African Journal of Biotechnology Vol. 9(5), pp. 612-619, 1 February, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1761
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Using molecular techniques for rapid detection of *Salmonella* serovars in frozen chicken and chicken products collected from Riyadh, Saudi Arabia

Moussa, I. M[1]*, Gassem, M. A.[2], Al-Doss, A. A[1], Mahmoud W. A. Sadik[3] and Abdel Mawgood, A. L.[1]

[1]Center of Excellence in Biotechnology Research, King Saud University, P.O. Box 2460 - Riyadh King Saudi Arabia.
[2]College of Food and Agricultural Sciences, King saud University, P.O. Box 2460 - Riyadh King Saudi Arabia.
[3]Ministry of Municipality and Rural Affair, Riyadh, Saudi Arabia.

Accepted 14 January, 2010

The current study was aimed to investigate the incidence of different *Salmonella* serovars in chicken products either from local or imported source. A total of 152 samples of chicken and chicken products were collected from different retail establishment markets in Riyadh, KSA including 38 local whole frozen chickens, 62 imported whole frozen chickens, 22 whole poultry eggs and 30 local chicken cuts samples and examined by standard microbiological techniques (SMT). *Salmonella* isolation revealed a total percentage of 5.92%; chicken cuts revealed a high incidence among the examined samples (10%), followed by local frozen chickens and imported frozen chicken samples with incidence of 7.89 and 4.83%, respectively. For this experiment, the whole chicken eggs were negative for *Salmonella* species by SMT. *Salmonella enteritidis* was dominating among the recovered *Salmonella* serovars, followed by *Salmonella typhimurium*, while only two strains of *Salmonella agona* and *Salmonella newport* were isolated. The PCR assay combined with Rappaport- Vassiliadis (RV) selective broth (PCR-RV) for the detection of *Salmonella* species in the collected field samples revealed the same positive samples directly from the imported frozen chickens and whole chicken eggs which gave negative results by SMT. Thus PCR-RV technique is rapid, time saving and applicable to detect *Salmonella* serovars directly from chicken samples.

Key words: Frozen chickens, *Salmonella* serovars, diagnosis, enrichment, selective, polymerase chain reaction.

INTRODUCTION

Salmonella species live in the intestinal tracts of warm and cold blooded animals. Some species are ubiquitous; other species are specifically adapted to a particular host. It is the major causes of food-borne disease throughout the world (Altekruse et al., 1999; Humphrey, 2002; Schlundt, 2002; Wang et al., 2008). *Salmonella* infected chickens represent a source of pathogens for humans, causing severe illness and even death. It is estimated that 16 million new cases of typhoid fever occur each year around the world, mostly in developing country (D'Aoust, 1994; Parry et al., 2002; Dimitrov et al., 2007); the infection is characterized by a variety of clinical manifestations ranging from high-grade fever to complications including "encephalopathy, peritonitis, perforation and hemorrhage". The commonest serotypes causing disease in humans are *Salmonella enteritidis* and *Salmonella typhimurium* (Baggesen et al., 2002; Aktas et al., 2007).

*Corresponding author. E-mail: imoussa1@ksu.edu.sa or moussaihab@gmail.com. Tel: 00966560749553. Fax: 00966-14678456.

Abbreviations: SMT, Standard microbiological techniques; **PCR,** polymerase chain reaction; **RV,** Rappaport- Vassiliadis; **PCR-RV,** PCR assay combined with Rappaport- Vassiliadis selective broth; **PCR-NS,** PCR-non selective test; **MPCR,** multiplex PCR; **BPW,** buffered peptone water; **XLD,** xylose lysine desoxycholate agar; **TSB,** tryptic soya broth; **EDTA,** ethylene diamine tetraacetic acid.

Table 1. Reference Standard strains used for quality assurance and PCR specificity.

S/N	Bacterial species	Source
1	*Escherichia coli*	NCIMB*-50034
2	*Enterococcus fecalis*	NCIMB-50029
3	*S. enteritidis*	ATCC**-13076
4	*S. enteritidis*	WHO***
5	*S. heidelberg*	WHO
6	*Escherichia coli* (O157:H7)	ATCC-35150
7	*Salmonella typhi*	ATCC-9992
8	*S. typhimurium*	ATCC-14028
9	*S. typhimurium*	NCIMB-50076
10	*Staphylococcus aureus*	NCIMB-50080

* NCIMB: National Collection for Industerial and Marine Bacteria.
** ATCC: American Type Culture Collection.
*** WHO: World Health Organization.

Multi-resistant *S. typhimurium* definitive phage type (DT) 104 strains are responsible for a high number of infections in humans and are primarily zoonotic in origin (Gatto et al., 2006). Today, it is widely spread and is considered pandemic.

