borne pathogens has become increasingly important for agricultural and food industries, as well as the legislative regulation of food safety as a basis control (Malorny *et al*., 2003). Laborious, time-consuming, and costly bacteriological, biochemical and serological tests have been used to detect Salmonellae based on the identification of O and H antigen variables (Kumar *et al*., 2006; Nori and Thong, 2010). In addition, phenotypic properties of the bacteria cause difficult classifications and interpretations because they are not always fixed, (Malorny *et al*., 2003). On the other hand, molecular methods detect serotypes rapidly and reliably, based on the identification of unique genes or gene arrangements (Maurer *et al*., 1999). Several genes are used to detect *Salmonella* genus or serovars including various chromosomal and plasmid virulence genes such as *inv*, *spv*, *int*, *fim*, *fliC*, etc, which play a major role in its pathogenesis (Wray and Wray, 2000).

The ability of *Salmonella* spp. to enter cells is an essential pathogenic feature of these organisms. *Inv* operon on the *Salmonella* chromosome is responsible for the invasion of epithelial cells and mediates intestinal pathogenesis (Brumme *et al*., 2007). The *invC* gene encodes a structural component of the type III secretion system (TTSS) from *Salmonella* pathogenicity island 1 (SPI-1) with a significant ATPase activity. Thus, it is probably an energy provider for the TTSS (Akeda and Galan, 2004).

During the past few decades, an increase in antibiotic resistance and especially multidrug resistance (MDR) has been observed in *Salmonella* isolated from foods with animal origins (Bacci *et al*., 2012). This increase has been attributed to the overuse of antibiotics in livestock including chickens, not only to treat or prevent diseases but also to promote growth. The link between the use of antimicrobial drugs in livestock and the emergence of antimicrobial drug resistance in human pathogenic bacteria has been well documented (Araque, 2009). People infected with antibiotic-resistant strains suffer from more prolonged and severe illnesses than those infected with susceptible strains (Cook *et al*., 2009). Thus, monitoring the resistance among bacteria isolated from clinical animal and human sources is vital to plan effective antimicrobial treatments in severe

salmonellosis cases and to warn about the consequences of antibiotic overuse.

Antibiotic resistance is a growing worldwide problem encoded by linked resistance genes that complicate attempts to control infectious diseases by transferring animal *Salmonella* serotypes to humans, (Bacci *et al*., 2012).

The transfer of antibiotic resistance genes between different species of bacteria can be facilitated by mobile DNA elements such as transposons and plasmids (Boyed *et al*., 1998; Gebreyes *et al*., 2004). Integrons, which have been identified on these mobile elements, often contain one or more genes that encode antibiotic resistance and are highly responsible in the evolution and dissemination of multidrug resistance Salmonellae (Boyd *et al*., 1998; Liebert *et al*., 1999; Gebreyes *et al*., 2004). Certain serotypes are particularly associated with systemic infections. A common feature of these serotypes is the presence of *spv* genes (Guiney *et al*., 1994). Another virulence factor, the *spv* operon, is transformed by plasmids as a mobile element containing five genes (*spvRABCD*) (Guiney *et al*., 1994; Guerra *et al*., 2002). Its main function is potentiating the systemic spread of pathogens (Heithoff *et al*., 2008). This potential is associated with the multidrug-resistance of *spv* operon demonstrated in *Salmonella* strains (Chu and Chiu, 2006).

Considering the importance of the mentioned genes in the pathogenesis of Salmonellae and the main role of the *spvA* and *int2* genes in *Salmonella* multidrug resistance, the aims of the present study were to detect *spvA*, *int2* and *invC* virulence genes of different *Salmonella* serotypes isolated from different clinical animal and human sources and to investigate antibiotic resistance patterns among these serotypes.

Materials and Methods

Bacterial isolates

A total of 64 *Salmonella* isolates were used in this study. All isolates originated from poultry, farm animals and humans from Shiraz, Iran. Thirty two isolates were from diseased poultry, 23 were from aborted sheep fetuses and 9 had human origins. All isolates were previously identified by conventional methods and serotyped by the *Salmonella* reference center (Hesarak, Iran). *Salmonella* origins and serotypes used in this study are listed in Table 1. *Salmonella typhimurium* ATCC 3589 was also used as a positive control for all experiments.

