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MOLECULAR CHARACTERIZATION BY USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS OF *SALMONELLA ENTERITIDIS* ISOLATES RECOVERED FROM AVIAN AND HUMAN SOURCES

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ABSTRACT

Random amplified polymorphic DNA (RAPD) analysis was applied for molecular characterization of five *Salmonella enteritidis* strains from different avian sources and human cases of infection. A total of 16 primers were used and only five primers showed good discriminatory power for all five isolates. Dendrogram showed a common lineage among all five isolates. There was a close genetic relationship among isolates of eggs and human sources, while there was less pronounced homology among isolates of broiler meat and human sources. On the basis of results we have found that an endemic strain of *S. enteritidis* is prevalent between the poultry derived food and humans which gives us an insight to genetic diversity of *S. enteritidis* from these sources.

Key words: *Salmonella enteritidis*, RAPD, dendrogram.

INTRODUCTION

Avian salmonellosis is of major economic concern in all phases of the poultry industry from production to marketing (Chansiripornchai *et al.,* 2000). Poultry and poultry products, in particular chicken meat and eggs, are considered important sources of human infection with this pathogen (Rampling, 1993). *Salmonella enterica* subsp. *enteica* ser. *enteritidis* (*S. enteritidis*) is a major cause of food-borne diseases (Brenner *et al.,* 2000), and during the last two decades it has been isolated world wide in increasing numbers (Rodrigue *et al.,* 1990; Herikstad *et al.,* 2002).

Global surveillance data indicate that incidence of gastroenteritis caused by *S. enteritidis* has increased massively during the last decade. Among the *S. enteritidis* isolates, those assigned to phage type 4 (PT4) and phage type 8 (PT8) have been isolated predominantly from poultry and are most frequent cause of human salmonellosis. Similar increase in *S. enteritidis* infections has also been reported in Europe, where PT4 has emerged as the predominant phage type, spreading rapidly through both poultry and human populations and virtually replacing all other phage types (Rampling, 1993). In Pakistan, *S. enteritidis* is an endemic organism and its regular outbreaks occur due to poultry derived food (Mansoor, 1997). In the present study, molecular characterization of *S. enteritidis* strains isolated from different poultry and human sources was performed using Random Amplified Polymorphic DNA (RAPD) analysis.

MATERIALS AND METHODS

Five isolates of *S. enteritidis* kept at the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan were selected for the present study. These isolates were collected from eggs, broiler meat and human faecal samples during 2004-2005. All the isolates were previously confirmed and identified by the standard bacteriological and serological methods. Three isolates were from egg sources, 1 from boiler meat and 1 from human (Table 1). DNA from all isolates was extracted by the alkaline lysis (Phenol:Chloroform:Isoamyl) method (Silhavy *et al.,* 1984).

Table 1: Distribution of collected samples on the basis of sources

Source	Total No. of samples	Strain designation
Eggs	3	E_3
		E_{12}
		E_{29}
Broiler	1	B_{15}
Human	1	H_9
Total	5	

The concentration of total genomic DNA was measured by spectrophotometer (CECIL, CE 2021, 2000 series) at 260 nm wavelength. Quality of DNA was checked by running 5 µl DNA on 0.8% agarose gel prepared in 0.5X TBE buffer. Furthermore, DNA quality was checked by measuring the ratio of absorbance (A_{260}/A_{280}); more than 1.8 indicates the

purity of DNA free from contaminants like proteins or phenol.

RAPD-PCR

Sixteen different primers (Genelink Company) were used for fingerprinting *S. enteritidis* isolates. The 25 µl PCR cocktail contained: 3 mM $MgCl_2$, 100 µM each of dATP, dCTP, dGTP, dTTP, 0.2 µM primer, 15 ng of genomic DNA and 1 unit of Taq polymerase. The thermolcycler (Eppendorf Mastercycler, USA) was programmed for 5 minutes initial denaturation at 95°C, 1 minute denaturation at 37°C and 2 minutes extension, 72°C for 40 cycles and final extension at 72°C for 10 minutes.

The PCR reaction products (10 µl each) were loaded on 1.2% agarose gel (Gibco BRL, Gaithersburg, Md.) containing 0.5 µg/ml ethidium bromide, at 80 V in 0.5X TBE buffer for approximately 1.5 hours. Two lanes of 1 kb ladder (Fermentas) were added in each gel for reference.

RAPD data analysis

The data obtained from amplification products by primers were used to estimate genetic similarity among different isolates on the basis of shared amplification products (Nei and Li, 1979). The RAPD patterns were scored on the basis of presence or absence of band. The similarity coefficients were utilized to generate dendrogram by using UPGMA (Unweighted Pair Group Method of Arithmetic means) through the programme, Popgene Version 1.31 (Microsoft windows based Freeware for population Genetic Analysis).

