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Vol. 9(9), pp. 651-661, 4 March, 2015
DOI: 10.5897/AJMR2014.7292
Article Number: 4318F9451093
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Molecular typing of *Salmonella enterica* serovars Typhimurium and Enteritidis isolated from Taif area of Saudi Arabia by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)

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Received 21 November, 2014; Accepted 9 February, 2015

Twenty three isolates of *Salmonella enterica* representing two serovars, *Salmonella* Typhimurium and *Salmonella* Enteritidis isolated from three hospitals in Taif province, Kingdom of Saudi Arabia, were allocated to 23 genomic types using random amplified polymorphic DNA RAPD-PCR employing four 10-mer arbitrary primers. Jaccard's similarity coefficients were used to reveal the genetic diversity among isolates. Random amplified polymorphic DNA-polymerase chain reactioin (RAPD-PCR) amplified a total of 55 distinct bands (54 polymorphic bands or 98%). Cluster analysis based on the combined data from the four primers indicated that each of the *S.* Enteritidis or *S.* Typhimurium isolates had a distinct RAPD type. Principal component analysis (PCA) showed that the first three components explained about 65 and 33% of the total variability among RAPD types belonging to serovars Enteritidis and Typhimurium, respectively. The isolates belonging to serovar Enteritidis exhibited considerable genetic variation between locations, while those belonging to serovar Typhimurium exhibited considerable genetic variation both within and between locations. RAPD-PCR yielded highly reproducible results that demonstrated the high genetic heterogeneity of *Salmonella* isolates. Several genovars were present among *Salmonella* serovars and no specific RAPD type (or genovar) was found to be predominantly circulating in Taif province. The results presented here indicated that RAPD analysis has a great discriminatory power for the differentiation of *Salmonella* isolates and, therefore, can be a useful tool for the analysis of *Salmonella* genovars.

Key words: *Salmonella* genovars, Taif province, random amplified polymorphic DNA (RAPD) profiles, RAPD types, discrimination index, cluster analysis, principal component analysis.

INTRODUCTION

Salmonella food poisoning is one of the most common and widely distributed diseases in the world (Rodrigues et al., 1990; WHO, 2005), estimated to cause 1.3 billion cases of gastroenteritis and 3 million deaths worldwide (Bhunia,

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2008). *Salmonella* is a Gram negative facultative rod-shaped bacterium in the same proteobacterial family as *Escherichia coli*, the family Enterobacteriaceae, trivially known as "enteric" bacteria (Andrews and Baumler, 2005; Parry, 2006; Bhunia, 2008). *Salmonella* is associated mainly with raw meats, poultry and dairy products (Yang et al., 2010). However, many other foods have been implicated in outbreaks caused by *Salmonella*, and these include mayonnaise, salads, milk, unpasteurized orange juice, seafood, sprouted seeds and eggs (Pui et al., 2011). It gets into other foods by cross contamination from contact with raw foods, utensils, equipment and hands. Infection by non-typhoid salmonella (NTS) is much more common and usually causes gastroenteritis, ranges from mild self-limiting diarrhea, abdominal pain, nausea and vomiting lasting from 1-7 days to severe diarrhea that requires hospitalization (Panhotra et al., 2004; Parry, 2006). Healthy adults rarely suffer other symptoms and the mortality rate is <1%. Children, the elderly and the immuno-compromised patients may develop much more severe infections, such as septicaemia (Grassl and Finlay, 2008). Salmonella infection has also been associated with wound infections, meningitis and urinary tract infections (CDC, 2011).

To decrease morbidity and mortality due to systemic salmonellosis, antimicrobial therapy must be started immediately using one of the three commonly used antibiotics in tropical countries for the treatment of systemic NTS infections, that is, ampicillin, co-trimoxazole and chloramphenicol (Crump et al., 2011).

There are over 2,500 different serotypes of *Salmonella*, but most commonly reported, *Salmonella* Typhimurium and *Salmonella* Enteritidis, together account for at last 70% of reported human infection in Europe (Pires et al., 2010; CDC, 2011). In Saudi Arabia, serovar Typhimurium and Enteritidis were the most frequently isolated serovars from humans and animals (Halawani and Shohyeb, 2006; Boyen et al., 2008; Abdullahi, 2010). The biochemical, serological and molecular techniques were employed to detect and characterize Salmonella isolates in human fecal samples obtained from the western region of Saudi Arabia by amplifying the *inv*A and *hil*A genes to understand the genetic links between those isolates which spread in Taif Province (Ohud et al., 2010). The commonest serovars associated with human disease are *S.* Typhimurium and *S.* Enteritidis, but many others have been shown to cause disease, such as *Salmonella* notably *Salmonella* Infantis, *Salmonella* Virchow and *Salmonella* Newport (Mishu et al., 1994; Andrews and Baumler, 2005).

One hundred and forty-two cases of Salmonella infection were seen in Asir region, Southern Saudi Arabia, during the period of 1989-1991(Malik et al., 1994). Thirty-six cases of Salmonella infection were reported in Taif region, Saudi Arabia during 2002-2003. The two main serotypes were *S.* Typhimurium (38.9%) and *S.* Enteritidis (36.1%) (Halawani and Shohayeb, 2006). Improperly prepared fruits, vegetables, dairy products and shellfishs have also been implicated as sources of *Salmonella*. In a wedding ceremony in 2010 in Riyadh, Kingdom of Saudi Arabia, 283 individual developed infection with *Salmonella enterica*, 88% developed gastroenteritis, most commonly manifested by diarrhea (100%), abdominal pain (94.31) and fever (86.4%).

Individual serovars can be further characterized (typed) by a number of methods, including an antibiotic resistance profiles (Hanes, 2003; Nayak et al., 2004; Bhunia, 2008, Halawani and Shohyeb, 2008).