Table 1: *Salmonella* origins, serotypes, and prevalence of *spvA*, *int2* and *invC* genes

Source/serotype	No. of isolates	No. positive for virulence genes		
		spvA	*Int2*	*invC*
Poultry				
S. enteritidis	17	12	3	13
S. typhimurium	8	4	4	4
S. infantis	5	1	2	3
S. colindal	1	0	1	0
Aborted sheep fetuses				
S. abortusovis	13	9	6	12
S. enteritidis	6	6	2	5
S. typhimurium	5	3	0	5
Human				
S. enteritidis	6	5	5	5
S. bardo	2	1	1	1
S. virchow	1	1	1	1
Total	64	42	25	49

DNA extraction

All isolates were grown on Luria Bertani agar (Merck, Germany). Following overnight incubation, 3-4 colonies of each isolate were suspended in 250 µl sterile distilled water. DNA extraction was performed by boiling for 10 min followed by centrifugation at 13000 rpm for 10 min. The supernatants were then stored at -20°C until use.

PCR assay

Salmonella isolates were examined for *invC*, *int2* and *spvA* genes using the following PCR protocol. Primer sequences and their target band sizes are listed in Table 2.

PCR was performed in a reaction of 25 µl containing 2.5 µl 10 X PCR buffer (CinaGen, Iran), 0.75 µl dNTPs (10 mM), 0.75 µl $MgCl_2$ (50 mM), 0.2 µl Taq DNA polymerase (5 U/µl) and 25 ng of DNA sample. For the PCR reaction, specific primers were used for each gene; 1 µl for *int2* and *invC* genes and 0.75 µl for the *spvA* gene. PCR was carried out by a thermocycler (miniMJ Model, BIORAD, USA) with an initial denaturation at 94°C for 4 min. Each PCR amplification program was performed in 35 cycles with different annealing temperatures of 57°C, 59°C and 60°C, for *spvA*, *int2* and *invC* genes, respectively. Afterwards, extension and final extension steps were carried out at 72°C for 1.5 min and 10 min. Amplified products were resolved in a 1% agarose gel and visualized under UV light.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed using

Table 2: PCR primers used for amplification of *spvA*, *int2* and *invC* genes

Gene name	Primer sequence	Amplicon size (bp)	Reference
spvA	F 5´-GTCAGACCCGTAAACAGT-3´ R 5´-GCACGCAGAGTACCCGCA-3´	641	Gebreyes *et al*., 2009
Int2	F 5´-CACGGATATGCGACAAAAAGGT-3´ R 5´-GTAGCAAACGAGTGACGAAATG-3´	789	Shibata *et al*., 2003
invC	F 5´-ATTGAGGCGGAATTGCGCGA-3´ R 5´-TGAATACTGCACTACCGGCT-3´	1189	This study

disk diffusion (Bauer *et al*., 1966). The isolates were tested against a panel of eight antibiotics: Amikacin, Co-Amoxiclav, Cefepime, Ceftizoxime, Ceftriaxone, Meropenem, Norfloxacin, and Piperacillin (Padtan Teb, Iran).

Statistical analysis

The rate of resistance regarding the prevalence of *spvA* and *int2* genes and the isolate origins were analyzed using Chi-square and Fisher's exact tests. The level of significance was considered to be $P<0.05$.

Results

Detection of virulence genes

PCR amplifications of the three main virulence genes revealed that *spvA* and *int2* genes were accounted for in 65.6% and 39.1% of the isolates, respectively (Figs. 1 and 2). The *InvC* virulence gene was also detected in 76.6% of the isolates (Fig. 3), which is more than the other mentioned genes. Four different serotypes *S. entritidis*, *S. typhimurium*, *S. infantis* and *S. colindal* in poultry, three various serotypes *S. abortusovis*, *S. entritidis* and *S. typhimurium* in aborted sheep fetuses and three serotypes *S. enteritidis*, *S. bardo* and *S. virchow* in humans were identified. Furthermore, 11 serotypes were found to be positive for the concurrent occurrence of these three virulence genes. However, one serotype (*S. typhimurium*) isolated from poultry was negative for these three virulent genes. Prevalence of the three virulence genes of the isolated serotypes are shown in Table 2.

Resistance patterns

Antibiotic susceptibility results showed that 20 isolates (31.25%) were susceptible to all tested antibiotics. Susceptibility was observed to Amikacin (84.4%), Co-Amoxiclav (81.2%), Cefepime (73.4%), Ceftizoxime (76.6%), Ceftriaxone (60.9%), Meropenem (50%), Norfloxacin (82.8%), and Piperacillin (75%). Prevalence of resistance and resistance patterns in isolated serotypes are shown in Tables 3 and 4, respectively.