Egg associated Salmonellosis is an important public health problem in the United States and several European countries. *S. enteritidis* silently infects the ovaries of healthy appearing hens and contaminates the egg before the shells are formed and if the eggs are eaten raw or undercooked, the bacterium can cause illness.

Imported birds and animals may serve to introduce different *Salmonella* species to the local area that can cause new and devastating outbreaks (Altekruse et al., 1999; Sareyyupoglu et al., 2007; Yu et al., 2008). The isolation and identification of salmonellae from clinical samples by traditional cultural techniques requires laborious procedures which can last up to 7 days (Stone et al., 1994), so there is a need for the development of innovative methods for the rapid identification of *Salmonella* food-borne pathogen to over come these drawbacks. Molecular techniques such as Polymerase Chain Reaction (PCR) especially by using selective broth culture have been invaluable tools for the detection of different *Salmonella* species (Oliveira et al., 2003). When multiple target genes need to be amplified, multiplex PCR (MPCR) can be performed and may provide a simple and sensitive tool for the simultaneous detection of multiple pathogenic bacteria (Soumet et al., 1999).

Investigation of *S. enteritidis* and *Salmonella typhimurium* among *Salmonella enterica* serovars in chickens and chicken products collected from Riyadh, King Saudi Arabia (KSA) using conventional and molecular techniques (PCR using selective broth culture) was the major strategy of this study.

MATERIALS AND METHODS

Bacteria and reagents

The bacterial reference strains used in this study were illustrated in (Table 1). The materials, chemicals and reagents used in this study were purchased from Sigma Chemicals Co. (St. Louis, MO. USA) unless otherwise specified. PCR reagents were purchased from Promega (Madison, WI, USA).

Samples collection

During the summer of 2009, a total of 152 samples of poultry and poultry products were collected from different retail establishment markets in Riyadh, KSA including, 38 local whole frozen chickens, 62 imported whole frozen chickens, 22 whole poultry eggs and 30 local chicken cut samples (Liver, framed and Giblets). We also used 10 negative control field samples (5 frozen chicken and 5 poultry eggs) collected from young birds a few hours after hatching, and these birds coming from breading flocks were continuously monitored for salmonella by standard microbiological techniques.

All samples were transported to the laboratory under refrigerated conditions where they were processed and bacteriologically examined immediately.

Standard microbiological techniques for *Salmonella* detection and identification (SMT)

The standard microbiological techniques for detection of different *Salmonella* serovars conducted according to ISO 6579 (2002); 25 g of poultry composite samples were homogenized in a stomacher (Bag Mixer 400, Interscience, France) for 1 to 2 min in 225 ml of buffered peptone water (BPW) and then incubated under aerobic conditions at 37°C for 16 - 20 h followed by selective enrichment of 0.1 in 10 ml of Rappaport –Vassiliadis (RV) broth. The RV broth was incubated at 42°C for 18-24 h. The broth was then sub cultured onto Xylose Lysine Desoxycholate agar (XLD) agar, Hektoen Enteric agar and Salmonella Chromogenic Agar then incubated at 37°C for 18 - 24 h. All media were supplied by Oxoid, Basingstoke, UK. Presumptive positive colonies (non lactose fermentative with suitable colony morphology) were identified morphologically, biochemically, serologically by slide agglutination test using polyvalent and monovalent somatic (O), virulence (Vi) and tube agglutination test for flageller (H) antigens (Difco Laboratories, Detroit, Michigan, USA) and Enzyme Linked Immuno-Sorbant Assay (ELISA) (Reveal Salmonella test kits systems, Neogen Corporation). 1 ml of BPW which had been incubated at 37°C was saved for the PCR-Non Selective test (PCR-NS) and 1 ml of the 37°C RV broth for the PCR-RV test.

Extraction of DNA

The standard and bacteriologically positive strains were grown in 10 ml Tryptic Soya Broth (TSB) at 37°C for 24 h. The overnight cultures were centrifuged at 3000 rpm for 5 min and the supernatant were decanted carefully. The bacterial pellets were washed three times with phosphate buffer saline pH 7.2 and resuspended in 400 µL tris-EDTA buffer (pH 8.0) and heated in water bath at 100°C for 20 min. There were left to cool at room temperature and centrifuged at 14,000 rpm for 10 min. An aliquot of 5 µL of the supernatant was used as template DNA in the PCR. While the extraction of DNA from the field samples enriched in RV broth was carried out by the same method reported by Oliveira et al. (2003).

Table 2. Oligonucleotide primers sequences used for amplification of DNA for the detection of *Salmonella* species according to (Oliveira et al., 2002).

