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Assessment of Intra-Serotypic Diversity in *Escherichia coli* from Dogs Using ERIC-PCR

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ABSTRACT

The scope of this study was to characterize the intra-serotypic genetic diversity in fecal *Escherichia coli* isolates obtained from dogs, using enterobacterial repetitive intergenic consensus (ERIC)-PCR. Serological typing of forty-seven *E. coli* isolates recovered from the rectal swabs (n=93) of different dog breeds in Andhra Pradesh revealed O141 (5 isolates), O128 (3 isolates), O126 (2 isolates), O9 (2 isolates), O15, O20, O35, O49, O63, O85, O101, O116, O117, O118 and O119 (1 isolate each) serotypes. A total of 10 and 14 isolates were found to be rough (R) and untypable (UT), respectively. ERIC-PCR genotyping allowed discrimination of 47 *E. coli* isolates into 46 ERIC-PCR genotypes. The numerical index of discrimination was 0.999. Dendrogram analysis discriminated 47 *E. coli* isolates into eight major clusters (C_1 to C_8) with 70% similarity between them and three isolates were found to be unclustered with other isolates. Our results showed high intra-serotypic diversity in fecal *E. coli* from dogs, as evidenced by the grouping of isolates of same 'O' serotype into different clusters. Furthermore, ERIC-PCR genomic fingerprinting was found to be a rapid, easy-to-perform genotyping tool to differentiate *E. coli* strains within serotype based on their genotype.

Keywords: Dogs, ERIC-PCR, *E. coli*, genotyping, serotyping

Enterobacterial repetitive intergenic concensus (ERIC) sequences, also described as 'selfish' DNA are imperfect palindromes interspersed throughout the genome of *E. coli* and other enteric bacteria (Wilson *et al.,* 2006). Individual bacterial strains vary with respect to the distance between these repetitive sequences as well as the frequency of repeated sequences (Wilson *et al.,* 2006). Polymerase chain reaction (PCR) based genomic fingerprinting techniques such as ERIC-PCR has been successfully applied in various studies as a powerful epidemiological tool for the identification and classification of bacterial strains (Mohapatra *et al.,* 2007; Prabhu *et al.,* 2010; Ardakani and Ranjbar, 2016). ERIC-PCR genotyping technique yields strain-specific unique multi-band patterns obtained by the amplification of conserved ERIC sequences, thus discriminating individual bacterial strains (Wilson *et al.,* 2006). *E. coli* strains of same 'O' serotype as well as untypable (UT) and rough (R) strains can be discriminated intra-serotypically into different genotypes using ERIC-PCR genotyping technique (Prabhu *et al.,* 2010). Perusal of the literature revealed the paucity of information related to the intra-serotypic diversity in *E. coli* of canine origin in India. Keeping this in view, the present study aimed at the characterization of intra-serotypic genetic diversity in fecal *E. coli* isolates obtained from different dog breeds in Andhra Pradesh using ERIC-PCR.

MATERIALS AND METHODS

Bacterial isolates

A collection of forty-seven *E. coli* isolates recovered from the rectal swabs (n=93) of different dog breeds in Andhra Pradesh were used in the present study. Isolation

and identification was done using nutrient broth, eosin methylene blue (EMB, HiMedia) medium. The isolates were confirmed to be *E. coli* by standard biochemical tests (Sneath and Holt, 2001) and also by the PCR amplification of the *E16S* gene of *E. coli* (Aghamiri *et al.,* 2014). Whole cell DNA extraction was carried out by the method of Wani *et al.* (2006). All the forty-seven *E. coli* isolates were serotyped at National *Salmonella* and *Escherichia* Centre (NSEC), Central Research Institute (CRI), Kasauli (Himachal Pradesh, India), on the basis of their 'O' antigen.

