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Asian Journal of Animal and Veterinary Advances

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Asian Journal of Animal and Veterinary Advances 9 (9): 543-555, 2014
ISSN 1683-9919 / DOI: 10.3923/ajava.2014.543.555
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Molecular Characterization of *Arcobacter* Isolates Using Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR)

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ABSTRACT

In modern years, *Arcobacters* are reflected as potential emerging food-borne zoonotic entero-pathogens. *Arcobacter* species displayed a wide variety of genetic diversity. The study was carried out to genotype and find molecular heterogeneity of *Arcobacter* spp. (*Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii*), isolated from different sources from Bareilly region, Uttar Pradesh, India by using randomly amplified polymorphic DNA - polymerase chain reaction (RAPD-PCR). RAPD-PCR was performed using genomic DNA of *Arcobacter* isolates (n = 56; 33 *A. butzleri*, 20 *A. cryaerophilus*, 3 *A. skirrowii*; recovered from chicken meat, pork, sheep faeces, goat faeces, poultry intestinal contents and human diarrhoeal stool samples) as template by employing two published primers. The RAPD profiling for primer 1 (HLWL85) yielded number of bands rangeing between 2-8 (500-3100 bp). Out of 56 isolates, 54 showed bands giving a typeability of 96.4%. These 54 typable strains were grouped to 35 types and giving discriminatory power of 0.9762. Primer 2 (OPA-11) yielded RAPD-PCR profiles comprising of 2-7 bands (210-2800 bp). Out of total 56 isolates, 54 were typable with a discriminatory power of 0.9336. This is the first report from India regarding RAPD profiling of *Arcobacter* spp. This study reveals epidemiological relationship of *Arcobacter* isolate from various sources and will help to design suitable prevention and control strategies for this important pathogen having public health significance.

Key words: *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirrowii*, enteropathogen, genotyping, RAPD-PCR, chicken, pork, human stool, poultry intestines

INTRODUCTION

The genus *Arcobacter* encompasses fastidious gam-negative, non-spore forming, spirally curved to 'S'-shaped rods and belongs to the family Campylobacteraceae that can grow microaerobically or aerobically. *Arcobacters* were first isolated from aborted bovine fetuses and later from porcine fetuses (Ellis *et al.*, 1977, 1978). *Arcobacters* have the ability to grow at 15 and 30°C, which is a distinctive feature that differentiates *Arcobacter* species from *Campylobacter* species

(Vandamme and De Ley, 1991; Lehner *et al.*, 2005). Nowadays, *Arcobacter* infections are reported from different parts of the world and *Arcobacters* have been recovered from a variety of foods of animal origin, water and human stool, hence *Arcobacters* are considered as emerging food-borne zoonotic pathogens (Douidah *et al.*, 2010; Patyal *et al.*, 2011; Ferreira *et al.*, 2013; Ramees *et al.*, 2014a, b; Mohan *et al.*, 2014). *Arcobacters* are associated with causing diarrhoea, mastitis and reproduction abnormalities in livestock animals and poultry (Houf *et al.*, 2002; Collado *et al.*, 2010; Bagalakote *et al.*, 2013). *Arcobacter* cause diarrhoea and intermittent septicaemia in human beings (Engberg *et al.*, 2000; Ramees *et al.*, 2014c).

To identify genetic diversity of microorganisms, various genotyping techniques have been reported to be useful viz., Randomly Amplified Polymorphic Dna-Polymerase Chain Reaction (RAPD-PCR), Amplified Fragment Length Polymorphism (AFLP), Repetitive extragenic palindromic-PCR (REP-PCR), Restriction Fragment Length Polymorphism (RFLP), Multilocus Sequence Typing (MLST), Pulsed Field Gel Electrophoresis (PFGE), Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR), gene sequencing based methods (Sanger method and pyrosequencing) and phylogenetic analysis (Rivas *et al.*, 2004; Figueras *et al.*, 2008; Merga *et al.*, 2011). The important utilities of the RAPD technique are reproducibility, typeability and discriminatory power (Power, 1996; Houf *et al.*, 2002). A significant genetic diversity among *Arcobacter* has been reported by many researchers from different sources (Collado *et al.*, 2010; Figueras *et al.*, 2012; Levican and Figueras, 2013). The present study was designed for assess the diversity and epidemiological relationship among *Arcobacter* spp. (*Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii*) isolated from different sources (chicken meat, pork, sheep faeces, goat faeces, poultry intestinal contents and human stool samples) from India using RAPD-PCR.

