Molecular characterization of *Salmonella* Typhimurium isolated from poultry

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Abstract

A total of 1500 *Salmonella* isolates were collected from chicken origin to detect ST. Twenty four ST isolates from chicken origin, one from duck, and one from infant diarrhea as well as standard ST strain (ATCC 14028) were investigated. PCR was applied among the collected ST strains using primers specific for STM4497 gene of ST. In the present study, RAPD analysis was used to establish serotype-specific markers for *Salmonella* serotypes, with emphasis on *Salmonella* Typhimurium. Three different randomly designed primers (23 L, P1245 & 9mer) were used in PCR. The SDS profile analysis of the extracted OMP of the examined ST isolates showing clearly that all isolates had 4 bands ranging in size 14-42kDa. Protein bands of 38 and 15 kDa appeared as major bands in 65% and 55% of the isolates, respectively. Similarly most of ST isolates had bands at 39, 37 and 36 (50% each). It is clear that ST from human, duck and chicken isolates showed similarity in the OMP profile analysis.

Key words: *Salmonella, Salmonella Typhimurium, RAPD, OMP, SDS.*

Introduction

Salmonellosis is one of the most widespread food-borne zoonoses in industrialized as well as developing countries (**Molla *et al*., 2003**). Foods of animal origin, especially poultry and meat, are the major vehicles for the transmission of human salmonellosis due to cross-contamination events or inadequate cooking (**Capita *et al*., 2007**). According to the RASFF 2009 (Rapid Alert System for Food and Feed) annual report, *Salmonella* is the most frequently notified microorganism, notifications relating to poultry (approximately 70) and to red meat (50) being the most common (**European Commission, 2010** and **Àlvarez-Fernández *et al*., 2012**)

The genus *Salmonella* consists of around 2200 serotypes based on their antigens (**Brenner, 1984**). Due to the large number of *Salmonella* serotypes, work towards finding DNA markers for general use in detecting *Salmonella* serotypes appears to be a challenge, especially when studies are based on methods that rely on the random amplifica-tion of DNA fragments. Thus, reports on the application of RAPD analysis for finding detection markers for *Salmo-nella* serotypes are rather limited (**Miyamoto *et al*., 1998**). Randomly amplified polymorphic DNA (RAPD) analysis, also known as arbitrarily primed-polymerase chain reaction (AP-PCR), is a PCR-based assay that has been developed to detect polymorphisms in genomic DNA (**Welsh and McClelland 1990; Williams *et al.* 1990**) The technique relies on the presence of priming sites on the genome, close enough to permit PCR amplification using single primers of arbitrary nucleotide sequence. The aim of our study is to characterize *Salmonella* Typhimurium isolated from poultry in Egypt.

Materials and Methods

A total of 1500 suspected *Salmonella* isolates were submitted to the central laboratory for veterinary quality control on poultry production-Dokki-Egypt to detect *Salmonella* Typhimurium from avian sources identified biochemically according to (**Quinn *et al*., 2002**) and serologically by (**Popoff, 2001**). Extraction of DNA by QIAGEN kits for bacterial DNA extraction.

Table (1). Primers sequences used for amplification of DNA for the detection of *Salmonella* Typhimurium **(Kim *et al*., 2006a)**

Primer	Target gene	Nucleotide sequence (5' - 3')	Amplification product (bp)
STM4497-f	STM4497	AACAACGGCTCCGGTAATGA	310
STM4497-r3		TGACAAACTCTTGATTCTGA	

Table (2). Primers used in RAPD-PCR.

Name of primer	Sequence of primer	Reference
P1254	5'CCGCAGCCAA3'	**Salehi *et al*. (2009)**
23L	5'CCGAAGCTGC3'	
9 mer	5'CGT GCA CGC3'	**Görakan *et al*. (2008)**

STM4497 gene: (Kim *et al*., 2006 a): An initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 45 s, annealing at 63 ° C for 30 s, 72°C for 30 s, and finishing with a final extension at 72°C for 3 min and storage at 4°C.

In 23L, P1254 gene: (Athena *et al*., 1996): Four cycles of 94 °C for 4 min, 35 °C for 4 min, and 72 °C for 4 min: 30 cycles of 94 °C for 30 s, 35 °C for 1 min, and 72 °C for 2 min; and 1 cycle of 72 °C for 5 min.

In 9 mer gene: (Görakan *et al*., 2008): 1x90 °C , 5 min; 35 x (89 °C, 1 min; 28 °C, 1 min; 72 °C, 1.5 min); and 1 x 50 °C , 3 min. Buffers and reagents used for agarose gel electrophoresis (**Sambrook *et al.*, 1989**).

Materials used for SDS-PAGE analysis: Media and reagents used for outer membrane protein extraction of *Salmonella* Typhimurium isolates: (**Nurminen *et al*., HYPERLINK "http://ps.fass.org/content/87/1/32.full" 1985**). Estimation of protein content of antigen, protein content of antigenic material was measured using the modified (**Lowry *et al*., 1951)** method. All chemicals were purchased from Sigma Chemicals Company. SDS-PAGE of different ST isolates **(Laemmli., 1970)**

Resaults:

1. Confirmation of *Salmonella* Typhimurium isolates.

Among 1500 investigated *Salmonella* isolates, 26 isolates were confirmed to be *Salmonella* Typhimurium. The source of the isolates was 7 from paper lining chick box, 1 from paper lining duckling box, 7 from slaughtered chicken, 1 from frozen chicken, 1 from food products (burger), 1 from infant diarrhea and 7 from chicken internal organs (liver, cecum). The 26 ST isolates were confirmed biochemically by APi 20E. (BIOMÈRIEUX) (photo:1) and serologically using poly and monovalent *Salmonella* antisera (photo:2). As well as by PCR using STM4497 primers specific for ST (photos 3,4).

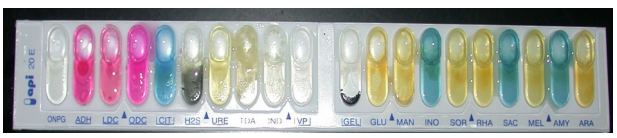

Photo (1). S*almonella* by API 20E.