Classical typing methods for identification of isolates involve serotyping based on the Kauffmann-White serological scheme that targets the cell surface O and H antigens. This system currently identifies more than 2500 serotypes worldwide (Grimont and Weill, 2007). Phage typing systems have been commonly used method of *Salmonella* typing since 1950, and developed for further discrimination of serovars commonly associated with disease such as *S. enterica* serovar Typhimurium and Enteritidis (Anderson et al., 1977; Ward et al., 1987). More recently, molecular typing methods have been assessed and adopted for further discrimination of *Salmonella* isolates (Weigel et al., 20004; Halwani and Shohayb, 2008; Bugarel et al., 2011).

In recent years, *S.* Typhimurium and *S.* Enteritidis have emerged as a major serovars in the world (Rodrigues et al., 1990; WHO, 2005). Because of the increasing role of *S. enterica* serovar Typhimurium and Enteritidis in salmonella infections in Saudi Arabia (Nabbut et al., 1982; Al-Nakhli et al., 1999; Panhotra et al., 2004; Halawani and Shohayeb, 2008; Moussa et al., 2010), establishment of molecular typing data for this *Salmonella* serovars is important.

A PCR-based typing method, random amplified polymorphic DNA (RAPD)-PCR, has been described as a simple and rapid method able to offer detailed fingerprinting of the genomic composition of the organism (Williams et al., 1990; Weigel et al., 2004; Ammari et al., 2009; Stella et al., 2011; Rezk et al., 2012). The success of this method is due to the fact that no prior sequence information about the target is needed and a single short 10- mer oligonucleotide primer can be used in the reaction. The amplification happens at low stringency, allowing the primers to anneal to several locations on the two strands of the DNA. These primers detect polymorphisms in the absence of specific sequence information and the DNA sequence variations may work as genetic markers that can be used in epidemiologic studies (Quintaes et al., 2002; Lim et al., 2005).

The molecular typing data may be useful in recognizing and identifying the infectious strains and the clonality of isolates which cause the food borne outbreaks and this would determine the epidemiologic and prevalence of strains of salmonella food poisoning infections (Terajima et al., 1998; Tsen and Lin, 2001; Halawani and Shohayeb, 2008; Moussa et al., 2010; Moussa et al., 2011). In the

present study, molecular typing method (RAPD-PCR) was performed in order to establish the genetic relationships among salmonella clinical isolates obtained from the Western region of Saudi Arabia and to understand the epidemiological links between these isolates which spread in this area.

MATERIALS AND METHODS

Bacterial samples

Twenty three *Salmonella* strains were isolated from human fecal samples obtained during 2010 from local general hospitals and private clinical laboratories in Taif province, KSA. *Salmonella* isolates were characterized biochemically using API 20E strip (bioMe´rieux®, Inc., France), serologically by using Salmonella O and H antisera (Remel Europe Ltd., UK), and molecularly by strain specific PCR (Ohud et al., 2010) and RAPD-PCR (This study). Twenty serovars were confirmed positive by using Salmonella O and H antisera while three isolates could not be classified as *Salmonella* spp (Ohud et al., 2010). The isolates were as follows: S1, S5, S15, S16, S17, S18, S18, S20, S21, S22 and S23: Pediatric hospital; S2, S3 and S4: King Faisal Hospital; S6, S7, S8, S9, S10, S11, S12, S13 and S14: Al Edwani Hospital, Taif Province.

Random primers

RAPD fingerprinting of *S.* Typhimurium and *S.* Enteritidis was carried out using four decamer random primers designated OPA-13(5'-CAGCACCCAC-3'), OPB-10 (5'-CGTCTGGGAC-3'), OPB-18 (5'-CCACAGCAGT-3') and OPJ-10 (5'-AAGCCCGAGG-3' (Un-Ho Jin et al., 2000; Ohud et al., 2010). The oligonucleotide primers were commercially synthesized by Operon, A Qiagen Company, Germany. All primers used were resuspended in dd H_2O and stored at -20°C in aliquots to be used in PCR.

Preparation of genomic DNA

Genomic DNA was extracted from cell suspensions of bacteria grown overnight on xylose-lysine-deoxycholate (XLD) broth at 37°C using QIAamp® DNA Mini kit from Qiagen according to Lee et al. (2009). To elute bacterial DNA, 100 µl elution buffer (AE) was added to the center of the column, and the column was incubated at 37°C for 5 min and centrifuged at 20,000 xg for 1 min. DNA purity and quantity was determined using a GeneSys 10 UV spectrophotometer (Thermo Scientific, USA). The quality of *Salmonella* DNA prepared in this study was verified by PCR amplification of the *inv*A and *hil*A genes from the bacterial genome.

RAPD-PCR

The reaction mixture (25 µl) contained 10 mM Tris–HCl, pH 7.5, 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM spermidine, 0.1 mM dNTPs, 15 pmol of the RAPD primer, 20 ng genomic DNA and 0.8 U of Taq DNA polymerase. Amplification was carried out in a heated-lid PXE 0.5 Thermal cycler (Thermo Electron Corporation, USA) for 40 cycles, each consisting of a denaturing step of 1 min at 94°C, followed by annealing step of 1 min at 36°C and an extension step of 2 min at 72°C. The last cycle was followed by 7 min of long extension at 72°C. The amplification products were separated by gel electrophoresis in 2% agarose (Ambion , USA) in 45 mM Tris-borate, 1 mM EDTA buffer (pH = 8.0), containing ethidium bromide at 0.5 g/ml at a constant voltage of 5 V/cm. The gels were photographed under UV transillumination (Biometra, Germany) using a digital camera.