Primer	Target gene	Specificity	Primer sequence (5'-3')	G+C content (%)
139	*invA*	*Salmonella* species	GTG AAA TTA TCG CCA CGT TCG GGC AA	50
141	*invA*		TCA TCG CAC CGT CAA AGG AAC C	55
Fli15	*fliC*	*S. typhimurium*	CGG TGT TGC CCA GGT TGG TAA T	55
Typ04	*fliC*		ACT GGT AAA GATGGC T	44
A058	*sefA*	*S. enteritidis*	GAT ACT GCT GAA CGT AGA AGG	48
A01	*sefA*		GCG TAA ATC AGC ATC TGC AGT AGC	50
sdiA1	*sdiA*	*Salmonella* species	AAT ATC GCT TCG TAC CAC	55
sdiA2	*sdiA*		GTA GGT AAA CGA GGA GCA	55

Table 3. Primers condition during PCR.

Primer	Forward primer	Reverse primer	Annealing temperature (°C)	Size of amplified product
invA	139	141	55	284
fliC	Fli15	Typ04	55	620
sefA	A058	A01	55	488
sdiA	SdiA1	SdiA2	60	274

Polymerase chain reaction

Oligonucleotide primers

Four sets of primer pairs were used; the first was 139 - 141, specific for the *invA* gene located on the salmonella pathogenicity island 1 which is highly conserved in *Salmonella* species and encodes a type III secretion system that exports proteins in response to bacterial contact with epithelial cells. (Galan et al., 1992; Rahn et al., 1992); the second was Fli15- Typ04, specific for the *fliC* gene found in *S. typhimurium* (Soumet et al., 1999), the third was A058-A01, specific for the *sefA* gene found in *S. enteritidis* (Doran et al., 1996). The fourth was SdiA1 and SdiA2 specific to genus *Salmonella* species according to Konstantia et al. (2006), Primer sequences and specificity, G+C content, annealing temperature and size of the amplified product were summarized in the Tables 2 and 3.

DNA amplification

Four independent reactions, each with one set of primers, were made for each DNA template. PCR amplifications were performed in a final volume of 50 µL in micro-amplification tubes (PCR tubes). The reaction mixtures consisted of 5 µL of the DNA template, 5 µL 10x PCR buffer (75 mM Tris-HCl, pH 9.0, 2 mM $MgCl_2$, 50 mM KCl, 20 mM $(NH_4)2SO_4$), 1 µL dNTPs (40 µM), 1 µL (1U Ampli Taq DNA polymerase), 1 µL (25 pmol) from the forward and reverse primers of both primer pairs and the volume of the reaction mixture was completed to 50 µL using DDW. The thermal cycler was adjusted as follows: Initial denaturation at 94°C for 5 min, followed by 35 cycles of (denaturation at 94°C for 1 min, annealing as shown in Table 3 for 1 min and extension at 72°C for 1 min). Final extension was carried out at 72°C for 10 min and the PCR products were stored in the thermal cycler at 4°C until they were collected.

Agarose gel electrophoresis

The PCR products were tested for positive amplification by agarose gel electrophoresis previously reported by Sambrook et al. (1989) using suitable molecular weight markers.

RESULTS

Standard microbiological techniques

The standard microbiological techniques revealed positive isolation of 9 *Salmonella* serovars (5.92%) out of 152 examined poultry samples and 3 of them (7.89%) were isolated from 38 local frozen chickens and identified biochemically and serologically as *S. enteritidis*. Also, 3 *Salmonella* serovars (4.83%) were isolated from 62 imported frozen chickens and identified as *Salmonella agona, Salmonella newport* and *S. enteritidis*. While the other 3 *Salmonella* serovars (10%) were isolated from 30 local chicken cuts and identified by SMT as *S. enteritidis* (one strain) and *S. typhimurium* (two strains). The whole chicken eggs were negative for isolation of *Salmonella* serovars by SMT (Table 4).

Molecular typing using PCR

The specificity of the oligonucleotid primers as well as typing of the recovered *Salmonella* serovars from SMT

Table 4. Results of standard microbiological techniques.

Types of samples	Origin	Number of samples	Standard microbiological techniques		
			Total positive	%	*Salmonella* serovars
Frozen chickens	Local source	38	3	7.89	*S. enteritidis*
Frozen chickens	Imported source	62	3	4.83	*S. agona* *S. newport* *S. enteritidis*
Chicken cuts	Local source	30	3	10.0	*S. enteritidis* *S. typhimurium* (2) strains
Whole eggs(10/each)	Local source	22	0	0.00	Negative
Total	Local/imported	152	9	5.92	*Salmonella* serovars