MATERIALS AND METHODS

***Arcobacter* isolates:** A total of 56 isolates (*A. butzleri* (33), *A. skirrowii* (03), *A. cryaerophilus* (20)), recovered from different sources (chicken meat, pork, sheep faeces, goat faeces, poultry intestinal contents and human stool samples), were used in this study (Table 1). All these isolates were maintained in Division of Veterinary Public Health, Indian Veterinary Research Institute Izatnagar, Bareilly, Uttar Pradesh, India.

DNA extraction: DNA extraction from *Arcobacter* isolates was done using commercially available DNA extraction kit, DNeasy Blood and Tissue Kit (QIAGEN, USA) following the manufacturer's protocol, isolated DNA were stored at -20°C till used.

RAPD-PCR profiling of *Arcobacter* isolates: RAPD-PCR was employed for genotyping and evaluating genetic diversity of the 56 isolates of three *Arcobacter* spp. (*A. butzleri, A. skirrowii, A. cryaerophilus*). Two primers, HLWL85: 5'-ACAACTGCTC-3' (Mazurier *et al.*, 1992) and OPA-11: 5'-CAATCGCCGT-3' (Hernandez *et al.*, 1995) were employed in the present study. RAPD-PCR was performed by slight modification of the protocol of Houf *et al.* (2002). Each PCR mixture consisted of 2.5 µL of 10x Taq buffer, 2.5 µL of 2 mM concentration of each dNTPs, 25 pmol of the primer, 1 µ of Taq polymerase, 3 µL of template DNA and nuclease-free water up to 25 µL. The amplification cycles included initial denaturation at 94°C for 5 min followed by 45 repeats of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 1 min 30 sec. Final extension was carried out at 72°C for 7 min.

Table 1: *Arcobacter butzleri, Arcobacter cryaerophilus* and *Arcobacter skirrowii* isolates used in the study

Arcobacter butzleri isolates	*Arcobacter cryaerophilus* isolates	*Arcobacter skirrowii* isolates
CM 10	CM 4	CM 1
CM 12	CM 6	SF 59
CM 61	CM 48	PI 6
CM 65	CM 70	
CM 76	CM 75	
CM 79	CM 83	
CM 86	CM 98	
CM 90	P 9	
CM 93	P 11	
CM 94	P 20	
CM 104	P 38	
P 4	P 48	
P 17	PI 1	
P 18	PI 47	
P 19	PI 48	
P 37	SF 23	
P 44	SF 62	
SF 12	SF 76	
SF 55	GF 15	
SF 15	GF 41	
SF 85		
SF 61		
PI 61		
PI 13		
PI 62		
PI 75		
PI 35		
GF 6		
GF 7		
GF 18		
HS 30		

CM: Chicken meat, P: Pork, SF: Sheep faeces, GF: Goat faeces, PI: Poultry intestinal contents, HS: Human stool

Gel electrophoresis and data analysis: The PCR products were characterized by gel electrophoresis on 1.5% agarose gel for 95 min at 80 V and stained with ethidium bromide (0.5 μg mL^{-1}) and visualized in gel documentation system. Approximately, 10 μL of the PCR product was loaded along with 100 bp plus ladder (GeneRuler 100 bp plus DNA Ladder, Fermentas, Canada) used as the molecular marker. The interpretation of results was done by pairwise binary band matching (Tenover *et al*., 1995). Only the distinct bands were considered for analysis by binary scoring pattern, wherein a score of 1 for the presence and 0 for absence of a band was assigned to each isolate. The dendrogram was created using the software TREECON for Windows v1.3b, Bioinformatics and Evolutionary Genomics, Belgium (Van de Peer and de Wachter, 1994). Dendrograms were constructed and analyzed separately for the three *Arcobacter* species. Numerical index of discrimination was calculated by Simpson's index of diversity (Hunter and Gaston, 1988).