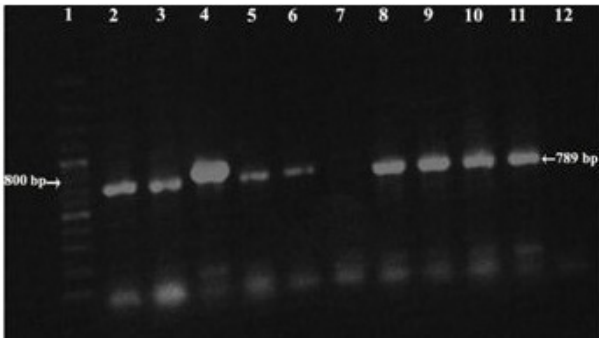

Fig. 1: PCR amplification of a 789 bp product of the gene *int2*. Lane 1: 100 bp ladder, lanes 2-6, and 8-10: positive samples, lane 7: negative sample, lane 11: positive control, and lane 12: negative control

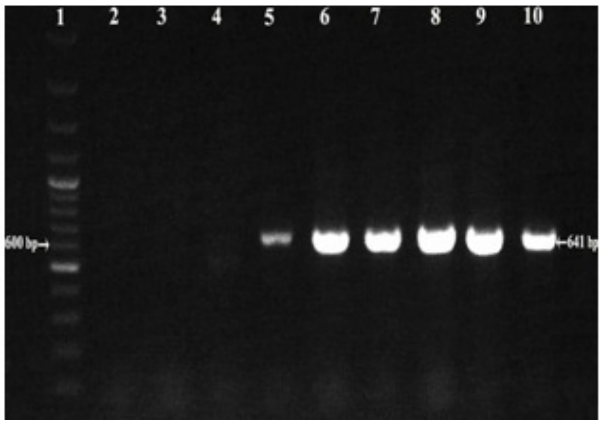

Fig. 2: PCR amplification of a 641 bp product of the gene *spvA*. Lane 1: 100 bp DNA ladder (Fermentas), lane 2: negative control, lanes 3 and 4: negative samples, lanes 5-9: positive samples, and lane 10: positive control

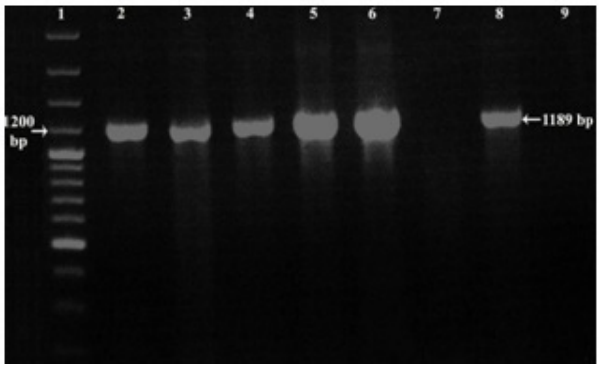

Fig. 3: PCR amplification of a 1189 bp product of the gene *invC*. Lane 1: 100 bp ladder, lanes 2-6: positive samples, lane 7: negative sample, lane 8: positive control, and lane 9: negative control

Statistical analysis indicated a significant difference between the presence of the *int2* gene and *Salmonella* resistance to Co-amoxiclav ($P=0.048$); however, no significant difference was found between the prevalence of *spvA* and *int2* genes and their resistance to other antibiotics. In humans, *Salmonela* resistance to Co-amoxiclav and Ceftizoxime was significantly higher than the other antibiotics ($P=0.007$ and $P=0.000$, respectively).

Discussion

Culture techniques are universally recognized as standard methods of detecting bacterial pathogens such as *Salmonella* in animal clinical diseases. However, to prevent economic loss and human transmission, a quicker method is needed to detect *Salmonella* serotypes. Accordingly, molecular methods have been developed to identify and distinguish different *Salmonella* serotypes based on specific virulence genes.