Analysis of RAPD-PCR data

Gel images were analyzed for genetic similarity among isolates using the AlphaEase Software (Alpha Innotech, CA, USA). Dendrograms were analyzed using MultiVariate Statistical Package software (MVSP 3.2., UK). RAPD bands were scored as discrete variables, using ‘‘1’’ to indicate presence and ‘‘0’’ to indicate the absence of a band in the profile. The PCR profiles are defined by the pattern of presence or absence of bands on the gel. The discrimination index (D) was calculated for each primer by using Simpson’s index of diversity as described by Hunter and Gaston (1988) as follows:

$$D = 1 - (\Sigma n_j(n_j - 1) / N(N - 1)$$

Where D is the diversity, N is the total number of strains, and n is the number of strains in each RAPD profile/type.

The similarities between DNA fingerprints were calculated with the band-matching Jaccard’s coefficient that ranges from 0 to 1.0, where 1.0 represents 100% identity (presence and position) for all bands in the two PCR fingerprints being compared. A pairwise similarity (or distance) matrix was developed and cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic averages (UPGMA) method. Principal component analysis (PCA), a mathematical procedure that uses orthogonal linear transformation, was used to recognize patterns in the RAPD-generated markers and to highlight the relationships between the genotypes examined.

RESULTS

In the present study, RAPD-PCR was used for characterizing *S. enterica* isolates in Taif province according to Ohud (2012). RAPD PCR using four 10-mer arbitrary oligonucleotide primers (OPA-13, OPB-10, OPB-18 and OPJ-10) was used in this study to evaluate the genetic diversity among *S. enterica* isolates (Figure 1). A total of 55 distinct and reproducible RAPD bands (54 polymorphic bands or 98 %) were amplified from the twenty three *Salmonella* isolates.

The total numbers of RAPD bands amplified were 10 (all polymorphic), 14 (all polymorphic), 14 (all polymorphic) and 17 (16 polymorphic), for RAPD primers OPA-13, OPB-10, OPB-18 and OPJ-10, respectively. Ten distinct polymorphic amplicons ranging from 400 to 1100 bp were generated by primer OPA-13. Cluster analysis based on RAPD primer OPA-13 and Jaccard's similarity coefficients distinguished 7 different RAPD profiles among *Salmonella* Enteritidis and 14 different RAPD profiles among *S.* Typhimurium. The index of discrimination (D) for RAPD primer OPA-13 was 0.94 for *S.* Enteritidis and 1 for *S.* Typhimurium according to Simpson's index of diversity. RAPD profiling using OPA-13 primer showed that the *S.* Enteritidis isolates S19 and S20 isolated from pediatric hospital were identical and had a RAPD profile #2. Isolates S12 and S14 isolated from Al-Edwani hospital were also identical and had a RAPD profile #6

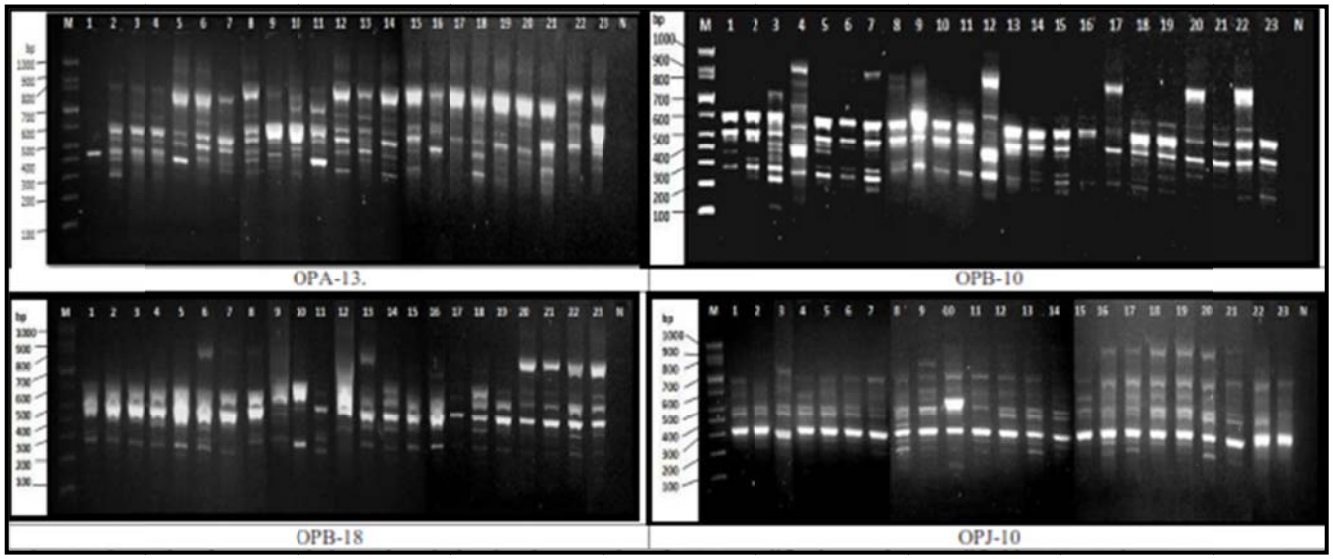

Figure 1. The results of agarose gel electrophoresis showing the separation of RAPD amplification products amplified by RAPD OPA-13, OPB-10, OPB-18, OPJ-10. The amplicons were electrophoresed onto 2% agarose in 0.5X TBE (45 mM Tris-borate, 1 mM EDTA) containing ethidium bromide (0.5 µg/ml) at constant voltage of 5 V/cm. Lanes (1-23): PCR produced from *Salmonella* isolates. M: Molecular size markers (100bp DNA ladder); N: Negative control (DNA-less, that is, ddH_2O instead of DNA).

while each of the remaining isolates produced a distinct RAPD profile. The RAPD primer OPA-13 produced an immense genetic variability to the maximum extent among the fourteen *S*. Typhimurium isolates and this is consistent with the discriminating ability of the primer (D = 1) according to Simpson's index of diversity. Cluster analysis based on RAPD primer OPB-10 and Jaccard's similarity coefficients distinguished 8 different RAPD profiles among *S.* Enteritidis and 13 different RAPD profiles among *S.* Typhimurium isolates. The index of discrimination (D) for RAPD primer OPB-10 was 0.97 for *S*. Enteritidis and 0.99 for *S*. Typhimurium according to Simpson's index of diversity. Cluster analysis based on RAPD primer OPB-18 and Jaccard's similarity coefficients distinguished 7 different RAPD profiles among *S.* Enteritidis and 9 different RAPD profiles among *S.* Typhimurium isolates. The index of discrimination (D) for RAPD primer OPB-18 was 0.94 for *S*. Enteritidis and 0.96 for *S*. Typhimurium according to Simpson's index of diversity.