Photo (2). slide agglutination test for *Salmonella* isolates

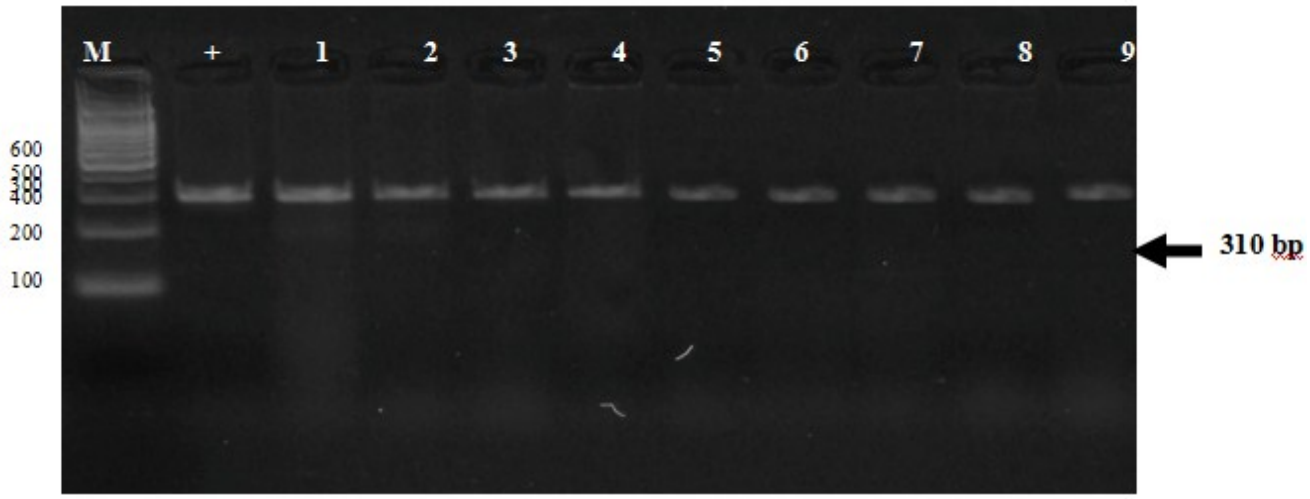

Photo (3). Agarose gel electrophoresis showing positive amplification of 310 bp fragment using STM4497-f, STM4497-r3 primers specific for STM4497 gene of ST isolates No: 1, 2, 3, 4, 5, 6,7,8 and 9. Lanes: 1-9 ST isolates. +: ST reference strain ATCC 14028
M: molecular weight marker 100bp ladder.

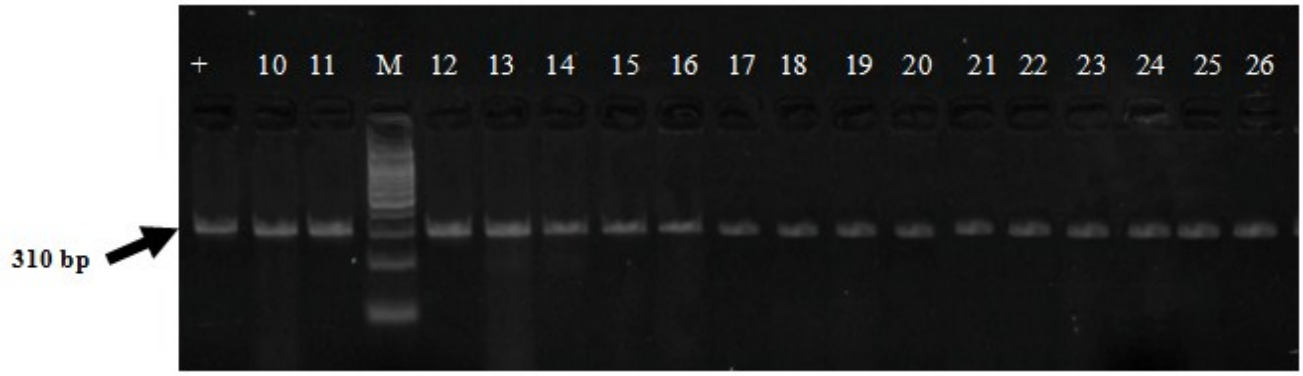

Photo (4). Agarose gel electrophoresis showing positive amplification of 310 bp fragment using STM4497-f, STM4497-r3 primers specific for STM4497 gene of the examined ST isolates No. 10, 11, 12, 13, 14, 15, 16,17,18,19, 20, 21, 22, 23, 24, 25 and 26.
Lanes: 10- 26 ST isolates. +: ST reference strain ATCC 14028
M: molecular weight marker 100bp ladder.

2. Results of RAPD-PCR of *Salmonella* Typhimurium isolates using.

2.1. 23L primer:

The data illustrated in Table (3) and photos (5 &6) shows 5 patterns: Isolates number 3, 11, 12, 13, 15, 16 and the standard reference strain ATCC 14028 produced 3 amplicons ranging from 800-1977 bp, Isolates number 14, 23, 24 and 26 produced 3 amplicons ranging from 811 -2193 bp, Isolates number 1, 2, 4, 5, 10, 17 and 24 produced 3 amplicons ranging from 709-1829 bp, Isolates number 6, 7, 19 and 20 produced 2 amplicons ranging from 727-1877 bp, Isolates number 8 and 9 produced 2 amplicons ranging from 854-1326 bp. On the other hand isolates number 18, 21 and 22 could not reacted with 23L primer.

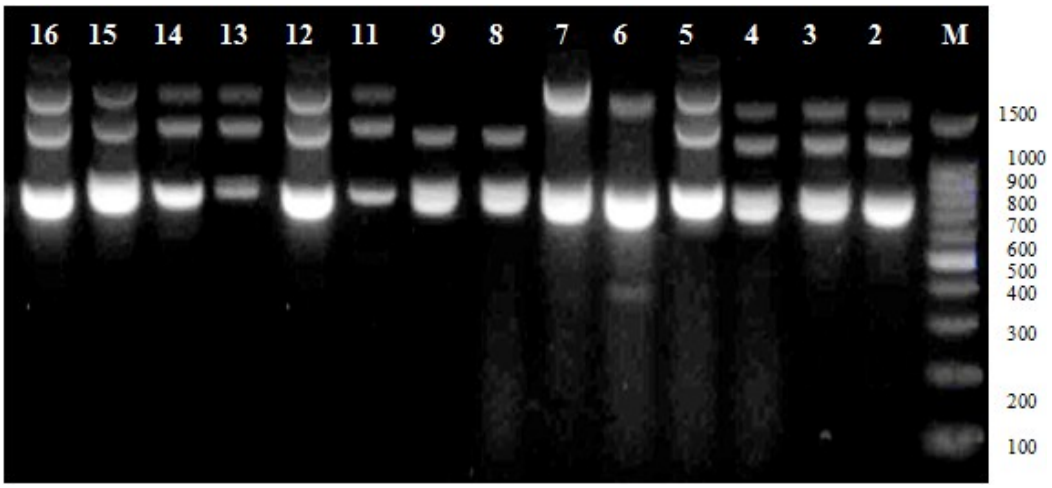

Photo (5). RAPD band patterns generated with 23L primer from ST isolates numbers 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15 and 16.
Lanes: 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15 and 16 show ST isolates.
M: DNA ladder marker 100 bp plus.