The *InvA* gene, a member of the chromosomal *inv* operon, has been recognized as an international standard used to detect *Salmonella* genus (Malorny *et al*., 2003). This gene is essential for full virulence and is thought to

Table 3: Antibiotic resistance in *Salmonella* serotypes isolated from animal and human sources

Serotypes	No. of isolates	AN	MEM	NOR	AMC	PIP	CT30	CRO	FEP
S. enteritidis	28	3	14	3	8	6	9	13	9
S. typhimurium	14	3	5	4	1	2	3	5	3
S. abortusovis	13	2	5	3	1	4	2	3	3
S. infantis	5	2	4	1	1	2	1	3	2
S. colindal	1	0	1	0	0	0	0	1	0
S. bardo	2	0	2	0	1	2	0	0	0
S. virchow	1	0	1	0	0	0	0	0	0
Total	64	10	32	11	12	16	15	25	17
(%)		(15.6%)	(50%)	(17.2%)	(18.7%)	(25%)	(23.4%)	(39.1%)	(26.6%)

AN: Amikacin, MEM: Meropenem, NOR: Norfloxacin, AMC: Co-amoxiclav, PIP: Piperacillin, CRO: Ceftriaxone, and FEP: Cefetizoxime

Table 4: Resistance patterns of *Salmonella* serotypes isolated from animal and human sources

No. of patterns	Antibiotic pattern	No. of isolates
1	CRO	3
2	PIP	2
3	AMC	1
4	MEM	6
5	NOR	2
6	MEM/CRO	2
7	MEM/AMC	2
8	MEM/FEP	1
9	MEM/PIP	3
10	MEM/PIP/CRO	1
11	NOR/CT30/CRO	1
12	MEM/AMC/PIP	1
13	MEM/AMC/CRO	2
14	AMC/CT30/CRO/FEP	2
15	AN/NOR/CRO/FEP	1
16	MEM/PIP/CT30/CRO/FEP	1
17	MEM/AMC/CT30/CRO/FEP	2
18	MEM/NOR/AMC/CT30/FEP	1
19	AN/MEM/NOR/CT30/CRO	1
20	AN/MEM/NOR/CRO/FEP	1
21	MEM/AMC/PIP/CT30/CRO/FEP	1
22	AN/MEM/PIP/CT30/CRO/FEP	3
23	AN/MEM/NOR/PIP/CRO/FEP	1
24	AN/MEM/NOR/PIP/CT30/CRO/FEP	3
Total		44

AN: Amikacin, MEM: Meropenem, NOR: Norfloxacin, AMC: Co-amoxiclav, PIP: Piperacillin, CRO: Ceftriaxone, and FEP: Cefetizoxime

trigger the internalization required for the invasion of deeper tissues (Khan *et al*., 2000). Eichelberg *et al*. (1994) showed that the *InvC* gene from the *inv* operon has an ATPase activity. Considering the importance of its function, for the first time in Iran we evaluated the prevalence of the *invC* gene to find out whether this chromosomal gene can be used to detect the genus *Salmonella* like the *invA* gene. In this study, the *invC* gene was detected only in 76.6% of the 64 *Salmonella* isolates. It seems, therefore, that this gene could not be considered as a suitable criterion for the detection of all *Salmonella* isolates. The prevalence of this gene as a chromosomal gene was more than other studied genes transferred by mobile elements.

SpvA is one of the most important virulence genes in the *spv* operon; however, it is not seen in all *Salmonella* isolates. Similar to the results of other studies (Woodward and Kirwan, 1996; Del Cerro *et al*., 2003), the present study revealed that the *spvA* gene was accounted for in 65.6% of the isolates. Gebreyes *et al*. (2009) investigated the association of *spvA* with multidrug resistance. They isolated the *spvA* gene from all their clinical cases of *S. typhimurium* and *S. heidelberg* serotypes and suggested that the existence of the *spvA* gene among MDR strains might increase its clinical importance. In the present study, the presence of the *spvA* gene in poultry was 70.59% in 17 isolates of *S. enteritidis*, which is less than that found by Gebreyes *et al*. (2009) and Amini *et al*. (2010). As mentioned by Heithoff *et al*. (2008), these results could be due to the carriage of the *spvA* gene in Salmonellae by self-transferrable virulence plasmids. Therefore, it appears that the presence of the *spvA* gene can be used to detect Salmonellae that contains virulence plasmids. The *spvA* gene was also detected in some serotypes such as *S. infantis*, *S. colindal*, *S. abortusovis*, *S. bardo* and *S. virchow*; however, this has not been reported previously in other studies.