Cluster analysis based on RAPD primer OPJ-10 and Jaccard's similarity coefficients distinguished 4 different RAPD profiles among *S.* Enteritidis and 7 different RAPD profiles among *S.* Typhimurium isolates. The index of discrimination (D) for RAPD primer OPJ-10 was 0.94 for *S*. Enteritidis and 0.95 for *S*. Typhimurium according to Simpson's index of diversity.

RAPD profiling using OPJ-10 primer showed that seven *S.* Typhimurium isolates S1 (pediatric hospital), S2 and S4 (King Faisal hospital), S5 (pediatric hospital), S6, S7 and S8 (Al-Edwani hospital) were identical and belonged to RAPD profile #11. These RAPD types were designated E1 to E9 (Figure 2A). Likewise, the fourteen *S.* Typhimurium isolates were clustered based on the combined data of the four primers and each isolate had a distinct RAPD type and fourteen RAPD types were designated (T1 to T14) (Figure 2B). Two RAPD types (T11 and T12) were found in King Faisal hospital, five RAPD types (T1, T2, T3, T9 and T14) were found in pediatric hospital, while seven RAPD types (T4, T5, T6, T7, T8, T10 and T13) were found in Al-Edwani hospital (Table 1).

Principal component analysis (PCA) showed that the first three components explained about 65 and 33% of the total variability among RAPD types belonging to serovars Enteritidis and Typhimurium, respectively (Tables 2 and 3) .

DISCUSSION

Four arbitrary RAPD primers were used with all twenty three *Salmonella* isolates genomes according to Ohud (2012). The four primers were found suitable for typing of *Salmonella* isolates on the basis of polymorphic RAPD markers after agarose gel electrophoresis. Interestingly, each of the studied *Salmonella* isolates had a distinct amplification pattern by RAPD-PCR with few exceptions. Cluster analysis calculated from the pairwise comparisons based on RAPD primer OPA-13 and Jaccard's similarity coefficients distinguished 7 different RAPD profiles among *S.* Enteritidis and 14 different RAPD profiles among *S.* Typhimurium. The results obtained by RAPD analysis revealed two identical RAPD profiles, profile #2 contained S19 and S20 isolated from pediatric hospital

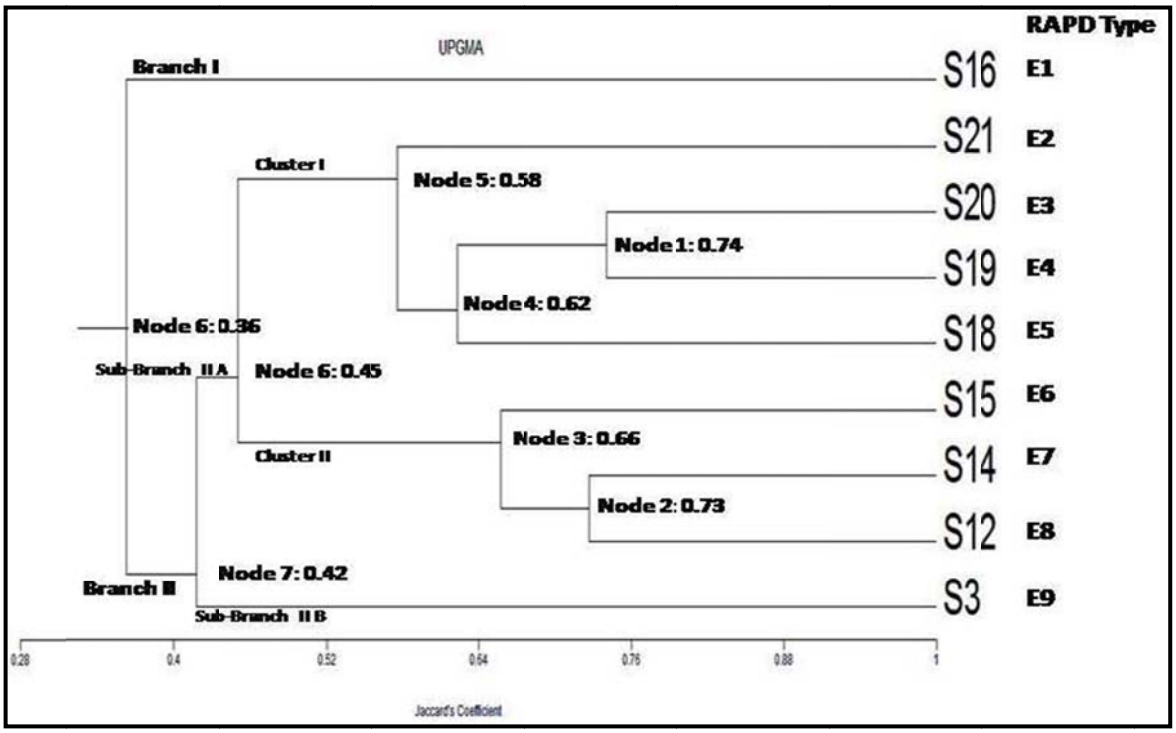

Figure 2A. A combined dendrogram constructed from the pairwise comparisons of Jaccard's similarity coefficients with clustering by UPGMA, based on the presence or absence of amplified product calculated identified nine RAPD type of *S.* Enteritidis. Similarity is given at each node.