RESULTS

Both the primers employed in this study yielded RAPD fragments in 54 out of 56 isolates giving a typeability of 96.4% (Table 2). Out of 33 *A. butzleri* isolates, primer 1 (HLWL85) yielded RAPD fragments in 31 isolates while 02 failed to show any RAPD fragments. RAPD fragments were present in all 20 *A. cryaerophilus* and 3 *A. skirrowii* isolates. The number of bands ranged between 2-8 and was compared between ~500-~3100 bp (Fig. 1-3). Distinct polymorphic bands at ~2200 bp were observed in CM 10, 12, 61, 68, 76, 79, 86, 90, 94, 104, P4, 19, 37, 17, 44, SF 12, 15, 55 *Arcobacter* isolates. Out of the 56 isolates, 54 typable strains and a total of 35 types were observed, giving a typeability of 96.4%. The discriminatory power of the primer was 0.9762 (Table 2). The profiles generated by RAPD-PCR using the primer 2 (OPA-11) comprised of 2-7 bands (Fig. 4-6), which were compared across the molecular weight of ~210-~2800 bp. Out of the total 56 isolates, 54 were typable, giving a typeability of 96.4%. Two isolates of *A. butzleri* failed to show any bands. There were a total of 29 RAPD types out of the 54 typable strains. A single largest cluster of 13 *Arcobacter* isolates was observed accounting for 23.21% of the isolates. The discriminatory power of this primer was 0.9336 (Table 2).

Table 2: Summary of RAPD typing technique

Primer	No. of RAPD types	Discriminatory value	Typeability (%)
PRIMER 1 (HLWL85)	35	0.9762	96.4
PRIMER 2 (OPA-11)	29	0.9336	96.4

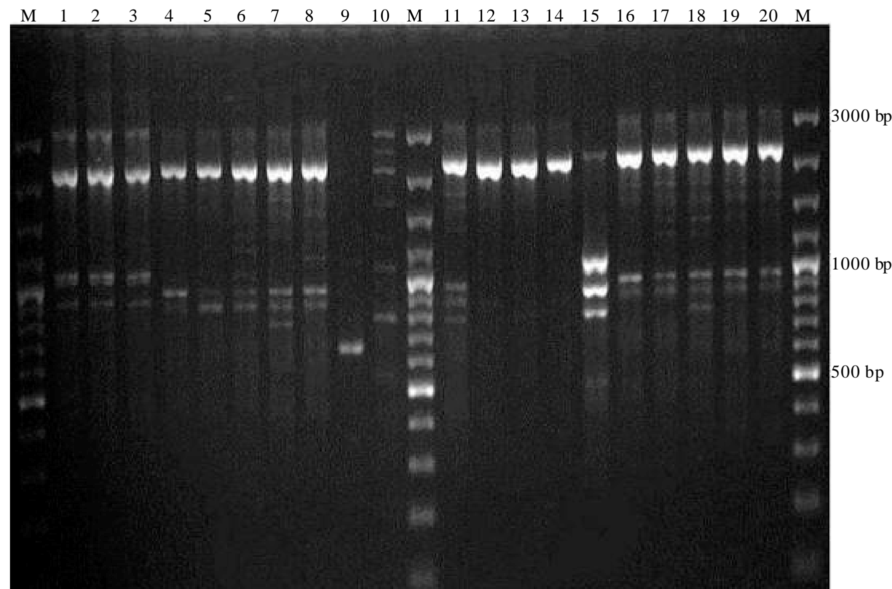

Fig. 1: The RAPD profiles obtained for *Arcobacter butzleri* isolates with HLWL85 primer. Lane M: 100 bp plus ladder, Lane 1: CM 10, Lane 2: CM 12, Lane 3: CM 61, Lane 4: CM 68, Lane 5: CM 76, Lane 6: CM 79, Lane 7: CM 86, Lane 8: CM 90, Lane 9: CM93, Lane 10: CM 94, Lane 11: CM 104, Lane 12: P 4, Lane 13: P19, Lane 14: P 37, Lane 15: P 18, Lane 16: P 17, Lane 17: P 44, Lane 18: SF 12, Lane 19: SF 15, Lane 20: SF 55