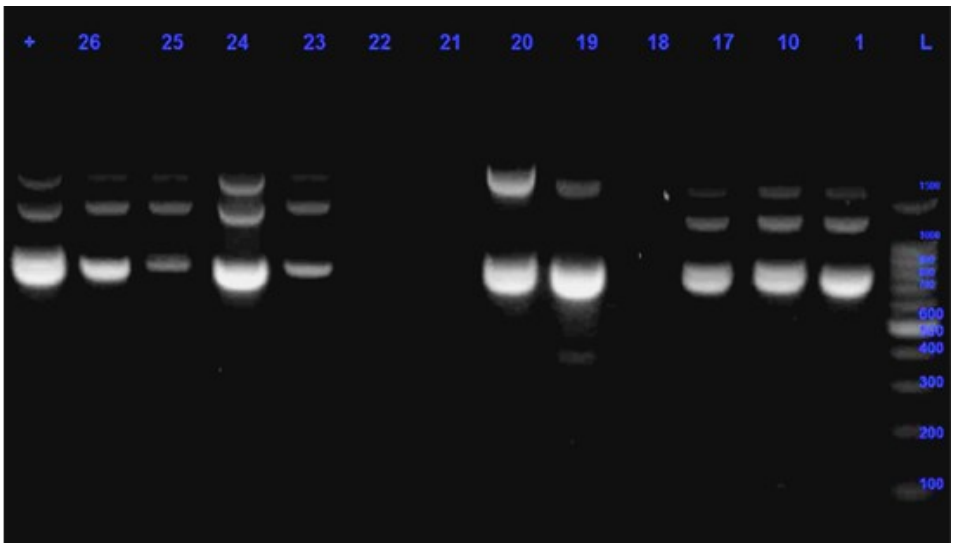

Photo (6). RAPD band patterns generated with 23L primer from ST isolates numbers 1, 10, 17, 18, 19, 20, 21, 22, 23, 24, 25, and 26
Lanes: 1, 10, 17, 18, 19, 20, 21, 22, 23, 24, 25,and 26 show ST isolates.
+: ST reference strain ATCC 14028
M: DNA ladder marker 100 bp plus.

Table (3). RAPD-PCR profiles analysis of ST isolates by 23L primer.

Isolate number	Source of isolates	Bands/bp		
1	Slaughtered chicken	1752	1199	709
2		1684	1261	733
3		1684	1252	800
4		1684	1261	733
5		1709	1270	779
6		1709		727
7	Paper lining chick box	1785		743
8		1326		860
9		1326		854
10	Human	1722	1209	732
11	Food (Burger)	1919	1426	843
12	Frozen chicken	1864	1405	849
13	Paper lining chick box	1891	1426	871
14		2004	1426	827
15		1850	1375	894
16	Chicken liver	1772	1355	816
17		1752	1199	741
18				
19	paper lining Ducklings box	1861		727
20	Paper lining chick box	1877		746
21	Chicken Liver			
22				
23	Slaughtered Chicken	2155	1539	811
24	Frozen Chicken	1829	1329	751
25	Chicken cecum	2118	1487	851
26		2193	1526	828
Reference strain	ATCC 14028	1977	1388	811

2.2. P1245 primer:

Table (4) and photos (7 & 8) illustrated that using P1245 primer isolates number 12 & 26 produced two bands at 523-652 & 832-905 bp, isolates number 2, 4 and 7 produced 3 bands at 570-654, 905-1183 & 1735-1873 bp; Isolate number 23 & 25 produced 3 bands at 320, 652 and 905 bp; isolates number 1, 3, 5, 8, 9, 10, 13, 14, 15, 16, 17 and the standard reference strain ATCC 14028 produced 4 bands at 253-323, 500-543, 758-946 and 1632 -2071 bp; isolates number 6, 11, 19 and 20 produced 5 bands at 284-323, 445-541, 791-894, 1383-1800 and 1735-2326 bp, while isolate number 24 produced 6 bands at 317, 641, 868, 1253, 1392 and 1907 bp. Isolate number 18, 21 and 22 could not produce amplicons.