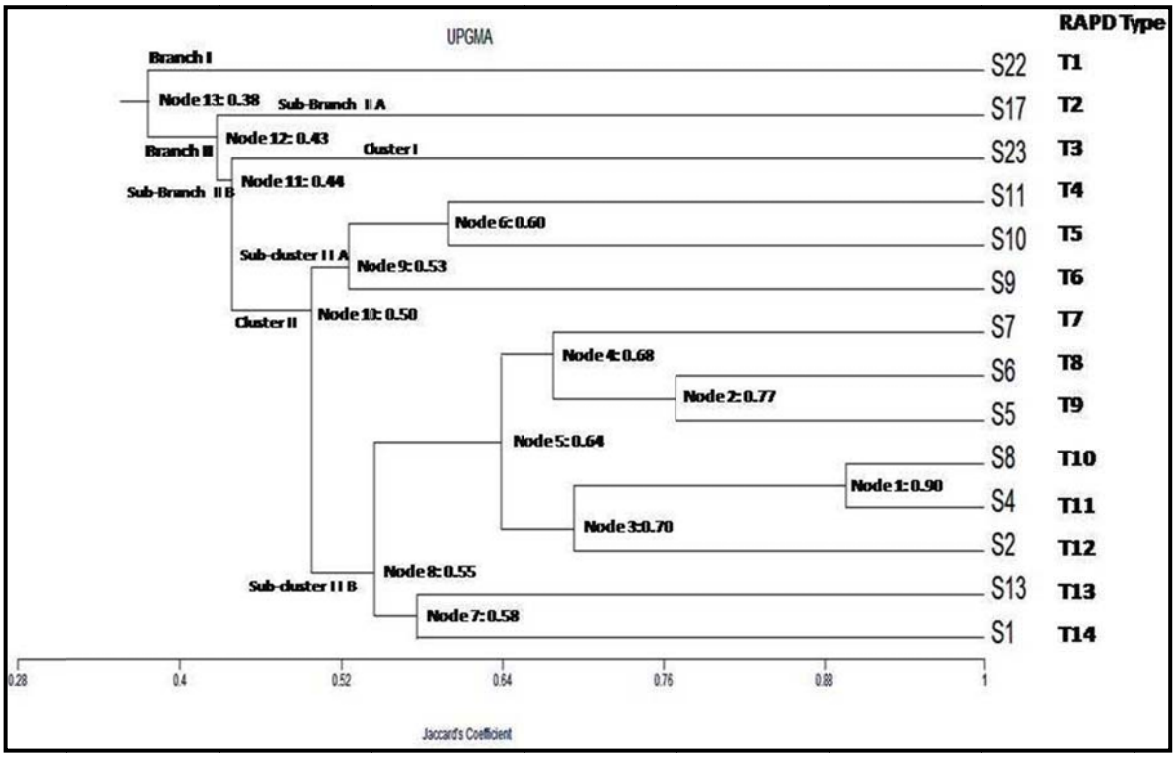

Figure 2B. A combined dendrogram constructed from the pairwise comparisons of Jaccard's similarity coefficients with clustering by UPGMA, based on the presence or absence of amplified product calculated identified 14 RAPD type of *S.* Typhimurium. Similarity is given at each node.

Table 1. RAPD PCR profiles and types generated from *Salmonella* enterica isolates using four RAPD primers.

RAPD type	RAPD profiles amplified by each primer				Isolate/Hospital	
	OPJ-10	OPB-18	OPB-10	OPA-13		
E9	4	7	5	7	S3- King Faisal	
E8	3	2	6	6	S12- Al-Edwani	
E7	3	5	2	6	S14 -Al-Edwani	
E6	3	1	2	5	S15- Pediatric	
E1	2	6	1	3	S16 -Pediatric	*Salmonella* Enteritidis
E5	2	5	3	4	S18 -Pediatric	
E4	2	3	4	2	S19- Pediatric	
E3	2	4	7	2	S20- Pediatric	
E2	1	4	8	1	S21- Pediatric	
T14	11	16	21	21	S1P-ediatric	
T12	11	14	20	20	S2 -King Faisal	
T11	11	11	16	18	S4- King Faisal	
T9	11	11	18	10	S5- Pediatric	
T8	11	10	11	9	S6- Al-Edwani	
T7	11	11	10	17	S7- Al-Edwani	
T10	11	12	15	19	S8- Al-Edwani	
T6	8	13	13	11	S9- Al-Edwani	*Salmonella* Typhimurium
T5	7	12	17	16	S10- Al-Edwani	
T4	10	13	19	8	S11- Al-Edwani	
T13	10	15	12	14	S13- Al-Edwani	
T2	9	13	9	12	S17- Pediatric	
T1	6	8	14	13	S22- Pediatric	
T3	5	9	12	15	S23- Pediatric	

Table 2. PCA analysis of nine RAPD profiles and 55 RAPD amplicons for *S.* Enteritidis isolates.

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7	Axis 8
Eigenvalues	2.845	1.672	1.412	0.960	0.826	0.789	0.376	0.259
Percentage	31.126	18.292	15.446	10.504	9.043	8.631	4.119	2.839
Cum. percentage	31.126	49.418	64.864	75.368	84.411	93.042	97.161	100.00

Table 3. PCA analysis of fourteen RAPD profiles and 55 RAPD amplicons for *S.* Typhimurium isolates.