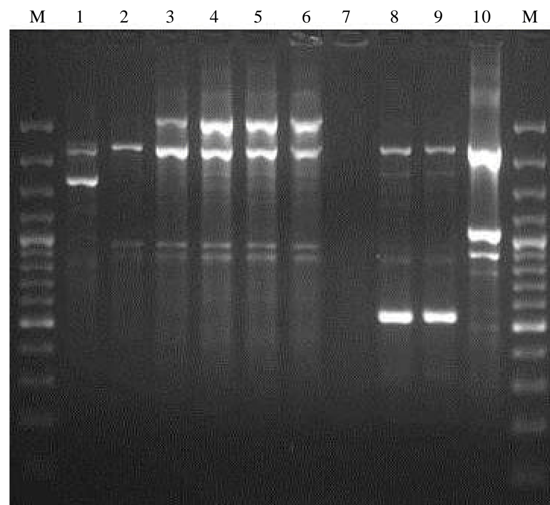

Fig. 2: RAPD profiles obtained for *Arcobacter cryaerophilus* isolates with HLWL85 primer. Lane M: 100 bp plus ladder, Lane 1: CM 4, Lane 2: CM 6, Lane 3: CM 48, Lane 4: CM 70, Lane 5: CM 75, Lane 6: CM 83, Lane 7: CM 98, Lane 8: P 9, Lane 9: P 11, Lane 10: P 20

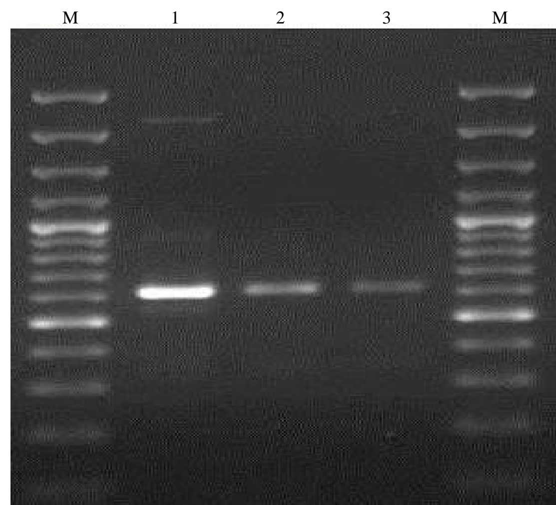

Fig. 3: RAPD profiles obtained for *Arcobacter skirrowii* isolates with HLWL85 primer. Lane M: 100 bp plus ladder, Lane 1: CM 1, Lane 2: SF 59, Lane 3: PI 6

Dendogram showed clustering of *Arcobacter* isolates in same group from different sources indicative of their epidemiological relationship. A significant genetic diversity was observed from the different and same sources of *Arcobacters* (Fig. 7-12).

DISSCUSION

In recent years, *Arcobacters* are considered as potential emerging food and water-borne pathogens. They are increasingly being isolated from a wide range of food products all over the world (Houf *et al.*, 2000; Patyal *et al.*, 2011; Dhama *et al.*, 2013; Ramees *et al.*, 2014a). In the last

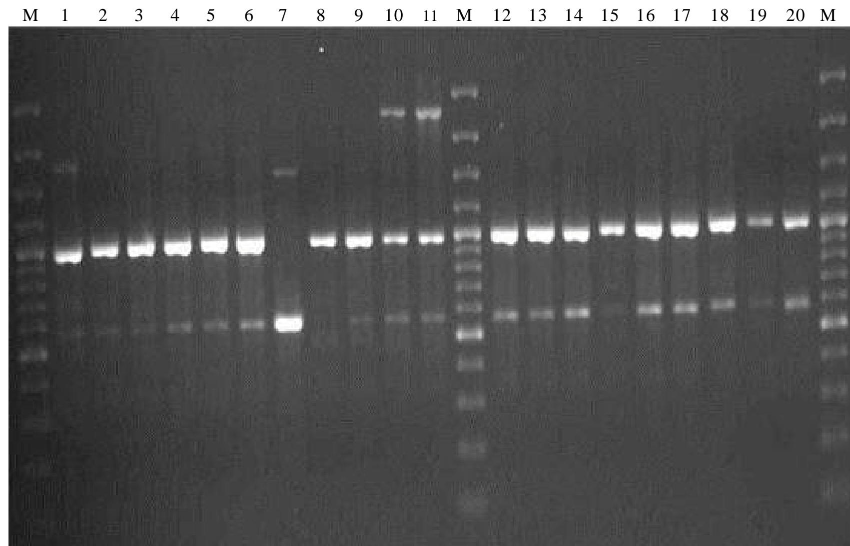

Fig. 4: RAPD profiles obtained for *Arcobacter butzleri* isolates with OPA-11 primer. Lane M: 100 bp plus ladder , Lane 1: CM 10, Lane 2: CM 12, Lane 3: CM 61, Lane 4: CM 65, Lane 5: CM 76, Lane 6: CM 79, Lane 7: CM 86, Lane 8: CM 90, Lane 9: CM93, Lane 10: PI 61, Lane 11: CM 104, Lane 12: P 4, Lane 13: P19, Lane 14: P 37, Lane 15: P 18, Lane 16: P 17, Lane 17: P 44, Lane 18: SF 12, Lane 19: SF 15, Lane 20: SF 55