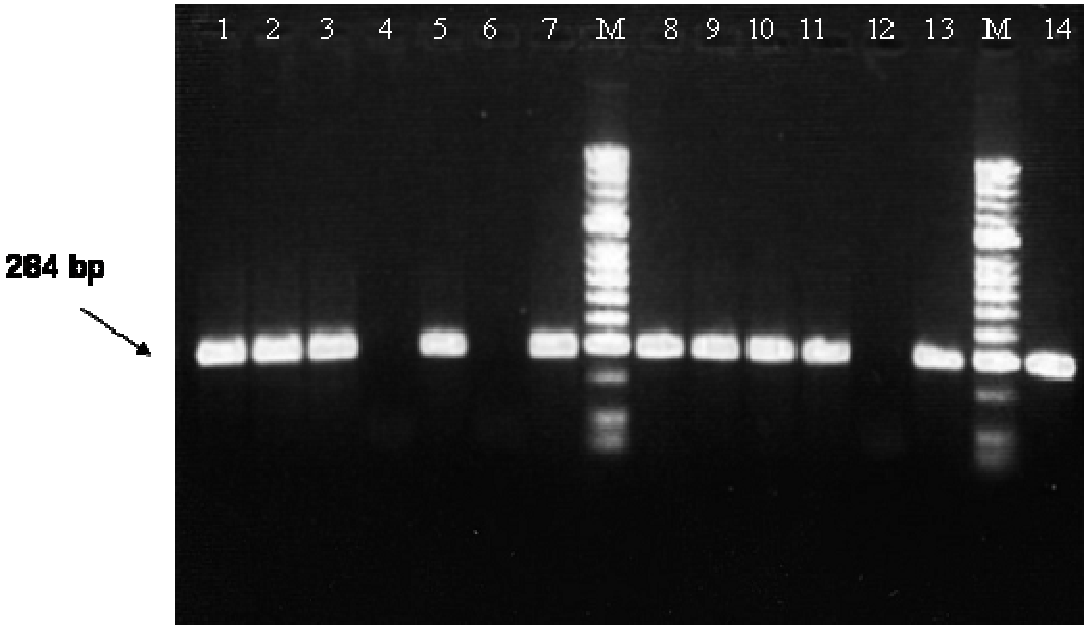

Figure 1. Agarose gel electrophoresis showing amplification of 284 bp fragments of *invA* genes. Lanes 1, 2, 3, 5, 7, 8, 9, 10, 11, 13 and14 showing positive amplification of 284 bp fragments of *Salmonella* sp*ecies*, while lanes 4, 6 and 12 showing no amplification. Lane M showsPCR markers.

were carried out by testing of all the recovered *Salmonella* strains in addition to the standard positive and standard negative strains with PCR, using four types of primer pairs targeting for (*invA*, *sdiA*, *sefA* and *fliC* genes). The specificity of such genes was mentioned in Tables 2 and 3. Whereas all *Salmonella* serovars were positive for amplification of 284 and 274 bp fragments of *invA* and *sdiA* genes, all non *Salmonella* serovars were negative (Figures 1, 2a and 2b). Only *S. enteritidis* gave positive amplification of 484 bp fragments of *sefA* gene; on the other hand, all *S. typhimurium* were positive for amplification of 620 bp fragments of *fliC* gene while all *Salmonella* serovars were negative for the presences of *sefA* and *fliC* genes (Figures 3a and b).

All the examined field samples with SMT as well as the negative control field samples were tested by PCR using the same primer pairs after selective enrichment on RV broth. All bacteriologically positive samples (100%) were positive by PCR and amplification of 284 and 274 bp fragments specific for *invA* and *sdiA* genes were observed. In addition, 2 samples (1.32%) previously identified as negative samples with SMT were positive with PCR using the two primer pairs (Tables 5 and 6). All *S. enteritidis* positive samples with SMT were positive for amplification of 484 bp fragments specific for *sefA* gene, moreover, PCR could detect *S. enteritidis* in 2 field samples (one imported frozen chickens and one whole chicken egg) previously identified as negative for *Salmonella* species with SMT (Tables 5 and 6). On the other hand, all *S. typhimurium* positive samples with SMT were positive for amplification of 620 bp fragments specific for *fliC* gene found in *S.* typhimurium (Tables 5 and 6). The