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7	Axis 8	Axis 9	Axis 10	Axis 11
Eigenvalues	1.557	1.056	0.982	0.930	0.715	0.693	0.527	0.402	0.363	0.295	0.212
Percentage	19.759	13.399	12.459	11.799	9.071	8.793	6.691	5.105	4.604	3.739	2.688
Cum. percentage	19.759	33.158	45.618	57.416	66.487	75.281	81.972	87.077	91.680	95.419	98.107

with 100% similarity and profile #6 contained S12 and S14 isolated from Al-Edwani hospital with 100% similarity. This two RAPD profiles may represent the most common RAPD profiles distributed among other different minor genotypes in Taif province while each of the remaining isolates produced a distinct RAPD profile. The discrimination index (D) for RAPD primer OPA-13 among *S.* Enteritidis was 0.94 according to Simpson's index of diversity. Cluster analysis based on this primer grouped *S.* Typhimurium into 14 different RAPD profiles. Among the 14 RAPD profiles, RAPD profile #20 isolate S2 found in King Faisal hospital and RAPD profile #19 isolate S8 found in Al-Edwani hospital had the highest similarity (86%) among the *S.* Typhimurium profiles. The index of

discrimination (D) for RAPD primer OPA-13 was 1 according to Simpson's index of diversity. Among all the 4 primers used, OPB-10 generated fourteen distinct fragments ranging from 145 to 1159 bp producing high genetic variability to the maximum extent and this is reflected in the high discriminating index of the primer (D=0.97). A report by Un-Ho et al. (2000) showed maximum genetic variability among *S*. Typhimurium isolates using the OPB-10 primer as compared to OPA-13, OPB-18 and OPJ-10. Cluster analysis based on OPB-10 grouped *S.* Enteritidis isolates into 8 different RAPD profiles and *S.* Typhimurium isolates with 13 RAPD profiles. Among the eight RAPD profiles found for *S*. Enteritidis isolates, RAPD profile #2 (S14) was found in Al-Edwani hospital and (S15) was found in Pediatric hospital with 100% similarity. The index of discrimination (D) for RAPD primer OPB-10 among *S.* Enteritidis was 0.97 according to Simpson's index of diversity. Among *S.* Typhimurium, 14 different RAPD profiles were elucidated by using OPB-10 RAPD primer. The two isolates S13 isolated from Al-Edwani hospital and S23 isolated from pediatric hospital were identical and belonged to RAPD profile #12, indicating that this profile might be more common among the remaining isolates in Taif province. This RAPD profile, therefore, was more frequent as compared to the other *S.* Typhimurium profiles. The index of discrimination (D) for RAPD primer OPB-10 among *S*. Typhimurium was 0.99 according to Simpson's index of diversity. Fourteen distinct polymorphic amplicons ranging from 200 to 850 bp were generated by OPB-18 primer. Cluster analysis distinguished 7 different RAPD profiles among *S.* Enteritidis, with isolates S20 and S21 (RAPD profile #4) and S14 and S18 (RAPD profile #5) being identical and each of the remaining isolates having a distinct RAPD profile. Isolate S19 (RAPD profile #3) was more close to isolate S20 and S21 (RAPD profile #2) as they had a similarity of 79%. The index of discrimination (D) for RAPD primer OPB-18 among *S.* Enteritidis isolates was 0.94 according to Simpson's index of diversity. Nine different RAPD profiles were resolved among *S.* Typhimurium isolates by using this primer. Four isolates, S4 (King Faisal hospital), S5 (pediatric hospital), S7 and S8 (isolated from Al-Edwani hospital), had the same RAPD profile (profile #11). Three isolates S9, S11 (Al-Edwani hospital) and S17 Pediatric hospital had the same RAPD profile (profile #13). Both profiles 11 and 13 were probably the most common profiles as compared to the other *S.* Typhimurium profiles. The index of discrimination (D) for RAPD primer OPB-18 among *S*. Typhimurium was 0.96 according to Simpson's index of diversity. Seventeen RAPD amplicons ranging in size from 160 to 1100 bp were generated by OPJ-10 RAPD primer, with only one monomorphic amplicon. Four different RAPD profiles were resolved among *S.* Enteritidis isolates by using OPJ-10 RAPD primer. Four isolates, that is, S16, S18, S19 and S20 (isolated from pediatric hospital) were identical and belonged to RAPD profile #2 and three isolates, that is, S12, S14 (Al-Edwani hospital) and S15 (pediatric hospital) were also identical and belonged to RAPD profile #3, indicating that the two RAPD profiles 2 and 3 were the most common profiles among *S.* Enteritidis isolates while each of the remaining isolates produced a distinct RAPD profile. The index of discrimination (D) for RAPD primer OPJ-10 among *S*. Enteritidis was 0.94 according to Simpson's index of diversity.

Cluster analysis distinguished seven different RAPD profiles among *S.* Typhimurium, with two common RAPD profiles #10 and #11. RAPD profile #10 contained two identical isolates, that is, S11 and S13 isolated from Al-Edwani hospital while RAPD profile #11 contained seven identical isolates, that is, S1 (pediatric hospital), S2 and S4 (King Faisal hospital), S5 (pediatric hospital), S6, S7 and S8 (Al-Edwani hospital). This might suggest the predominance of this two RAPD profiles in Taif province. The index of discrimination (D) for RAPD primer OPJ-10 among *S.* Enteritidis isolates was 0.95 according to Simpson's index of diversity.

All the four primers, that is, OPA-13, OPB-10, OPB-18 and OPJ-10 produced reproducible results and useful as efficient RAPD primers regardless of their short oligonucleotide sequence. Similar results have been found by Hilton et al. (1996), Chansiripornchai et al. (2000) and Mare et al. (2001). These results revealed that RAPD-PCR can be used as an epidemiological tool in combination with other molecular and phenotypic typing techniques. Similar interpretations have been reported by several investigators on the same serotypes as well as other serotypes (Meenu, 2002; Jin et al., 2006; Yaqoob et al., 2007; Maripi et al., 2007; Dos Santos et al., 2008; Morshed and Peighambari, 2010).