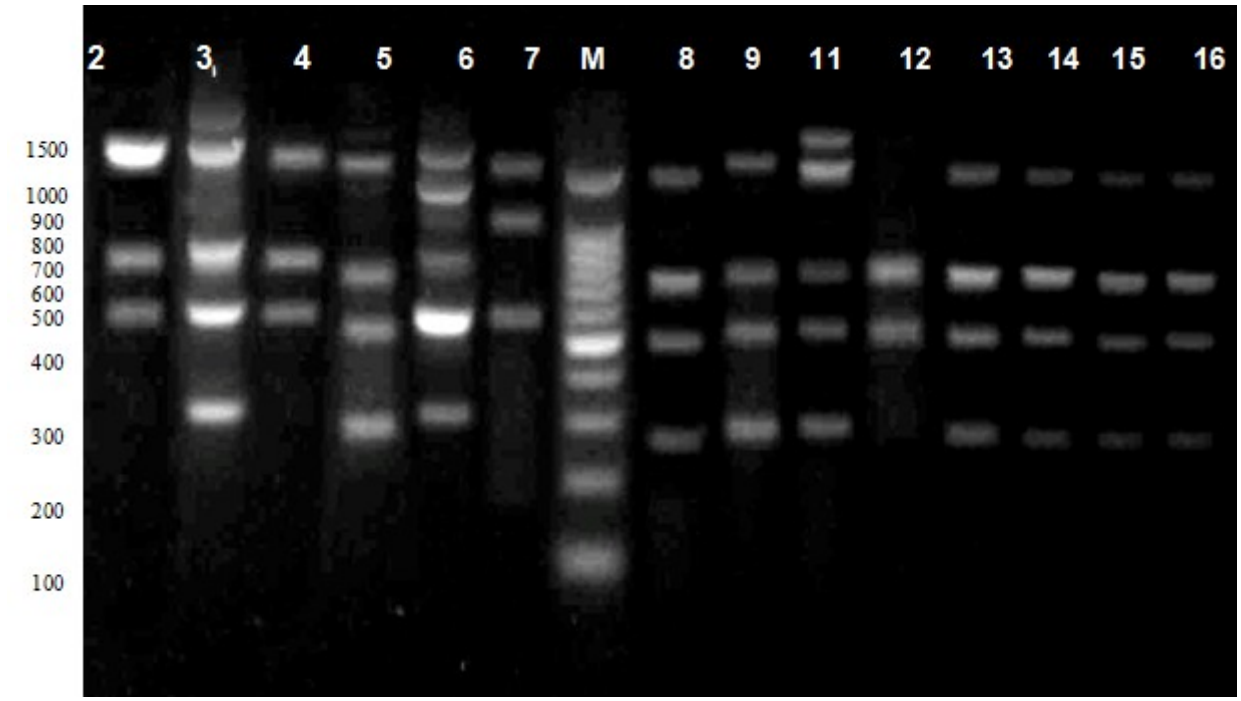

Photo (7). RAPD band patterns generated with P1245 primer from ST isolates numbers 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15 and 16. Lanes: 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15 and16 show ST isolates. M: DNA ladder marker 100 bp plus.

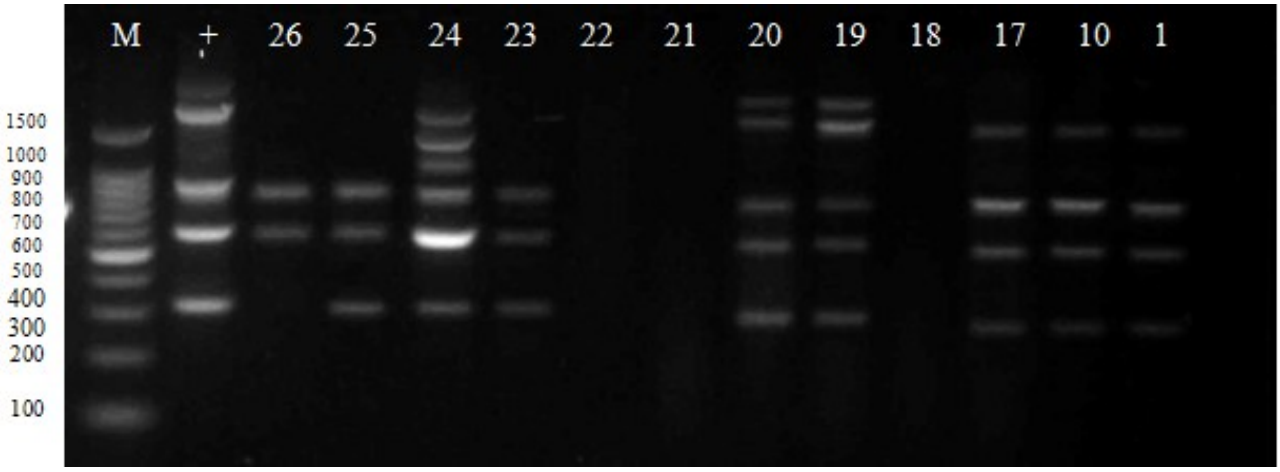

Photo (8). RAPD band patterns generated with P1245 primer from ST isolates numbers 1, 10, 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26. Lanes: 1, 10, 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26 show ST isolates.
+: ST reference strain ATCC 14028 M: DNA ladder marker 100 bp plus.

Table (4). RAPD-PCR profiles analysis of ST isolates by P1245 primer.

Isolates number	Source of isolates	Bands/bp							
1	Slaughtered chicken		1670				758	500	255
2			1873			919		654	
3			1961			929		543	323
4			1858			919		601	
5			1748				832	534	306
6			1735	1383			894	541	323
7	Paper lining chick box		1735		1183			570	
8			1632				788	510	268
9			1844				843	523	292
10	Human		1670				775	513	253
11	Food (Burger)	2084	1682				821	521	310
12	Frozen chicken						832	523	
13	Paper lining chick box		1670				816	510	285
14			1657				811	510	285
15			1632				788	500	276
16	Chicken liver		1657				788	510	276
17			1698				775	513	271
18									
19	Paper lining Ducklings box	2269	1727				791	445	284
20	Paper lining chick box	2326	1800				783	530	284
21	Chicken Liver								
22									
23	Slaughtered Chicken					905		652	320
24	Frozen Chicken		1907	1392	1253		868	641	317
25	Chicken cecum					905		652	320
26						905		652	
Reference strain	ATCC 14028	2071				946		652	320

3. 9 mer primer:

As shown in Table (5) and photos (9 & 10) 6 patterns could be detected among ST isolates using 9 mer primer, isolates number 15, 16 and the standard reference ST strain (ATCC14028) produced one band at 587-658 bp, isolates number 13 & 14 produced 2 bands at 811 & 636 bp; isolates 8, 9, 11, 12 and 20 produced 2 bands at 1044- 1402 and 647-873 bp; isolates 1,4, 5, 10, 17, 18, 19, 21, 22, 23, 24, 25 and 26 produced 3 bands at 1029-1171, 629-910 and 479-529 bp; isolate number 2 produced 4 bands at 672, 535, 391 and 294; isolates number 3 and 6 produced 4 bands at 1022-1831, 828-863, 671-676 and 529 bp while isolate number 7 produced 5 bands at 1000, 616, 519, 391 and 261 bp.