The presence of integrons in multi-drug-resistant *Salmonella* isolates has been reported by Gebreyes and Altier (2002). Four classes of integrons have been described, but the most clinical and widely disseminated isolates of the animals belong to class 1 among the members of *Enterobacteriaceae* in the USA (Recchia and Hall, 1995; Goldstein *et al*., 2001). Goldstein *et al*. (2001) concluded that class 2 integrases were present only among *E. coli* and *Salmonella* isolates; with a 5.43% prevalence in 129 *Salmonella* samples. A 1% occurrence of the *int2* gene was also reported in *S. java* and *S. typhimurium* serotypes in the Netherlands by Van Essen-Zandbergen (2007), which is less than that found by the present study. We therefore suggest that the transfer of this gene by mobile elements might have increased in the recent years. Nevertheless, more regional studies are needed to determine the increasing prevalence of these genes in restricted areas.

In the present study, the highest prevalence of the *int2* gene was detected in *S. abortusovis* and serotypes other than *S. typhimurium* and *S. java* were found to carry this gene. Since *Int2* and *spvA* genes express antibiotic resistance, the presence of both *spvA* and *int2* genes may be used as an indicator to detect the multidrug resistance of Salmonellae.

An increasing rate of *Salmonella* antimicrobial resistance has been reported in several developing and developed countries (Ashtiani *et al*., 2009). Singh *et al*.

(2012) reported *Salmonella* strains isolated from clinical cases (human and animal) and meat to be resistant to all tested antibiotics of the cephalosporin, quinolone β-lactam, cabapenem, aminoglycoside groups; however, in the present study, 31.25% of all *Salmonella* isolates were susceptible to all mentioned antibiotics.

Resistance to combinations of several classes of antimicrobials has led to the emergence of multidrug resistance strains (White *et al*., 2001). The present study determined 34.4% MDR (resistance to more than 3 antibiotics) strains and similar to Akbarmehr (2012), we found MDR Salmonellae in 25.5% of the isolates. Nevertheless, other reports have expressed lower percentages.

In this study, similar to Singh *et al*. (2012), multidrug resistance exhibited higher rates in *Salmonella* isolated from animals compared to those from humans. This fact is presumably due to the overuse of antibiotics for growth promotion and treatment of food-born infections in veterinary medicine.

Although the use of carbapenems is not recommended, they are likely to have a role in the final stage of treatment of quinolone-resistant and extended-spectrum β-lactamase (ESBL)-producing multidrug resistant Salmonellae (Capoor *et al*., 2009). While lower rates of carbapenem resistance have been recorded for many countries (Firoozeh *et al*., 2011; Singh *et al*., 2012); the 50% resistance rate of meropenem found in the present study might have been caused by the increasing prescription of carbapenems in Iran, especially for human infections. The lowest resistance was exhibited by amikacin which could be a potentially effective suggestion for salmonellosis treatments in southern Iran. Referring to Firoozeh *et al*. (2011) and Hur *et al*. (2011), Hopkins *et al*. (2008) also demonstrated the increasing resistance of quinolone.

In this study, the presence of *int2* and *spvA* genes in MDR Salmonellae were 40.9% and 68.2%, respectively. These findings show that the two genes do not support all resistant phenotypes observed among *Salmonella* isolates, and as Firoozeh *et al*. (2011) also mentioned earlier, might be related to the transport of resistance genes via other integron classes and *spv* genes or other genetic elements like transposons.

In conclusion, for the direct detection of *Salmonella,* PCR can represent an alternative to time consuming culture methods. Bearing in mind the fact that *spvA* is a plasmid gene and the *int2* gene has been identified on mobile elements, and that the chromosomal *invC* gene is associated with the type III secretion system (TTSS; not present in all Salmonellae), it is concluded that for the detection of these genes one can rely on genus *Salmonella*, nevertheless, their absence does not definitively reject the genus *Salmonella* as a possible option. The increase in MDR *Salmonella* serotypes is related to the high prevalence of transportable genetic elements such as *int* and *spv* genes which cause serious problems for public health; hence, mandatory laws should be set by the authorities aiming at making a more prudent use of antibiotics in both human and veterinary medicine.

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References

Akbarmehr, J (2012). Antimicrobial resistance in *Salmonella* isolated from broiler chicken carcasses. Afr. J. Microbiol. Res., 6: 1485-1488.

Akeda, Y and Galan, JE (2004). Genetic analysis of the *Salmonella enterica* type III secretion-associated ATPase *InvC* defines discrete functional domains. J. Bacteriol., 186: 2402-2412.