The RAPD profiles generated by the four primers were combined and the combined dendrograms were highly branched, suggesting a genetically diverse population. The majority of *Salmonella* isolates within a cluster belonged to different RAPD types. The differences in the percent similarity could be the result of strain variation, as described by Hilton et al. (1996) and Franklin et al. (2011). Similar results have been described by Millemann et al. (1996), Chansiripornchai et al. (2000) and Mare et al. (2001). Dendrogram branch lengths were proportional to genetic distance between isolates. Phylogenetic comparisons of *S. enterica* isolates from different geographical regions have been useful in taxonomical and epidemiological investigations.

The nine *S.* Enteritidis isolates were clustered based on the results of the four primers. Each of the nine *S.* Enteritidis isolates had a distinct RAPD type. The nine RAPD types (E1 to E9) were designated No E1 to E9 (Figure 1A). One RAPD (E9) was found in King Faisal hospital, two RAPD types (E7 and E8) were found in Al-Edwani hospital, while six RAPD types (E1, E2, E3, E4, E5 and E6) were found in pediatric hospital. These results suggested that several genovars were present

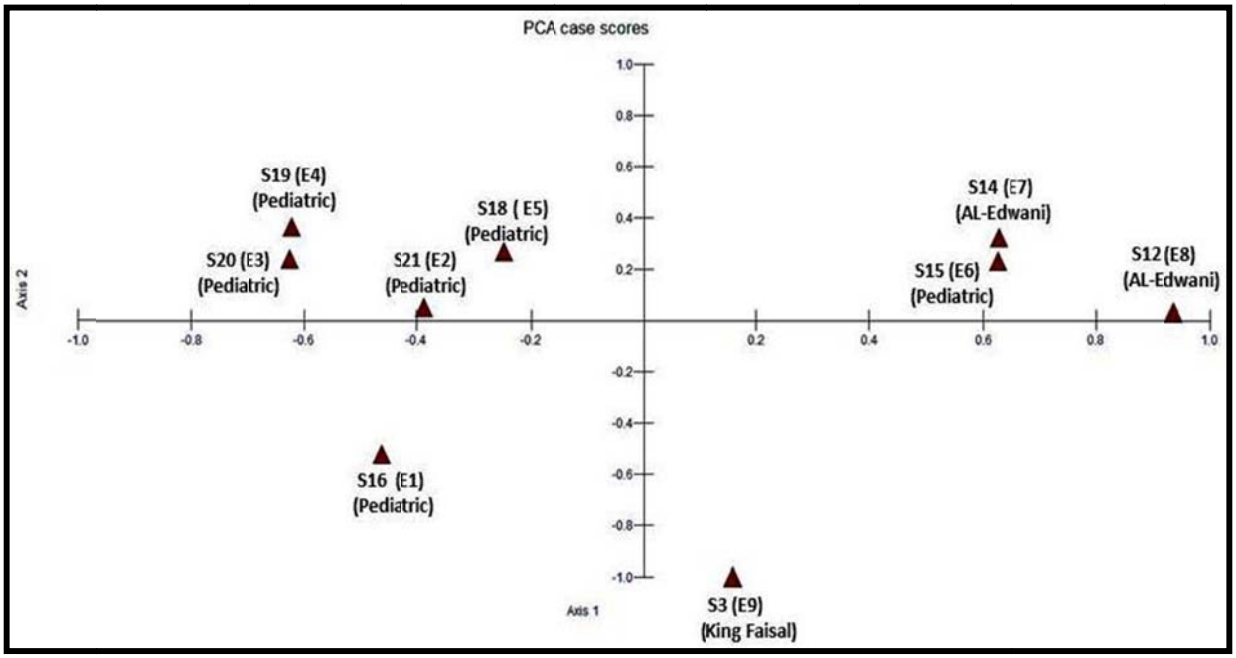

Figure 3A. Scatter plots showing two dimensional distribution of *S.* Enteritidis isolates derived from principal component analysis (PCA) of RAPD-PCR data. *Salmonella* codes are shown on the arrows.

among *Salmonella* serovars. This result indicates that there is no specific *S.* Enteritidis strain that circulates in Taif province. Likewise, the fourteen *S.* Typhimurium isolates were clustered based on the four primers and each isolate had a distinct RAPD type. The fourteen *S.* Typhimurium RAPD types were designated T1 to T14 (Figure 1B). Two RAPD types (T11 and T12) were found in King Faisal hospital, five RAPD types (T1, T2, T3, T9 and T14) were found in pediatric hospital, while seven RAPD types (T4, T5, T6, T7, T8, T10 and T13) were found in Al-Edwani hospital.

The results presented in this study indicated that RAPD analysis is a useful tool for the analysis of *Salmonella* genovars, provided that strict experimental protocols are maintained and might be used as a cost-effective tool for molecular epidemiology research (Garaizar et al., 2000).

The principal component analysis (PCA) based on RAPD data of the nine RAPD Types of *S.* Enteritidis showed four components with Eigenvalues more than one. The first two components explained about 49.4% of the RAPD variation, while the first three components explained about 65% of the total variance (Table 2). The remaining components accounted for about 35% of the total variance. The scatter plot defined by the first two principal components (Axis 1 and 2) distinguished the RAPD Type E9 (found in King Faisal hospital) from the other RAPD Types (Figure 2A). The two RAPD Types E7 and E8 (found in Al-Edwani hospital), and the six RAPD Types E1 to E6 (found in Pediatric hospital) were also distinguishable in the scatter plot (Figure 2B). This result indicated that *S. enterica* subspecies *enterica* serovar Enteritidis may exhibit considerable genetic variation between locations (Figure 3A).