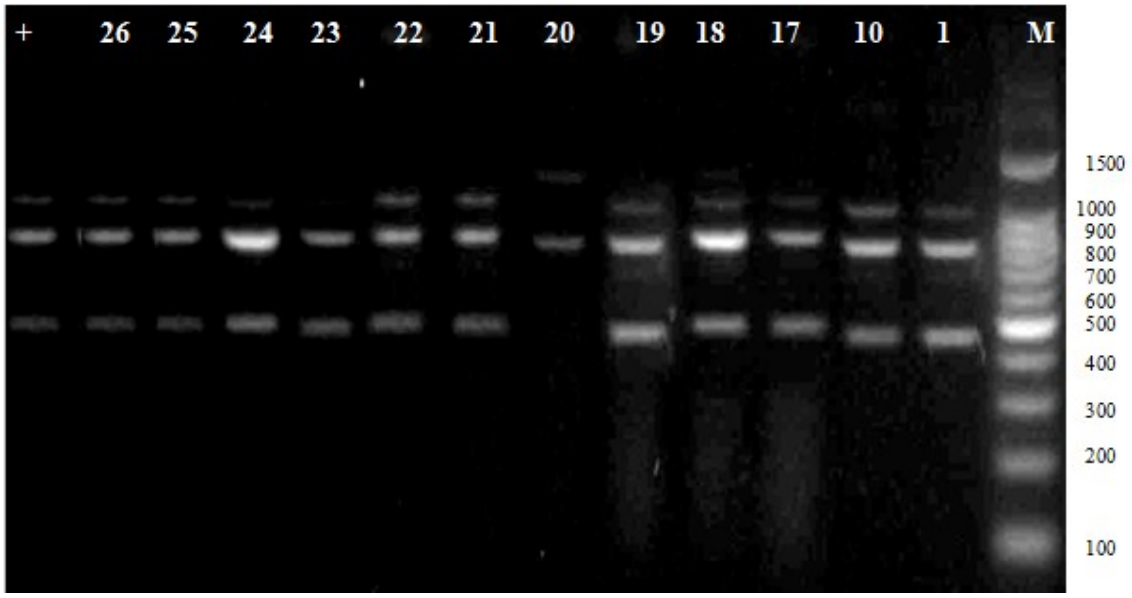

Photo (9). RAPD band patterns generated with 9mer primer from ST isolates numbers 1, 10,17, 18, 19, 20, 21, 22, 23, 24, 25 and 26
Lanes: 1, 10,17, 18, 19, 20, 21, 22, 23, 24, 25 and 26 show ST isolates.

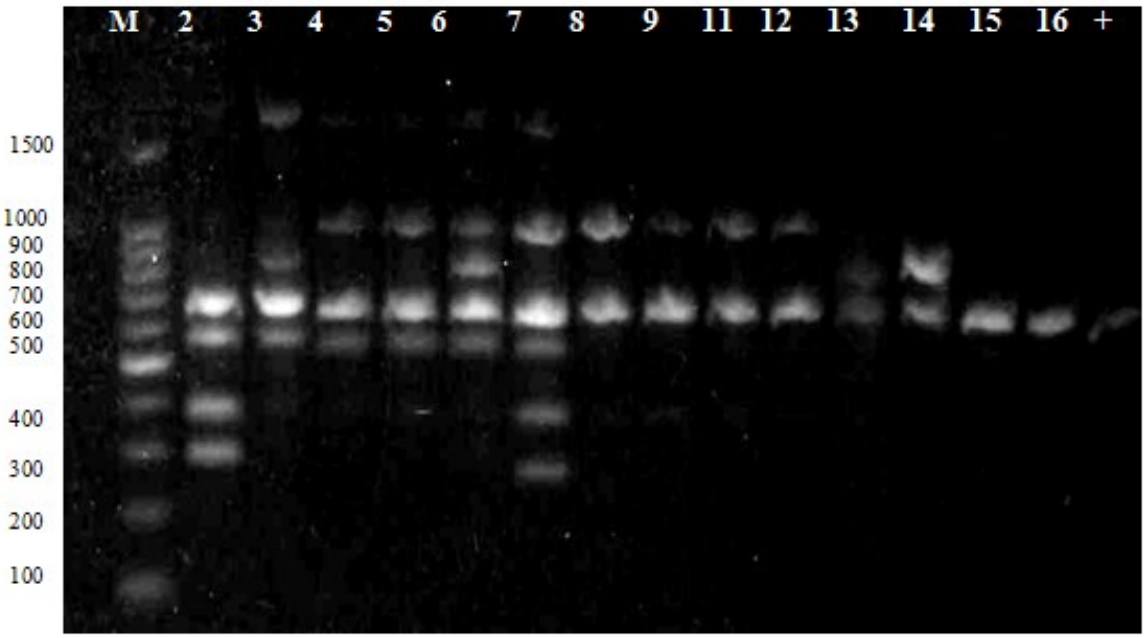

Photo (10). RAPD band patterns generated with 9mer primer from ST isolates numbers 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15 and 16 Lanes: 2 , 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15 and 16
+: ST reference strain ATCC 14028 M: DNA ladder marker 100 bp plus.

Table (5). RAPD-PCR profiles analysis of ST isolates by 9mer primer.

Samples	Source of isolates	Bands/bp							
1	Slaughtered chicken	1078		835			479		
2					672	535		391	294
3		1831		863	676	529			
4		1036			652	529			
5		1029			629	529			
6		1022		828	671	529			
7	Paper lining chick box	1000			616	519		381	261
8		1022			658				
9		1044			679				
10	Human	1078		841			480		
11	Food (Burger)	1036			647				
12	Frozen chicken	1044			668				
13	Paper lining chick box			806	636				
14				811	658				
15						587			
16	Chicken liver				606				
17		1136		894		500			
18		1136		878		500			
19	Paper lining Ducklings box	1078		856			480		
20	Paper lining chick box	1402		873					
21	Chicken Liver	1171	906			500			
22		1171	906			523			
23	Slaughtered Chicken	1145		889		500			
24	Frozen Chicken	1136		878		518			
25	Chicken cecum	1171	906			515			
26			910			519			
Reference strain	ATCC 14028				658				