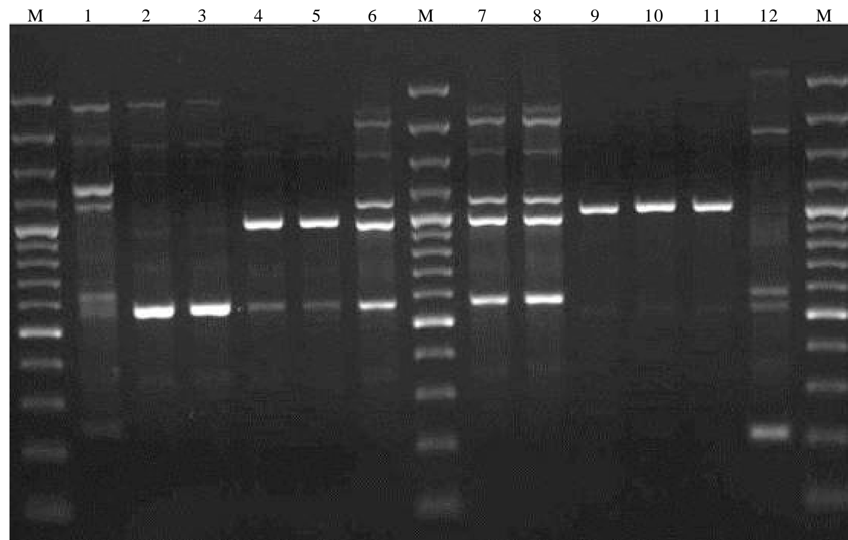

Fig. 5: RAPD profiles obtained for *Arcobacter cryaerophilus* isolates with OPA-11 primer. Lane M: 100 bp plus ladder, Lane 1: CM 4, Lane 2: CM 6, Lane 3: CM 48, Lane 4: CM 70, Lane 5: CM 75, Lane 6: CM 83, Lane 7: CM 98, Lane 8: P 9, Lane 9: P 11, Lane 10: P 20, Lane 11: P 38, Lane 12: P 48

5 years, the number of new species has risen exponentially due to the application of molecular techniques such as multiplex-PCR, 16S rRNA gene-RFLP, sequencing of the 16S rRNA gene, ERIC-PCR, AFLP and Pulsed-Field Gel Electrophoresis (PFGE) (Hume *et al.*, 2001; On *et al.*, 2004;

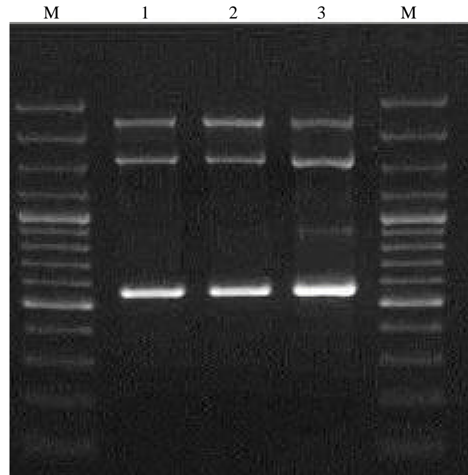

Fig. 6: RAPD profiles obtained for *Arcobacter skirrowii* isolates with OPA-11 primer. Lane M: 100 bp plus ladder, Lane 1: CM 1, Lane 2: SF 59, Lane 3: PI 6