ERIC-PCR genotyping of *E. coli* isolates

The oligonucleotide primers used in this study were ERIC-1 (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and ERIC-2 (5'-AAG TAA GTG ACT GGG GTG AGC G-3') (Mohapatra *et al.,* 2007). DNA amplification was carried out in 25 µl optimized reaction mixture using 12.5 µl of Master Mix (Takara), 2 µl of DNA template (100 ng/µl), 1.0 µl of each primer (20 pmol/µl) and 8.5 µl of nuclease free water. The PCR amplifications were performed in the Eppendorf thermal cycler (USA) with an initial denaturation at 95ºC for 7 min, followed by 30 cycles of denaturation at 94ºC for 30 sec, annealing at 50ºC for 1 min, elongation at 65ºC for 8 min and 16 min of final elongation at 65ºC. The DNA from reference strain *E. coli* (ATCC 25922) was used as positive control for the standardization of the ERIC-PCR reactions.

Analysis of DNA fingerprinting patterns

ERIC-PCR products were resolved by 1.5% agarose gel electrophoresis under 110V for 2 h (Sambrook and Russell, 2001). The banding patterns of the ethidium-bromide stained gel were photographed using BIO-RAD Gel Documentation system (USA) and the DNA fingerprints were analyzed both by visual inspection and image lab software (BIO-RAD). The position of bands was compared using 100 bp and 1 kb DNA ladder as an external reference. Binary matrix was generated based on the presence (1) or absence (0) of a particular band in the given strain. Patterns with at least one different band were considered as different genotypes. The similarity of band patterns was determined using the Pearson's correlation coefficient. Dendrogram was constructed by "branch-and-bound method" using dollop programme of phylip software, version 3.6. Clusters were considered at a 70% similarity cut-off value. The numerical index of discrimination was calculated by the application of Simpson's index of diversity, $D = 1\text{-}1/N\ (N\text{-}1)\ \sum n_j\ (n_j\text{-}1)$ where D corresponds to the Discriminatory power, N corresponds to the total number of strains, n_j corresponds to the number of strains belonging to the j^{th} type (Ramees *et al.,* 2014).

RESULTS AND DISCUSSION

Serological typing of *E. coli* (47) done at NSEC (Kasauli) on the basis of 'O' antigen showed following serotypes – rough (10 isolates), O141 (5 isolates), O128 (3 isolates), O126 (2 isolates), O9 (2 isolates), O15, O20, O35, O49, O63, O85, O101, O116, O117, O118, O119 (1 isolate each) and the rest 14 isolates were found to be untypable (UT). ERIC-PCR typing revealed 3-20 fragments resolved per isolate, ranging in size from slightly less than 100 bp to 2500 bp (Fig. 1). The binary data of ERIC-PCR profiles showed highly polymorphic DNA fragments in these 47 isolates; viz., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 amplicons detected in 1, 1, 2, 4, 1, 1, 2, 3, 5, 4, 3, 5, 3, 4, 2, 2, 2 and 2 isolates, respectively. ERIC PCR profiles revealed 46 ERIC-PCR genotypes discriminated among 47 *E. coli* isolates. Two *E. coli* isolates (V5 and V6) displayed a single ERIC-PCR profile (Fig. 1).

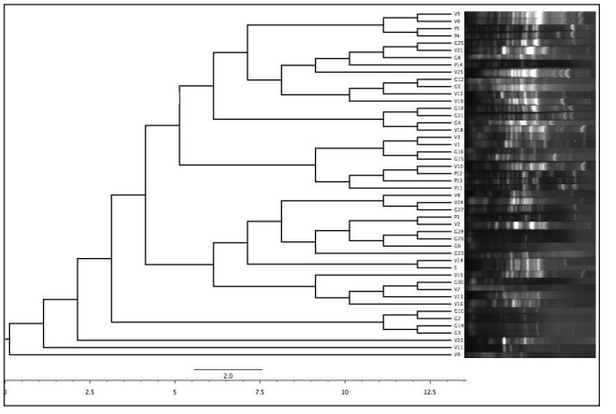

Fig. 1: ERIC-PCR cluster analysis of dog fecal *E. coli* isolates (S: *E. coli* ATCC 25922)

Prabhu *et al.* (2010) reported discrimination of 37 *E. coli* isolates recovered from bovine mastitis cases into 19 different genotypes using ERIC-PCR. Other studies also reported high sensitivity of ERIC-PCR genomic