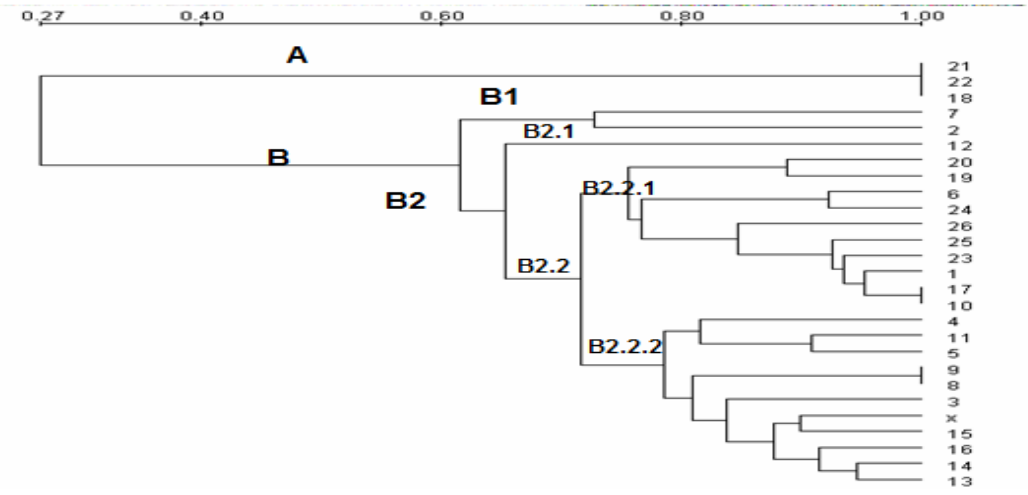

Fig. (1). Dendogram showing RAPD patterns and relationship of 26 ST Isolates and reference strain ATCC 14028 by using of 3 primers (23L, p1245, 9mer)

2. Results of outer membrane protein using SDS-PAGE among *Salmonella* Typhimurium isolates.

The SDS profile analysis of the extracted OMPs of the examined ST isolates illustrated in Table (6) and photos (11-14). It is clear that all isolates had 4 bands ranging in size from 42-14 kDa. Protein bands of 38 and 15 kDa appeared as major bands in 65% and 55% of the isolates respectively. Similarly most of ST isolates had band at 39, 37 and 36 kDa(50% each). It is clear that ST human, duck and chicken isolates showed similarity in the OMP profile analysis.

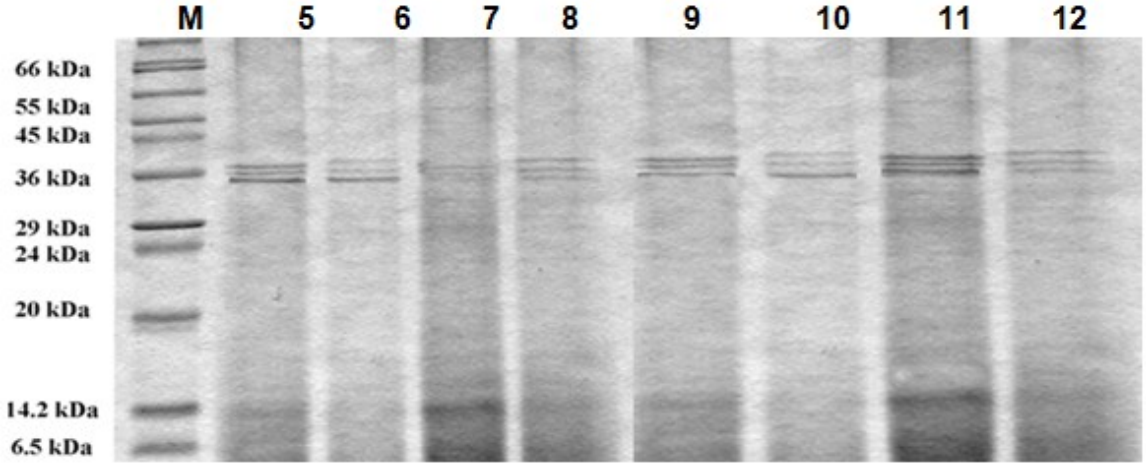

Photo (12). Outer membrane protein profile patterns of ST isolates Number 5, 6, 7, 8, 9, 10, 11 and 12.
M: Protein marker. Lanes 5-12: ST isolates.

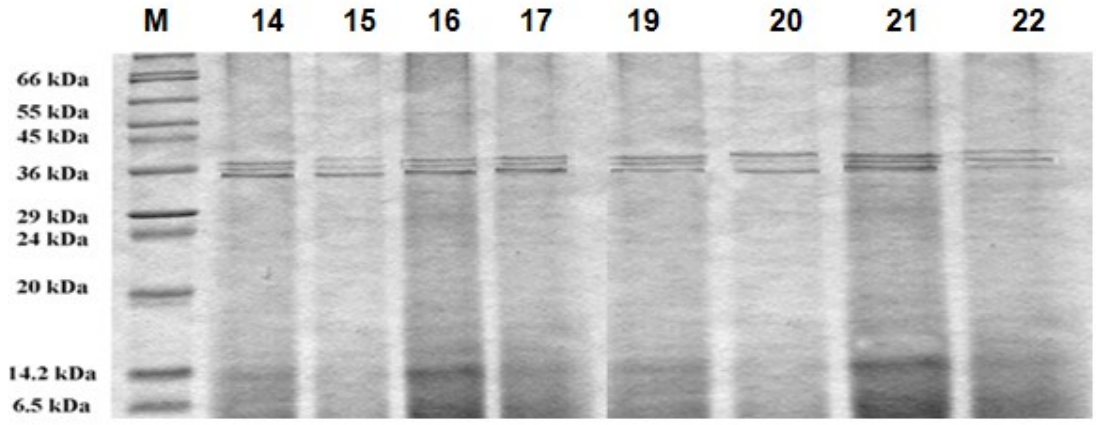

Photo (13). Outer membrane protein profile patterns of ST isolates 14, 15, 16, 17, 19, 20, 21 and 22.
M: Protein marker. Lanes 14, 15, 16, 17, 19, 20, 21 and 22: ST isolates.

Photo (14). outer membrane protein patterns of ATCC 14028
M: protein marker

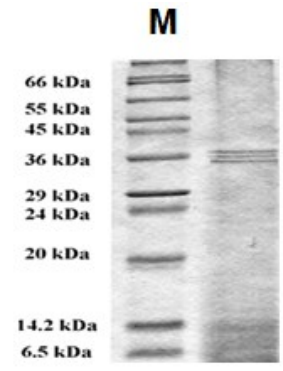

Table (6). SDS PAGE outer membrane protein analysis for ST isolates.