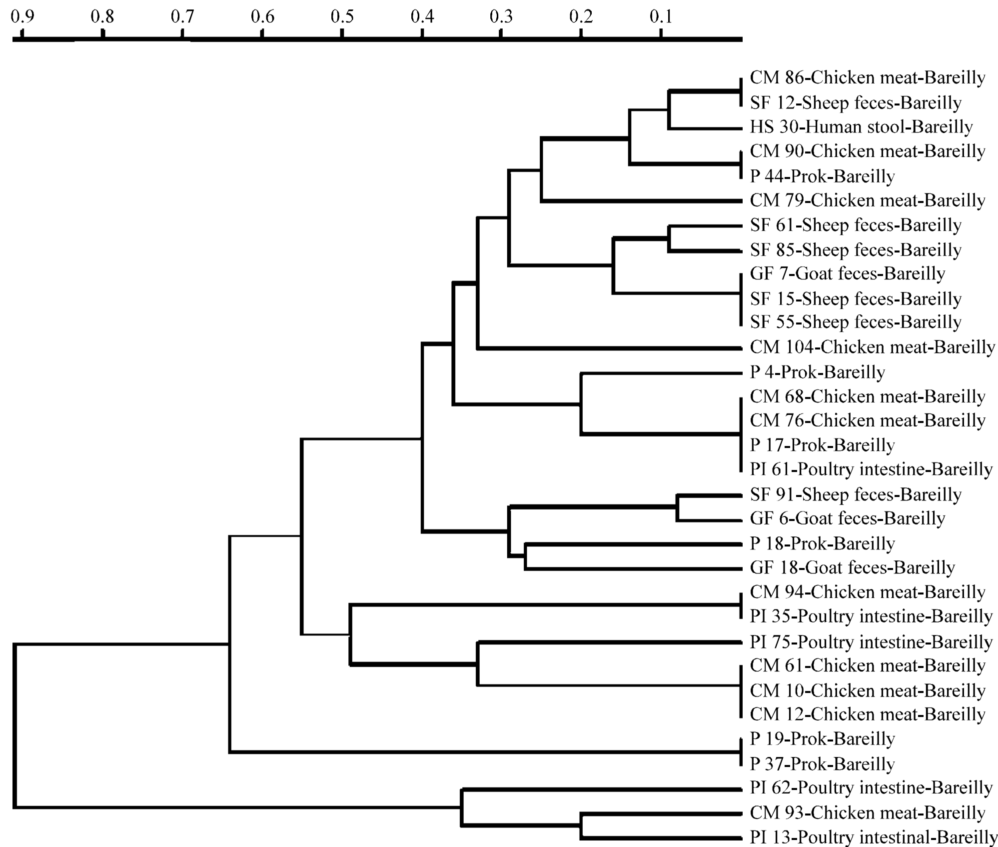

Fig. 7: Dendrogram showing RAPD types obtained for *Arcobacter butzleri* isolates amplified with HLWL85 primer

Quinones *et al*., 2007; Collado and Figueras, 2011; Kayman *et al*., 2012). The present study reports the diversity and epidemiological relationship among *Arcobacter* spp. isolated from different sources from India using RAPD-PCR.

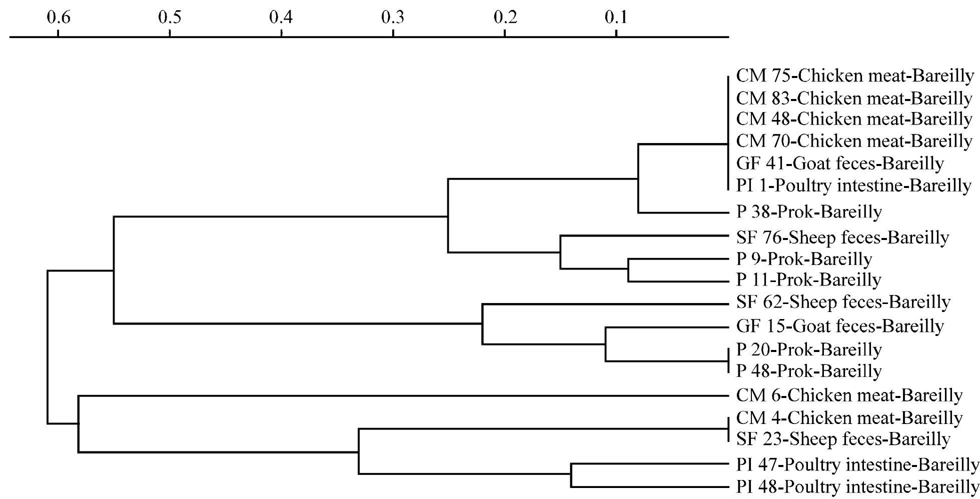

Fig. 8: Dendrogram showing RAPD types obtained for *Arcobacter cryaerophilus* isolates amplified with HLWL85 primer