RESULTS

Out of 16 primers used, 8 were found totally non polymorphic and produced no polymorphic band. While out of the remaining 8 primers, only 5 primers namely: A-10 (5′-GTGATCGCAG-3′), A-13 (5′-CAGCACCCAC-3′), A-16 (5′-AGCCAGCGAA-3′), C-02 (5′-GTGAGGCGTC-3′) and C-15 (5′-GACGGATCAG-3′) provided good discriminatory power among different isolates of *S. enteritidis*. Primer C-02 provided unique results among all the five polymorphic primers (Fig. 1). No amplification products were seen in reaction performed in the absence of primer or in the absence of DNA template.

The dendrogram obtained from RAPD data showed a single lineage pattern (Fig. 2). There was less genetic variation among isolates of poultry eggs and humans. However there was about 28.57% genetic relatedness among poultry eggs and broiler sources; and 73.47% genetic relatedness was found between human and layer sources (Table 2).

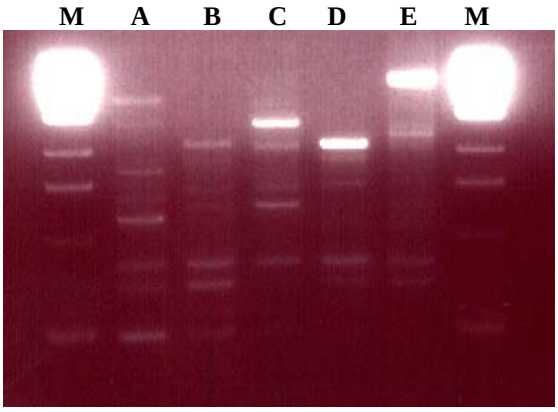

Fig. 1: Amplification of five RAPD products by *Salmonella enteritidis* isolates with primer C-02.
Lane A = B_{15}, Lane B = E_3, Lane C = E_{29}, Lane D = H_9, Lane E = E_{12}, Lane M = DNA Marker (1 kb)

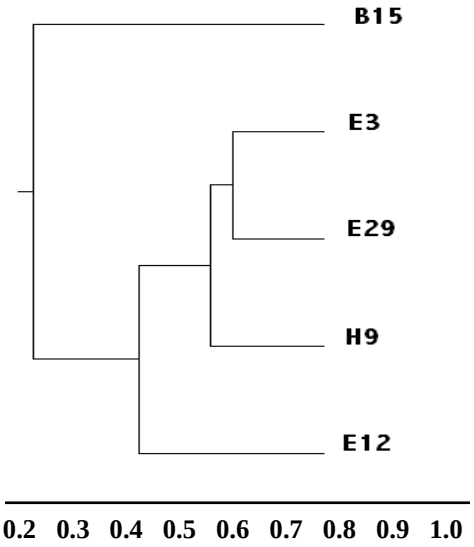

Fig. 2: Dendrogram among five isolates of *Salmonella enteritidis* generated through RAPD data using UPGMA method.

DISCUSSION

Salmonella enteritidis is among the major etiological agents of the human gastroenteritis and is also most frequently isolated from poultry products. To our knowledge, the present manuscript is the first molecular study of *S. enteritidis* isolates in Pakistan to look for insight into the genetic diversity of strains that have been circulating locally between the human and poultry.

Table 2: Similarity matrix of five *Salmonella enteritidis* isolates obtained from RAPD markers

Strains	B_{15} (Broiler)	E_3 (Eggs)	E_{29} (Eggs)	H_9 (Human)	E_{12} (Eggs)
B_{15}	1				
E_3	0.4490	1			
E_{29}	0.2857	0.6327	1		
H_9	0.4286	0.7347	0.5714	1	
E_{12}	0.3673	0.6735	0.4286	0.6939	1

In this study, maximum numbers of amplified loci were obtained using primer C-02 sequence code (5'-GTGAGGCGTC-3') that is nine times repetition was found in the entire genome of *S. enteritidis* (Fig. 1). Similar results have been found by Hilton *et al.* (1996), Chansiripornchai *et al.* (2000) and Mare *et al.* (2001). The same primer C-02 was found unique for all isolates as it produced a unique common locus in all isolates. The genetic similarity matrix of RAPD data for 5 isolates was constructed based on coefficient of similarity utilized for the construction of dendrogram using the UPGMA, as described by Chansiripornchai *et al.* (2000). Dendrogram was highly branched, suggestive for a genetically diverse population (Fig. 2). Similar results have been described by Millenman *et al.* (1996), Chansiripornchai *et al.* (2000) and Mare *et al.* (2001). The diversity in phylogenetic tree proved that the field isolates were distinctive with regard to biochemical profiles and serotyping.

The phylogenetic tree constructed from RAPD data showed a single lineage pattern, which indicated that all isolates are descendents of a single species of *Salmonella enterica.* The differences in the percent similarity could be the result of strain variation, as described by Hilton *et al.* (1996). On the basis of phylogenetic analysis of field isolates, *S. enteritidis* were found 28.57 to 73.47% similar among each other. There was very little (28.57%) genetic homology of isolates taken from the broiler sources, suggesting that there is genetically less related strain circulating in broilers. While more closely related (73.47%) strains are circulating between layers and humans.

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