Isolates number	Source of isolate	Protein bands/KDa			
1	Slaughtered chicken	38	37	35	14
2		39	37	35	14
3		38	37	35	14
4		39	37	36	15
5		38	36	35	14
6		39	37	35	14
7	Paper lining chick box	38	36	35	14
8		39	37	35	14
9		39	38	36	15
10	Human	40	38	36	15
11	Food (Burger)	40	38	36	15
12	Frozen chicken	40	38	37	15
14	Paper linsing chick box	38	37	35	14
15		39	37	35	14
16	Chicken liver	39	38	36	15
17		40	38	36	15
19	Paper lining Ducklings box	40	38	36	15
20	Paper lining chick box	41	39	36	15
21	Chicken Liver	41	39	37	15
22		42	39	38	15

Discussion

Salmonellosis is the most important foodborne disease, in both animals and man (**Brenner *et al*., 2000**), causing over 1400 human cases a year in the USA (**Mead *et al*., 1999**). *Salmonella enterica* is a zoonotic species that can acquire its resistance in livestock. The resulting animal food products are important vectors for the transfer of resistant bacteria from animals to humans (**Majtaá-novaá *et al*., 2010**).

Salmonella enterica is a leading cause of human gastroenteritis in both developed and developing countries, causing millions of human and animal illnesses and significant economic losses world□wide (**Alvarez-Fernandez *et al*., 2012**). In the European Union, the reported incidence of *Salmonella* in-fections in 2009 was 23.7 per 100,000 people, with a total of 108,614 confirmed cases, this being the second most often reported zoonotic disease in humans, following campylobacteriosis (**EFSA, 2011**). There are several routes for contracting salmonellosis, but the con-sumption of contaminated foods is by far the most frequent cause of human infections (**Lestari *et al*., 2009**). During 2009, *Salmonella* was responsible for 1722 outbreaks of food-based infection (31.0% of all reported food-borne outbreaks and 33.2% of all verified outbreaks) in the EU, remaining the most commonly recognized causative agent in the food-borne outbreaks reported. *S. enterica* serotype Enteritidis and *Salmonella* Typhimurium were the predominant sero-types associated with these infections (59.6% and 15.7% of the out-breaks, respectively) (**EFSA, 2011**).

In the present study a total of 1500 *Salmonella* isolates were collected from avian origin to detect ST between 2009-2011. Twenty four ST isolates from avian origin, one from duck, and one from infant diarrhea as well as standard ST strain (ATCC 14028) were investigated. All ST isolates were confirmed to be salmonellae biochemically by API 20E as shown in photo (2) and serologically using poly and mono valent antisera photo (3). Serotype identification is necessary for investigations of foodborne outbreaks and provides the examination of the overall changes in the prevalance of a particular serotype. *Salmonella* Enteritidis and *Salmonella* Typhimu-rium are the 2 most common *Salmonella* food-poisoning serotypes

in the United Kingdom (**Plummer *et al*., 1995**). It was recently reported that most of the clinical isolates of foodborne diseases from different hospitals in Ankara, Turkey, were *Salmonella* Typhimurium (**Yildirmak *et al*., 1998**). Although serotyping has shown that *Salmonella* Enteritidis displaced *Salmonella* Typhimurium as the most frequently isolated serotype; the identification of *Salmonella* Typhimurium serotype is still important in some countries (**Eley, 1996**).

Since genetic differences between isolates may not encode differences in phenotypic markers (i.e. phage types, antigens, enzymes or isozymes, antimicrobial susceptibility, or metabolic profiles), phenotypic techniques have limited utility for sub-typing of bacterial isolate (**Gürakan *et al*., 2008**). As subtypes must, by definition, differ in DNA, genotype subtyping approaches are characterized by the greatest discriminatory power (**Lin *et al*. 1996**). A variety of DNA-based typing methods has been applied to characterize *Salmonella* species (**Schmidt 1994; Farber 1996**). Among these are ribotyping and random amplified polymorphic DNA (RAPD). The application of random amplified polymorphic DNA (RAPD) analysis (**Williams *et al*., 1990; Caetano-Anolles *et al*., 1991**) based on the random amplification of genomic DNA fragments through short arbitrarily designed primers is an attractive alternative for the detection and identification of microor-ganisms (**Meunier and Grimont, 1993; Cave *et al*., 1994; Stephan, 1996**), especially where previous sequence information is not available. Random amplified polymorphic DNA analysis has the potential to detect polymorphism throughout the entire genome as com-pared to other techniques. It also allows one to start a blind walk through the genomic DNA of an organism and to possibly discover regions of interest that would otherwise not be easily established (**Gürakan *et al*., 2008**). Furthermore, RAPD is more suitable than other techniques, because it does not require prior knowledge of target DNA and it uses short (9 to 10 bases) primers with a small amount of starting DNA.

In the present study, RAPD analysis was used to establish serotype-specific markers for *Salmonella* serotypes, with emphasis on *Salmonella* Typhimurium, due to its impor-tance in causing diarrhea. Three different randomly designed primers (23 L, P1245 & 9mer) were used in PCR in the presence of ST DNA. Tables (3-5) and photos (5-10) summarized the RAPD pattern for ST strains. It could be concluded that 23L primer produced amplicons ranging from 727-2193 bp, P1245 primer produced amplicons ranging from 253-2326 bp and 9mer primers produced amplicons ranging from 261-1171 bp. Study of (**Smith *et al*., 2011**) revealed that the use of the primers 784 and 1254 did not produce any discrimination amongst the isolates as a lot of isolates did not show any amplification band even after four PCR repeats. This result is different from that obtained by **Tekeli *et al***. (**2006**) and **Quintaes *et al.*** (**2002**); using primers 1254 and 784 respectively for *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhi. In fact primer 784 by **Quintaes *et al.,*** (**2002**) gave the highest discrimination amongst their human *S*. Typhi isolates from Brazil. Reproducibility of RAPD fingerprints was confirmed for each primer by comparison between profiles obtained in three different, analyses (**De Cesare *et al*., 2001**).

Our results show in Table (5) and photos (9 & 10) 6 patterns could be detected among ST isolates using 9 mer primer, isolates number 15, 16 and the standard reference ST strain (ATCC14028) produced one band at 587-658 bp, isolates number 13 & 14 produced 2 bands at 811 & 636 bp; isolates 8, 9, 11, 12 and 20 produced 2 bands at 1044- 1402 and 647-873 bp; isolates 1,4, 5, 10, 17, 18, 19, 21, 22, 23, 24, 25 and 26 produced 3 bands at 1029-1171, 629-910 and 479-529 bp; isolate number 2 produced 4 bands at 672, 535, 391 and 294; isolates number 3 and 6 produced 4 bands at 1022 -1831, 828-863, 671-676 and 529 bp while isolate number 7 produced 5 bands at 1000, 616, 519, 391 and 261 bp. All *Salmonella* Typhimurium isolates used by **Gürakan *et al*., 2008**) gave strong amplification product at about 700

bp, an additional band at about 300 bp, and rarely faint bands at 800, 900, and over 1,000 bp in size. They concluded that a 700- amplification product was found as a specific polymorphic region for *Salmonella* Typhimurium, and this band can be used as a specific marker for differentiation of *Salmonella* Typhimurium from the other serotypes.