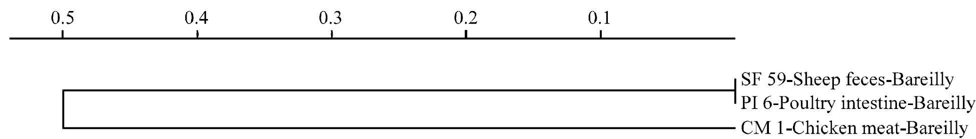

Fig. 9: Dendrogram showing RAPD types obtained for *Arcobacter skirrowii* isolates amplified with HLWL85 primer

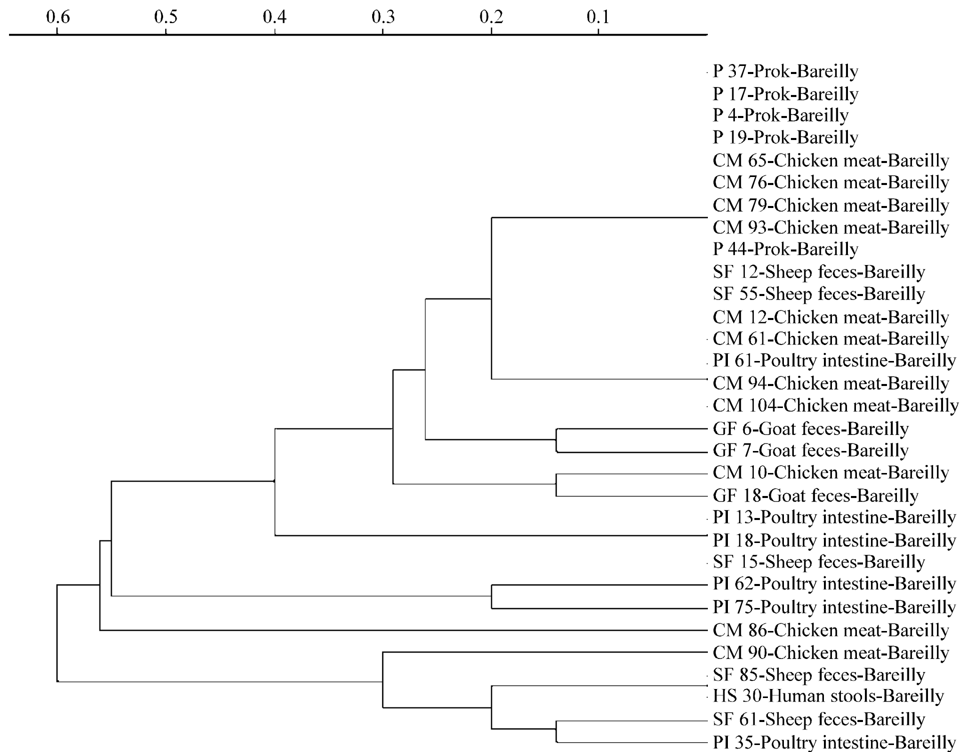

Fig. 10: Dendrogram showing RAPD types obtained for *Arcobacter butzleri* isolates amplified with OPA-11 primer

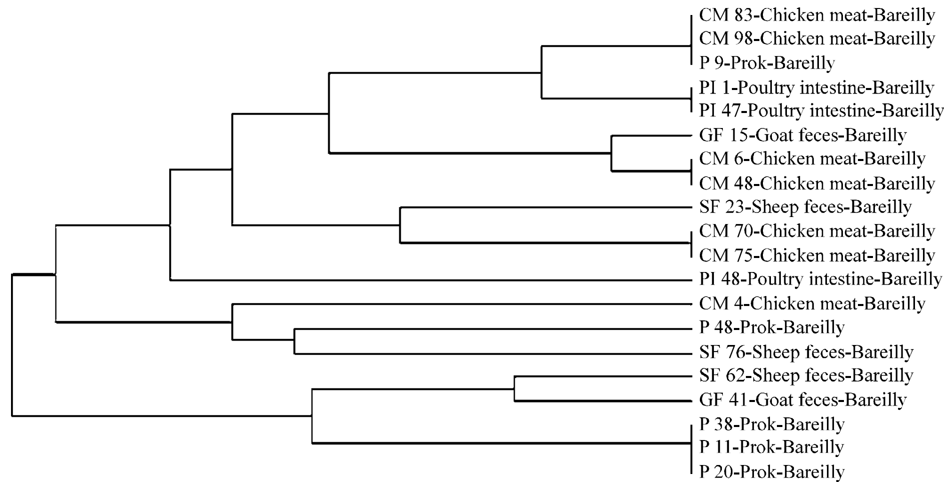

Fig. 11: Dendrogram showing RAPD types obtained for *Arcobacter cryaerophilus* isolates amplified with OPA-11 primer