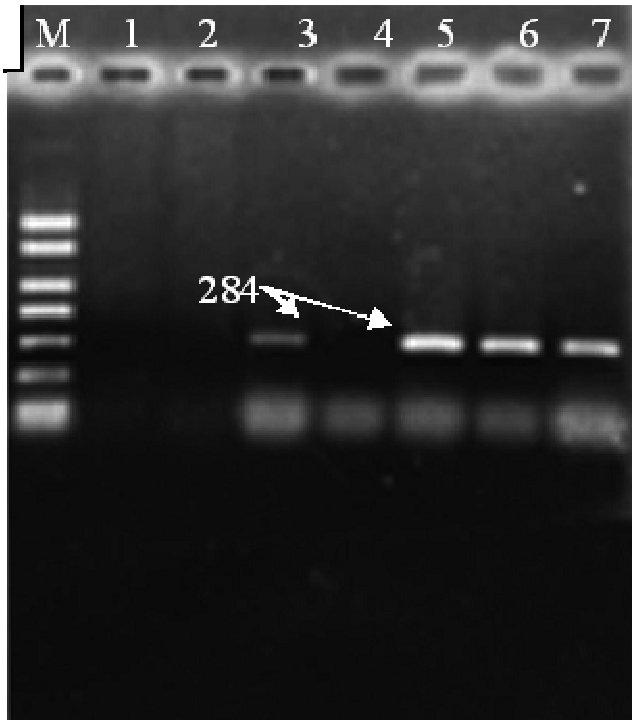

Figure 2a. Agarose gel electrophoresis showing amplification of 284 bp fragments of *invA* gene in lanes 3, 5, 6 and 7, while lanes 1, 2 and 4 show negative results. Lane M shows PCR markers.

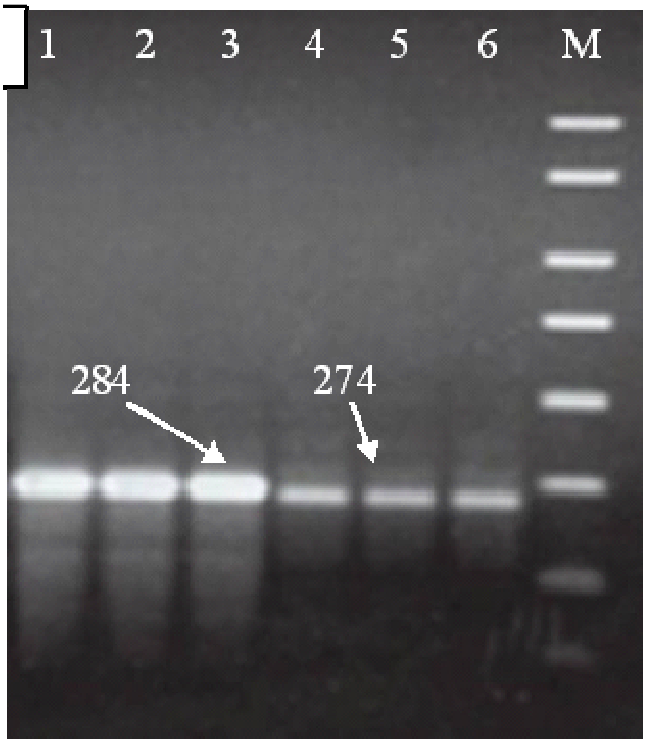

Figure 2b. Agarose gel electrophoresis showing amplification of 284 bp fragments of *invA* gene in lanes 1, 2 and 3, while lanes 4, 5 and 6 show amplification of 274 bp fragments of *sdiA* gene specific for *Salmonella* species. Lane M shows PCR markers.

negative control field samples were negative for the PCR assay and no amplification could be detected with the four primer pairs.

DISCUSSION

Poultry are one of the most important reservoirs of *Salmonellae* that can be transmitted to humans through the food-chain. The commonest serotypes causing disease in humans are *S. enteritidis* and *S. typhimurium* (Aktas et al., 2007). The detection of *Salmonella* species by regulatory agencies is still primarily based on standard microbiological techniques which may take up to 7 days to confirm the results (Stone et al., 1994). The earlier a foodborne outbreak is suspected, the faster the source of the pathogen can be identified, and the sooner the public can regain confidence in the food supply (Bhagwat and Lauer, 2004). The current study was aimed to investigate the incidence of different *Salmonella* serovars in chicken and chicken products from both local and imported source, therefore, 152 samples collected from frozen chickens and chickens products were examined by SMT. *Salmonella* isolation revealed a total percentage of 5.92% from imported and local frozen chicken and chicken products. The results observed in Table 4 revealed a high incidence of *Salmonella* serovars isolation among chicken cuts (10%), followed by local frozen chickens and imported frozen chickens samples with incidence of 7.89 and 4.83%, respectively. On the other hand, the whole chicken eggs revealed negative results for isolation of *Salmonella* species by SMT. The results of SMT revealed that *S. enteritidis* was dominating among the recovered *Salmonella* serovars with incidence of 55.56% (5 out of the 9 strains recovered by SMT), followed by *S. typhimurium* (two strains) with incidence of 22.22%. While the other two strains were *S. agona* and *S. newport*. These results indicated the health hazard of poultry as a major source of *Salmonella* foodborne pathogens (Altekruse et al., 1999; Humphrey, 2002; Schlundt, 2002). The *S. agona* and *S. newport* isolated from imported frozen chickens only are indications of the ability of imported chickens to introduce different *Salmonella* species to the local area that can cause new and devastating outbreaks (Altekruse et al., 1999). The SMT used in these study reported by ISO 6579 (2002) was characterized by very good analytical parameters which allow the detection of low numbers of potentially stressed cells of various *Salmonella* serovars through the use of pre-enrichment in BPW followed by selective enrichment in RV broth and finally plated into three different *Salmonella* selective agars.