Table 1: Intra-serotypic genetic diversity in fecal *E. coli* isolates from dogs

Serotype (number of isolates)	Cluster number (C_1-C_8)	Number of isolates in each cluster	Number of ERIC-PCR genotypes	Isolates code
Rough, R (10)	C_2	3	3	G25, G8, G5
	C_3	2	2	G21, G4
	C_4	3	3	G15,P13, P11
	C_5	1	1	G23
	UC*	1	1	V9
O141 (5)	C_1	1	1	P5
	C_2	2	2	V21, G12
	C_3	1	1	V18
	C_4	1	1	V10
O128 (3)	C_5	2	2	G29, G28
	C_8	1	1	G2
O126 (2)	C_3	1	1	G18
	C_5	1	1	G6
O9 (2)	C_4	1	1	V3
	C_7	1	1	V15
O15 (1)	C_7	1	1	V16
O20 (1)	C_7	1	1	G30
O35 (1)	C_8	1	1	G10
O49 (1)	C_4	1	1	G16
O63 (1)	C_4	1	1	P12
O85 (1)	C_8	1	1	G14
O101 (1)	C_2	1	1	P14
O116 (1)	C_8	1	1	G3
O117 (1)	C_4	1	1	V1
O118 (1)	C_5	1	1	V24
O119 (1)	C_5	1	1	G27
Untypable, UT (14)	C_1	3	2	V5, V6, P4
	C_2	3	3	V25, V12, V19
	C_5	3	3	V8, V2, P3
	C_6	1	1	V14
	C_7	2	2	V7, V13
	UC*	2	2	V23, V11
Total (47)	**8**	**47**	**46**	**47**

*UC – unclustered

fingerprinting technique for the discrimination of *E. coli* strains of diverse origin (Sanchez *et al.,* 2002; Ardakani and Ranjbar, 2016).

Dendrogram analysis of ERIC-PCR profiles discriminated *E. coli* isolates into eight major clusters (C_1 to C_8) for a 70% similarity cut-off, with C_2 and C_5 being the major ones (9 isolates each) followed by C_4 (8 isolates), C_7 (5 isolates), C_1, C_3, C_8 (4 isolates each) and C_6 (an isolate and reference standard) (Fig. 1). Three isolates were found to be unclustered (UC) with other isolates. Cluster analysis indicated wide intra-serotypic genetic diversity among the

isolates affiliated to the same serotype, as evidenced by their grouping under different clusters in the dendrogram (Table 1). For example, isolates of O141 serotype viz., P5, V21, V18 and V10 were grouped under clusters C_1, C_2, C_3 and C_4 respectively (Table 1). ERIC-PCR genotyping discriminated strains of same "O" serotype into different genotypes. Furthermore, compared to serotyping, different genotypes were discriminated among the untypable (UT) and rough (R) strains of *E. coli* in the present study.

The present observations were in accordance with the findings of Prabhu *et al.* (2010), who assessed the utility of ERIC-PCR technique for the intra-serotypic differentiation of *E. coli* strains isolated from episodes of bovine mastitis. PCR based DNA fingerprinting was reported to be easiest, quickest and low cost method for the discrimination of *E. coli* O157:H7 strains from other *E. coli* serotypes (Hahm *et al.,* 2003). The numerical index of discrimination calculated for 47 isolates, using Simpson's index of diversity was 0.997, which corroborate with earlier studies (De Moura *et al.,* 2001; Mohapatra *et al.,* 2007), where ERIC-PCR was shown to have highly significant discriminatory power in characterizing the genetic diversity of *E. coli* strains. ERIC-PCR was reported to have more discriminatory power than 16S rRNA gene analysis in the discrimination of non-pathogenic groups of *E. coli* from pathogenic groups (A'yun *et al.,* 2015).

This is the first study in India to elucidate the genetic diversity of fecal *E. coli* isolates from dogs and ERIC-PCR was proved to be an efficient genotyping tool for intra-serotypic differentiation of strains based on their genotype. In summary, the results presented in this paper and past studies emphasize the utility of combination of serotyping with genotyping in the epidemiological investigations related to *E. coli* strains.

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