Álvarez-Fernández, E; Alonso-Calleja, C; García-Fernández, C and Capita, R (2012). Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from poultry in Spain: comparison between 1993 and 2006. Int. J. Food Microbiol., 153: 281-287.

Amini, K; Zahraei Salehi, T; Nikbakht, Gh; Ranjbar, R; Amini, J and Ashrafganjooei, SB (2010). Molecular detection of *invA* and *spv* virulence genes in *Salmonella entritidis* isolated from human and animals in Iran. Afr. J. Microbial. Res., 4: 2202-2210.

Araque, M (2009). Nontyphoid *Salmonella* gastroenteritis in pediatric patients from urban areas in the city of Merida, Venezuela. J. Infect. Dev. Ctries. 3: 28-34.

Ashtiani, MT; Monajemzadeh, M and Kashi, L (2009). Trends in antimicrobial resistance of fecal Shigella and *Salmonella* isolates in Tehran, Iran. Indian J. Path. Microbiol., 52: 52-55.

Bacci, C; Boni, E; Alpigiani, I; Lanzoni, E; Bonardi, S and Brindani, F (2012). Phenotypic and genotypic features of antibiotic resistance in *Salmonella* enteric isolated from chicken meat and chicken and quail carcasses. Int. J. Food Microbiol., 160: 16-23.

Bauer, AW; Kirby, WM; Sherris, JC and Turck, M (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Boyed, EF and Hartl, DL (1998). *Salmonella* virulence plasmid modular acquisition of the *spv* virulence region by an F-plasmid in *Salmonella enterica* subspecies I and insertion into the chromosome of subspecies II, III a, IV and VII Isolate. Gen. Soc. Am., 149: 1183-1190.

Brumme, S; Arnold, T; Sigmarsson, H; Lehmann, J; Scholz, HC; Hard, WD; Hensel, A; Truyen, U and Roesler, U (2007). Impact of *Salmonella typhimurium* DT104 virulence factors *invC* and *sseD* on the onset, clinical course, colonization patterns and immune response of porcine salmonellosis. Vet. Microbiol., 124: 274-285.

Capoor, MR; Nair, D; Posti, J; Singhal, S; Deb, M; Aggarwal, P and Pillai, P (2009). Minimum inhibitory concentration of carbapenems and tigecycline against *Salmonella* spp., J. Med. Microbiol., 58: 337-341.

Carli, KT; Unal, CB; Caner, V and Eyigor, A (2001). Detection of Salmonellae in chicken feces by a combination of tetrathionate broth enrichment, capillary PCR, and capillary gel electrophoresis. J. Clin. Microbiol., 39: 1871-1876.

Chu, C and Chiu, CH (2006). Evolutions of the virulence plasmid of non-typhoid *Salmonella* and its association with antimicrobial resistance. Microb. Infect., 8: 1931-1935.

Cook, A; Reid-Smith, R; Irwin, R; McEwen, SA; Valdivieso-García, A and Ribble, C (2009). Antimicrobial resistance in *Campylobacter*, *Salmonella*, and *Escherichia coli* isolated from retail turkey meat from Southern Ontario. J. Food Prot., 72: 473-481.

Del Cerro, A; Soto, SM and Mendoza, MC (2003). Virulence and antimicrobial-resistance gene profiles determined by PCR-based procedures for *Salmonella* isolated from samples of animal origin. Food Microbiol., 20: 431-438.

Eichelberg, K; Ginocchio, CC and Galan, JE (1994). Molecular and functional characterization of the *Salmonella typhimurium* invasion genes *invB* and *invC*: homology of *InvC* to the F0F1 ATPase family of proteins. J. Bacteriol., 176: 4501-4510.

Firoozeh, F; Shahcheraghi, F; Zahraei Salehi, T; Karimi, V and Aslani, MM (2011). Antimicrobial resistance profile and presence of class I integrons among *Salmonella enteric* serovars isolated from human clinical specimens in Tehran, Iran. Iranian J. Microbiol., 3: 112-117.

Gebreyes, WA and Altier, C (2002). Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* isolates from swine. J. Clin. Microbiol., 40: 2813-2822.

Gebreyes, WA; Thakur, S; Davies, P; Funk, J and Altier, C (2004). Trends in antimicrobial resistance, phage types and integrons among *Salmonella* serotypes from pigs, 1997-2000. J. Antimicrob. Chemother., 53: 997-1003.