Traditional methods of identification of food-borne pathogens, which cause disease in humans, are time-

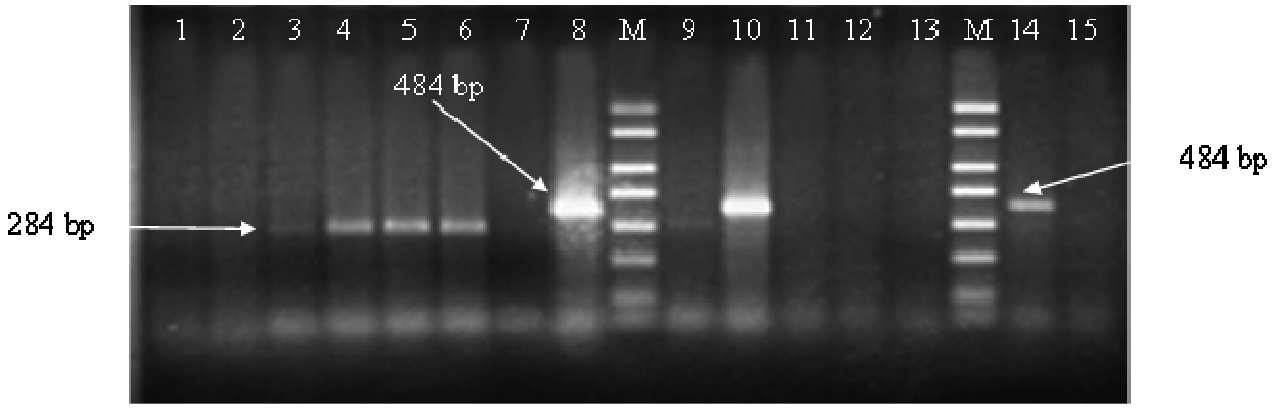

Figure 3a. Agarose gel electrophoresis showing amplification of 284 bp fragments of *invA* gene in lanes 3, 4, 5 and 6, while lanes 1, 2, 7, 11, 12, 13 and 15 show negative results. Lanes 8, 10 and 14 reveal amplification of 484 bp fragments of *sefA* gene specific for *Salmonella enteritidis*. Lane M shows PCR markers.

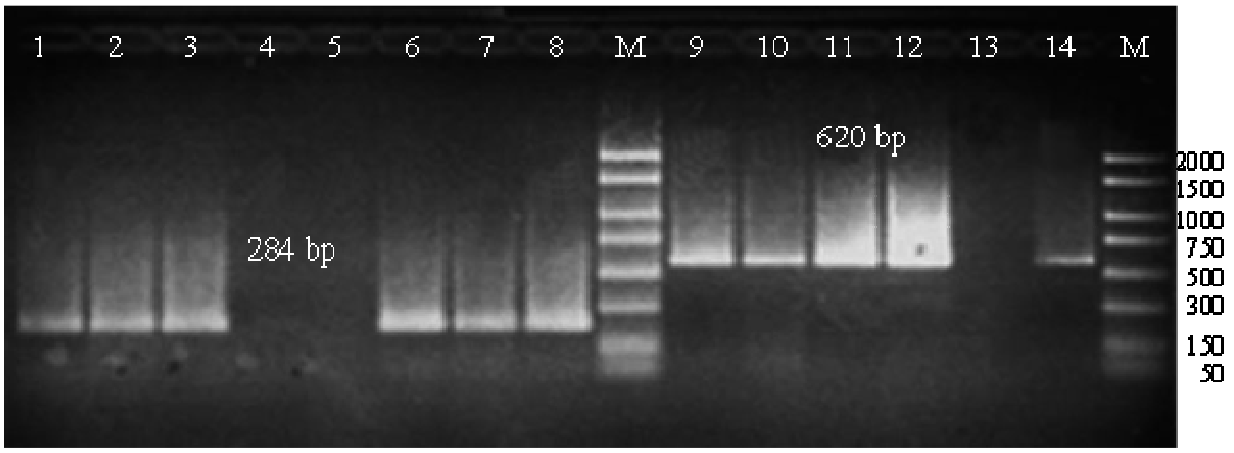

Figure 3b. Agarose gel electrophoresis showing amplification of 284 bp fragments of *invA* gene in lanes 1, 2, 3, 6, 7 and 8, while lanes 4, 5 and 13 show negative results. Lanes 9, 10, 11, 12 and 14 reveal amplification of 620 bp fragments of *fliC* gene specific for *Salmonella* Typhimurium. Lane M shows PCR markers.