The PCA based on RAPD data of the fourteen RAPD types of *S.* Typhimurium showed that there were two components with Eigenvalues more than one. The first component explained about 20% of the RAPD variation, while the first two components explained about 33% of the total variance (Table 3). The remaining components accounted for about 67% of the total variance. No RAPD types could be appropriately grouped in the scatter plot defined by the first two principal components (Axis 1 and 2). This result confirmed the results of cluster analysis and showed that *S. enterica* subspecies *enterica* serovar Typhimurium exhibit considerable genetic variation within and between locations (Figure 3B).

The results of PCA for the nine RAPD types of *S.* Enteritidis indicated that *S. enterica* subspecies *enterica* serovar Enteritidis may exhibit considerable genetic variation between locations. The results of principal component analysis (PCA) for fourteen RAPD types of *S.* Typhimurium confirmed the results of cluster analysis and showed that *S. enterica* subspecies *enterica* serovar Typhimurium exhibit considerable genetic variation within and between locations (Figure 3B). The results of PCA (Tables 2 and 3) indicated that the isolates belonging to serovar Enteritidis exhibited considerable genetic variation between locations, while the isolates belonging to serovar Typhimurium exhibited considerable genetic variation both within and between locations. In conclusion, RAPD-PCR yielded highly reproducible results that demonstrated the high genetic heterogeneity of

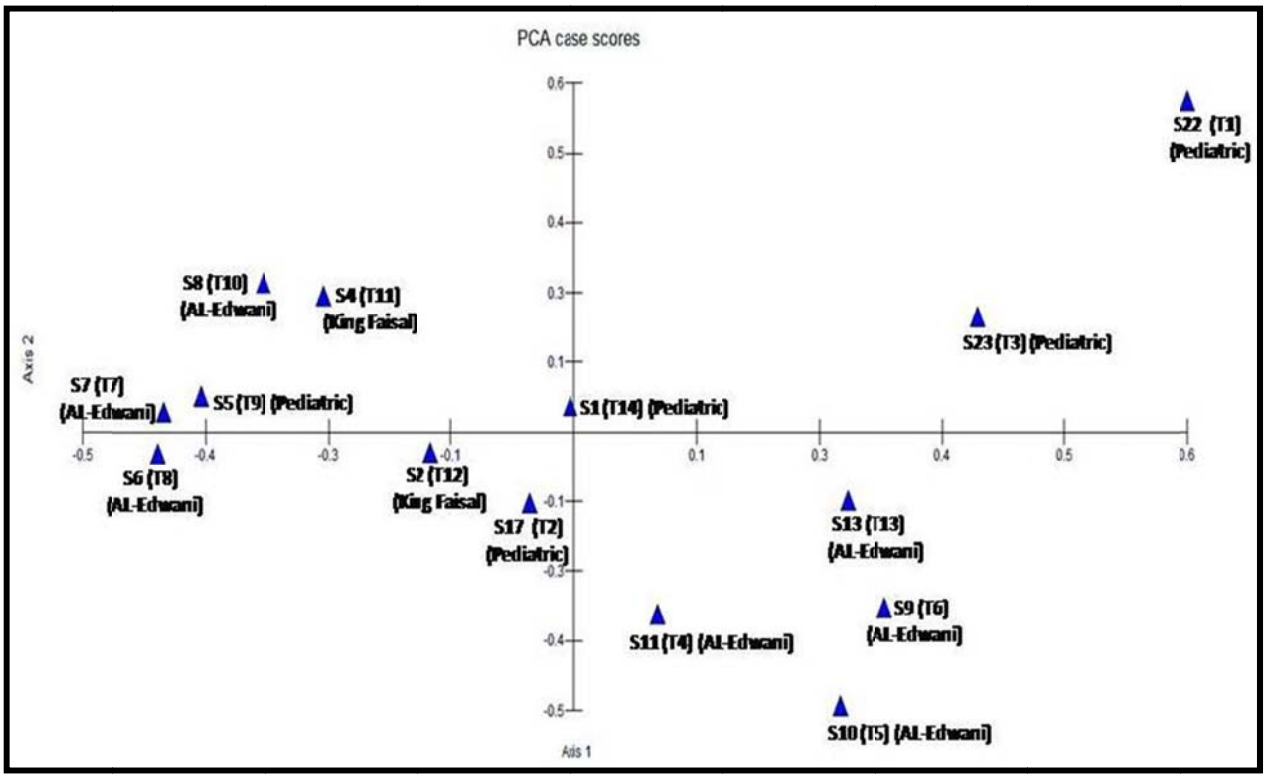

Figure 3B. Scatter plots showing two dimensional distribution of *S.* Typhimurium isolates derived from principal component analysis (PCA) of RAPD-PCR data. *Salmonella* codes are shown on the arrows.

Salmonella isolates. Several genovars were present among *Salmonella* serovars and no specific RAPD type (or genovar) was found to be circulating predominantly in Taif province.

Our results were in agreement with the work of Un-Ho Jin et al. (2000) and Jegadeeshkumar et al. (2010) in identifying and differentiating *S.* Typhimurium from other Gram negative bacteria by using DNA (RAPD) fingerprinting of *S.* Typhimurium. Un-Ho Jin et al. (2000) used the same andom primers designated OPA-13, OPB-10, OPB-18 and OPJ-10, and its patterns compared with 6 representive intestinal, Gram negative bacterial strains. The results showed that *S.* Typhimurium had unique and distinct fingerprinting patterns and the RAPD fingerprinting is thus concluded to be a rapid and sensitive method for the identification of *S.* Typhimurium as compared to conventional culturing procedures or immunoassays.

The results shown in the present study was not reported elsewhere about the genetic diversity among *Salmonella* isolates using RAPD-PCR in Saudi Arabia. The results presented in this study indicated that RAPD analysis has a great discriminatory power for the differentiation of *Salmonella* isolates. RAPD analysis, therefore, can be a useful tool for the analysis of *Salmonella* genovars. Of course, this should have important implications in the fields of food industry, human health and epidemiology.

Conflict of interests

The authors did not declare any conflict of interest.

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