Table (3) and photos (5 & 6) show examined isolates in this study number 1, 2, 3, 4, 5, 10, 11, 12, 13, 14, 15, 16, 17, 23, 24, 25, 26 and the standard strain produced 3 bands at 709-894, 1199-1684 and 1709-2193 bp with 23L primer, while isolates number 6, 7,8 ,9 ,19 and 20 produced 2 bands at 1326-1877 and 727-860 bp. On the other hand isolates number 18, 21 and 22 could not reacted with 23L primer using RAPD PCR. Among P1245 primer Table (4) and photos (7 & 8) illustrated that using P1245 primer isolates number 12 & 26 produced two bands at 523-652 & 832-905 bp, isolates number 2, 4 and 7 produced 3 bands at 570-654, 905-1183 & 1735-1873 bp; isolates number 23 & 25 produced 3 bands at 320, 652 and 905 bp; isolates number 1, 3, 5, 8, 9, 10, 13, 14, 15, 16, 17 and the standard reference strain ATCC 14028 produced 4 bands at 253-323, 500-543, 758-946 and 1632-2071 bp; isolates number 6, 11, 19 and 20 produced 5 bands at 284-323, 445-541, 791-894, 1383-1800 and 1735-2326 bp, while isolate number 24 produced 6 bands at 317, 641, 868, 1253, 1392 and 1907 bp. Isolates number 18, 21 and 22 could not produce amplicons.

In RAPD analysis, although the P-1245 primer generated a more monotonous DNA profile than that associated with the OPB-17 primer, this primer allowed for dis-crimination into only 18 genotypes, and evidenced a far lower DA (SID; 0.941) than did the OPB-17 pri-mer (SID; 0.946) (**Woo and Lee, 2006**)

Outer membrane proteins (OMPs) of pathogenic bacteria exhibit immunogenic and virulent properties and have been investigated for diagnostic application of monoclo-nal or polyclonal antibodies (**Udhayakumar and Muthukuruppan, 1987; Kerr *et al.* 1992; Charles *et al.*, 1994**). OMP profiles of many bacterial species have been characterized using SDS-PAGE (**Barenkamp *et al.* 1981; Buchanan and Hildebrandt, 1981** and **Achtman *et al.*, 1983**). The SDS profile analysis of the extracted OMPs of the examined ST isolates illustrated in Table (6) and photos (11-14). It is clear that all isolates had 4 bands ranging in size 42-14 KDa. Protein bands of 38 and 15 kDa appeared as major band in 65% and 55% of the isolates respectively. Similarly most of ST isolates had band at 39, 37 and 36 (50% each). It is clear that ST human, duck and chicken isolates showed similarity in the OMP profile analysis.

Kim *et al.* (**2006 b**) detected 53.7, 51.3 & 48.7 kDa from the extracted ST OMP. SDS-electrophoretic analysis of the outer membrane proteins (OMP) revealed common antigen protein bands, especially between *S.* Typhimurium and *S.*Enteritidis, all serovars showed intense protein bands in the range from 20 to 45 kDa (**Zoo El-Fakar and Rabie, 2009**). The analysis of isolated OMPs by **Hamid and Jain (2008**) using SDS-PAGE showed a complex electrophoretic profile having more than 15 proteins with molecular masses be-tween 15 and 100 kDa, the molecular masses of 14 main proteins were calculated to be 78, 75, 70, 68, 65, 55, 49, 43, 37, 35, 32, 30, 25, and 15 kDa.

The only identifiable differences in migrational patterns of six *Salmonella* OMP extracts from SDS-PAGE were found by **Kim *et al.* (2006b)** between 48 and 54 kDa on the gel, the patterns were largely classified into two groups: three major OMP bands (Typhimurium, Enteritidis, Hadar and Brandenburg) and two major OMP bands (Seftenberg and Thomasville). These results are consistent with published data by (**Helmuth *et al.*, 1985; Kerr *et al.*, 1992**). A 37 kDa protein was found to be present in all septicemic isolates and in most isolates, from clinically healthy animals and might be considered for vaccine production given its high immunogenicity (**Bergeron *et al.*, 2010**).

OMP pattern analysis of *Salmonella* species revealed a high level of homogenicity between ST serotypes (**Kim *et al.*, 2006b**). **Helmuth *et al.*** (**1985**) reported that the similarit-ies of *Salmonella* OMP profiles might be attributed to

high development of the *Salmonella* serology. This phenomenon was confirmed by **Malik *et al.*** (**1999**).

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التوصيف الجزيئى للسالمونيلا التيفيموريم المعزولة من الدواجن

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الملخص العربى

في هذه الدراسة تم تجميع عدد 1500 معزولات من السالمونيلا من أصل داجنى للكشف عن السالمونيلا تيفيموريم. وقد تم التعرف على أربع و عشرين معزولة سالمونيلا تيفيميوريم من الدجاج، ومعزولة من البط، بالإضافة إلى معزولة من إسهال الرضع، والعترة المرجعية (ATCC 14028). وقد تم تصنيف معزولات السالمونيلا تيفيموريم كيميائيا(API (20 E و سيرولوجيا. وكذلك باستخدام اختبار تفاعل البلمرة المتسلسل (PCR). وتم توصيف هذه العترات باستخدام اختبار RAPD-PCR باستخدام ثلاثة أنواع مختلفة من البادئات (23L، P1245 ، 9mer). و سجلت النتائج نسبة التشابه والاختلاف بين المعزولات. وتم أيضا التحليل الكهربائى لبروتينات الغشاء الخارجى لمعزولات السالمونيلا تيفيموريم وأثبتت النتائج أن جميع المعزولات أنتجت 4 أوزان تتراوح في حجمها من 42-14 كيلو دالتون. وتم تسجيل نسبة 65٪ و 55٪ من المعزولات تحتوى الحزم البروتينة 38 و 15 كيلو دالتون على التوالي. فمن الواضح أن السالمونيلا تيفيموريم المعزولة في الإنسان، والبط والدجاج أظهرت تشابها فى التحليل الكهربائى لبروتينات الغشاء الخارجى.