consuming and laborious although control of the infection depends increasingly on the availability of rapid and precise diagnostic tests for monitoring. Therefore, the present study was aimed to investigate the sensitivity of PCR protocol in conjunction with selective enrichment in Rappaport Vassiliadis broth and compared with standard microbiological techniques using field chicken and chicken products collected from Riyadh, KSA. In the present study, the PCR produced positive amplification of 284 and 274 bp fragments of *invA* and *sdiA* genes (100%), specific for all members of *Salmonella* species, respectively, while all non *Salmonella* serovars (100%) were negative (Figures 1, 2a and 2b). These results were parallel to those obtained by Oliveira et al. (2002) and Malorny et al. (2003), who reported that 139 - 141 primers, which target the *invA* gene were able to identify all the examined *Salmonella* serovars, whereas all non *Salmonella* serovars gave negative results. Our results concluded that, all *Salmonella* carry the *invA* gene, which is not carried by any other bacterial species (Lin et al., 2007). Regarding detection at the serovar level, the PCR assay for the identification of *S. typhimurium* was very specific because it could amplify 620 bp fragments of *fliC* gene in all standard *S. typhimurium* strains, two isolated *S.* typhimurium and in none of the other *Salmonella* serovars (Figure 3a). These data support the work of Oliveira et al. (2002) who correctly identify all *S. typhimurium* strains but none of the strains from other *Salmonella* serovars. Moreover, PCR amplify 480 bp fragments of *sefA* gene in all standard *S. enteritidis* strains and 5 isolated strains of *S. enteritidis*; whereas, DNA from other *Salmonella* serovars and other bacterial genera did not produce any amplification product (Figure 3b). These results confirm the results of somatic serogrouping with polyvalent antisera. Moreover, PCR has several advantages over the slide agglutination test with polyvalent antisera, because serogrouping is not possible when *Salmonella* isolates lack O- antigen (rough strain) or lack

Table 5. Results of PCR with 4 sets of primers directed against 4 genes.

Types of samples	Origin	Number of samples	Results of PCR with 4 sets of primers directed against 4 genes			
			invA	*sdiA*	*sefA*	*fliC*
Frozen chickens	Local source	38	3	3	3	0
Frozen chickens	Imported source	62	4	4	2	0
Chicken cuts	Local source	30	3	3	1	2
Whole eggs(10/each)	Local source	22	1	1	1	0
Total	Local/ imported	152	11	11	7	2

Table 6. Comparison between SMT and PCR for detection of *Salmonella* serovars.

Types of samples	Origin	Number of samples	SMT		PCR	
			Number*	%	Number*	%
Frozen chickens	Local source	38	3	7.89	3	7.89
Frozen chickens	Imported source	62	3	4.83	4	6.45
Chicken cuts	Local source	30	3	10.0	3	10.0
Whole eggs(10/each)	Local source	22	0	0.00	1	4.54
Total	Local/ imported	152	9	5.92	11	7.23

both O and H antigen (Hoorfar et al., 1999).

The specificity and sensitivity of PCR assay combined with Rappaport- Vassiliadis (RV) selective enrichment broth (PCR-RV) for the detection of *Salmonella* species in the collected field samples were tested in this study. The same positive samples detected using SMT were also detected by PCR-RV. In addition, 2 excess positive results only were obtained by PCR-RV assay with a percentage of 5.92 and 7.23% for SMT andPCR-RV, respectively (Tables 5 and 6). The recorded results confirmed that the PCR-RV assay could detect more positive samples of *Salmonella* species than SMT; these results also confirm the finding of Oliveira et al. (2003) that the PCR test combined with RV selective enrichment is more sensitive in detecting *Salmonella* at genus level than bacteriological methods.

In conclusion, the PCR assay unequivocally proved to be a highly specific, sensitive and time saving method for detecting *Salmonella*. The combination of a routine PCR test in conjunction with SMT could be effective in providing a more accurate profile of the prevalence of *Salmonella* in poultry and poultry related samples.

REFERENCES

Aktas Z, Martin D, Kayacan CB, Diren S and Threlfall EJ (2007). Molecular characterization of Salmonella Typhimurium and Salmonella Enteritidis by plasmid analysis and pulsed-field gel electrophoresis. Inter. J. Antimicrob. Agent, 30: 541-545.

Altekruse SF, Cohen ML, Swerdlow DL (1999). Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Baggesen LS, Aarestrup FM (2002). Characterization of Salmonella enterica Serovar Typhimurium DT104 Isolated from Denmark and Comparison with Isolates from Europe and the United States. J. Clin. Microbiol. pp. 1581-1586.

Bhagwat AA, Lauer W (2004). Food borne outbreaks in raw product can be prevented. Food Quality, 11: 62-63.

D'Aoust JY (1994). *Salmonella* and the international food trade. Int. J. Food Microbiol. 24: 11-31.