Gebreyes, WA; Thakur, S; Dorr, P; Tadesse, DA; Post, K and Wolf, L (2009). Occurrence of *spvA* virulence gene and clinical significance for multidrug-resistant *Salmonella* strains. J. Clin. Microbiol., 10: 777-780.

Guerra, B; Soto, S; Helmuth, R and Mendoza, MC (2002). Characterization of a self-transferable plasmid from *Salmonella enterica* serotype *Typhimurium* clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes. Antimicrob. Agents Chemother., 46: 2977-2981.

Guiney, DG; Fang, FC; Krause, M and Libby, S (1994). Plasmid mediated virulence genes in non-typhoid *Salmonella* serovares. FEMS Microbiol. Lett., 124: 1-9.

Heithoff, DM; Shimp, WR; Lau, PW; Badie, G; Enioutina, EY; Daynes, RA; Byrne, BA; House, JK and Mahan, MJ (2008). Human *Salmonella* clinical isolates distinct from those of animal origin. Appl. Environ. Microbiol., 10: 1757-1766.

Hopkins, KL; Day, M and Threlfall, EJ (2008). Plasmid-mediated quinolone resistance in *Salmonella enterica*, United Kingdom. Emerg. Infect. Dis., 14: 340-342.

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

Khan, AA; Nawaz, MS; Khan, SA and Cerniglia, CE (2000). Detection of multidrug resistant *Salmonella typhimurium* DT104 by multiplex polymerase chain reaction. FEMS Microliol. Lett., 182: 355-360.

Kumar, S; Balakrishna, K and Batra, HV (2006). Detection of *Salmonella enterica* serovar *Typhi* (*Salmonella typhi*) by selective amplification of *invA*, *viaB*, *fliC*-d and *prt* genes by polymerase chain reaction in multiplex format. Appl. Microbiol., 42: 149-154.

Liebert, CA; Hall, RM and Summers, AO (1999). Transposon Tn21, flagship of floating genome. Microbiol. Mol. Biol. Rev., 63: 507-522.

Lim, YH; Hirose, K; Izumiya, H; Arakawa, E; Takahashi, H; Terajima, J; Itoh, KI; Tamura, K; Kim, S and Watanabe, H (2003). Multiplex polymerase chain reaction assay for selective of *Salmonella enterica* serovar *typhimurium*. Jpn. J. Infect. Dis., 56: 151-155.

Malorny, B; Hoorfar, J; Bunge, C and Helmuth, R (2003). Multicenter validation of the analytic accuracy of *Salmonella* PCR: toward an international standard. Appl. Environ. Microbiol., 69: 290-296.

Maurer, JJ; Schmidt, D; Petrosko, P; Sanchez, S; Bolton, L and Lee, MD (1999). Development of primers to O-antigen biosynthesis genes for specific detection of *Escherichia coli* O157 by PCR. Appl. Environ. Microbiol., 65: 2954-2960.

Nori, MEE and Thong, KL (2010). Differentiation of *Salmonella enterica* based on PCR detection of selected somatic and flagellar antigen. Afr. J. Microbiol. Res., 4: 871-876.

Ranjbar, R; Giammanco, GM; Aleo, A; Plano, MRA; Naghoni, A; Owlia, P and Mammina, C (2010). Characterization of the first extended-spectrum beta lactamase-producing non thyphoidal *Salmonella* strains isolated in Tehran, Iran. Foodborne Pathog. Dis., 7: 91-95.

Recchia, GD and Hall, RM (1995). Gene cassettes: a new class of mobile element. Microbiol., 141: 3015-3027.

Singh, S; Agarwal, RK; Tiwari, SC and Singh, H (2012). Antibiotic resistance pattern among the *Salmonella* isolated from human, animal and meat in India. Trop. Anim. Health Prod., 44: 665-674.

Van Essen-Zandbergen, A; Smith, H; Veldman, K and Mevius, D (2007). Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in the Netherlands. J. Antimicrob. Chemother., 59: 746-750.

White, DG; Zhao, S and Sudler, R (2001). The isolation of antibiotic resistant *Salmonella* from retail ground meats. New Engl. J. Med., 345: 1147-1154.

Woodward, MJ and Kirwan, SE (1996). Detection of *Salmonella enteritidis* in eggs by the polymerase chain reaction. Vet. Rec., 138: 411-413.

Wray, C and Wray, A (2000). *Salmonella* in domestic animals. 1st Edn., Wallingford, UK, CABI Publishing. PP: 57-58.