Dimitrov T, Udo EE, Albaksami O, Kilani AA, Shehab MR (2007). Ciprofloxacin treatment failure in a case of typhoid fever caused by *Salmonella* enterica serotype Paratyphi A with reduced susceptibility to ciprofloxacin. J. Medical Microbiol. 56: 277-279.

Doran JL, Collinson SK, Clouthier SC, Cebula TA, Koch WH, Burian J, Banser PA, Todd ECD, Kay WW (1996). Diagnostic potential of sefA DNA probes to *Salmonella* enteritidis and certain other O-serogroup D1 *Salmonella* serovars. Mol. Cell. Probes, 10: 233-246.

Galan JE, Ginocchio C, Costeas P (1992). Molecular and functional characterization of the *Salmonella* invasion gene invA: homology of invA to members of a new protein family. J. Bacteriol. 174: 4338-4349.

Gatto AJ, Peter TM, Green J (2006). Distribution of molecular subtypes within *Salmonella* enterica serotype Enteritidis phage type 4 and *Salmonella* Typhimurium definitive phage type 104 in nine European countries, 2000-2004: results of an international multi-centre study. Epidemiol. Infect. 25: 1-8.

Hoorfar J, Baggesen DL, Porting PH (1999). A PCR-based strategy for simple and rapid identification of rough presumptive *Salmonella* isolates. J. Microb. Method 35: 77-84

Humphrey T (2002). Public-health aspects of Salmonella infection. In C. Way and A. Way, Editors, Salmonella in Domestic Animals, CABI Publishing, Oxon, UK. pp. 245-263.

ISO 6579 (2002). Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spescies. (4th ed.) International Organization for Standardization.

Konstantia H, Ioannis O, Maria L, Georgia M, Alkiviadis V, Adamantini K (2006). PCR detection of *Salmonella* spp. using primers targeting the quorum sensing gene sdiA. Int. Sci. 259: 201-207.

Lin CL, Chiu CH, Chu C, Huang YC, Lin TY, Ou JT (2007). A multiplex chain reaction method for rapid identification of *Citrobacter freundii* and *Salmonella* species, including *Salmonella* Typhi. J. Microbiol. Immunol. Infect. 40: 222-226.

Malorny B, Hoorfar J, Hugas M, Heuvelink A, Fach P, EllerbroekBunge C, Dorn C, Helmuth RL (2003). Interlaboratory diagnostic accuracy of a *Salmonella* specific PCR-based method. Int. J. Food Microbiol. 89:

241-249.
Oliveira SD, Rodenbusch CR, Rocha SLS, Canal CW (2003). Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. Appl. Microbiol. 36: 217-221.
Oliveira SD, Santos LR, Schuch DMT, Silva AB, Salle CTP, Canal CW (2002). Detection and identification of S*almonellas* from poultry by PCR. Vet. Microbiol. 87: 25-35.
Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ (2002). Typhoid fever. N. Engl. J. Med. 347: 17-82.
Rahn K, De Grandis SA, Clarke RC, McEwen, Gala´n JE, Ginocchio C, Curtiss III, R, Gyles CL (1992). Amplification of an invA sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol. Cell. Probes, 6: 271-279.
Sambrook J, Fritscgh EF, Meniates (1989). Molecular Colning: A laboratory manual. Vol. 1. Cold Spring Harbor Laboratory press, New York.
Sareyyupoglu B, Çelik AA, Cantekin AZ, Yard AH, Akan AM, Akçay A (2007). Polymerase Chain Reaction Detection of *Salmonella* spp. in fecal samples of pet birds. Avian Dis. 52(1): 163-167
Schlundt J (2002). New directions in foodborne disease prevention. Int. J. Food Microbiol. 78: 3-17.
Soumet C, Ermel G, Rose V, Rose N, Drouin P, Salvat G, Colin P (1999). Identification by a multiplex PCR-based assay of *Salmonella* Typhimurium and *Salmonella* Enteritidis strains from environmental swabs of poultry houses. Appl. Microbiol. 29: 1-6.
Stone GG, Oberst RD, Hays MP, McVey S, Chengappa MM (1994). Detection of *Salmonella* serovars from clinical samples by enrichment broth cultivation-PCR procedure. J. Clin. Microbiol. 32: 1742-1749.
Wang L, Shi L, Alam MJ, Geng Y, Li L (2008). Specific and rapid detection of foodborne *Salmonella* by loop-mediated isothermal amplification method. Food Res. Int. 41: 69-74.
Yu CY, Chou SJ, Yeh CM, Chao MR, Huang KC, Chang YF, Chiou CS, Weill F, Chiu CH, Chu CH, Chu C (2008). Prevalence and Characterization of Multidrug-Resistant (Type ACSSuT) *Salmonella enterica* Serovar Typhimurium Strains in Isolates from Four Gosling Farms and a Hatchery Farm. J. Clin. Microbiol. 46(2): 522-526.