Fig. 12: Dendrogram showing RAPD types obtained for *Arcobacter skirrowii* isolates amplified with OPA-11 primer

Using primer 1 (HLWL85) RAPD-PCR, yielded bands ranging between 2-8 (500-3100 bp) and of the 56 *Arcobacter* isolates, 54 typable strains and a total of 35 types were observed, e with discriminatory power of 0.9762. Recently, Suelam (2012) reported 9 genotypes out of 10 *Arcobacter* isolates recovered from rabbit. In an earlier study by Houf *et al.* (2002), using a universal random primer 5'-GGTGCGGGAA-3' high genetic diversity among *Arcobacter* spp. was reported. AFLP profiling of 73 isolates of *A. butzleri* from different sources (human infections, chickens, turkeys, ducks, sheep and poultry abbatoir effluent) distinguished 51 subtypes and the similarity level was 91%, of which 39 included single strains. The remaining 34 isolates were scattered among 12 subtypes, each of which contained strains homogeneous with respect to their respective source of isolation (On *et al.*, 2004). In a total of 72 strains of *Arcobacter* subjected to AFLP profiling 62 distinct types were defined, with evidence of clonal lineages within *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* and a new taxon identified (On *et al.*, 2003). Among *Arcobacter* isolates, there was a significant level of variation reported from a Farrow-to-Finish Swine Facility (Hume *et al.*, 2001). In dendogram analysis, the clustering pattern of some of the *A. butzleri* isolates (CM 68, 76, P 17 and 61) of chicken meat, pork and poultry intestinal content origin in the same group indicated possible homogeneity and their phylogenic relationship. Similarly, some of the *A. cryaerophilus* isolates (CM 48, 7, 75, 83, GF 41 and PI 1) of chicken meat, goat fecal sample and poultry intestinal content showed origin in the same group. A significant level of homogeneity among *Arcobacter* species from same sources has also been reported previously (Aydin *et al.*, 2007). Isolate of *A. butzleri* from cattle of beef and dairy farms in the North West of England showed significant diversity using multilocus sequence typing (Merga *et al.*, 2013).

A. cryaerophilus isolates from pork, sheep faeces and goat faeces did not cluster with other isolates, indicating the possibility of heterogeneity as per the source of isolates. Shah *et al.* (2012) reported12 different clusters consisting of 29 different PFGE patterns within the *Arcobacter* species using PFGE technique, which is indicative of diversity among the *Arcobacter* isolates. Fingerprints data generated by RAPD-PCR in the present study showed 21 genotypes of *A. butzleri* and 14 genotypes of *A. cryaerophilus*, which showed genomic diversity within the *Arcobacter* species. Earlier studies have also reported *Arcobacers* to be having high genetic diversity within and between the species (Atabay *et al.*, 1998, 2006; Collado *et al.*, 2010; Kayman *et al.*, 2012). However, a study using PFGE reported homogeneity among the *A. butzleri* isolates, indicative of common source of contamination (Rivas *et al.*, 2004). A number of closely related *A. butzleri* and *A. cryaerophilus* isolates were found from chicken meat samples which indicate cross contamination of common type of *Arcobacter*. A high genetic diversity among *Arcobacter* spp. and their continuous evolving nature has been reported recently from United Kingdom, using MLST technique (Merga *et al.*, 2011). Characterization of 13 *A. cryaerophilus* and 10 *A. butzleri* isolates by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) resulted in 10 and 5 different genotypes, respectively (De Smet *et al.*, 2010).

Primer 2 (OPA-11) yielded RAPD-PCR profiles comprising of 2 to 7 bands (210-2800 bp) and of total 56 isolates, 54 were typable, giving a typeability of 96.4%. Two isolates of *A. butzleri* failed to show any band. There were a total of 29 RAPD types out of the 54 typable strains. A single largest cluster of 13 *Arcobacter* isolates was observed accounting for 23.21% of the isolates is showing homogeneity among *Arcobacter* spp. The discriminatory power of this primer was 0.9336. Both the primers yielded a satisfactory typeability and discriminatory power, which indicated RAPD-PCR to be a highly desirable genotyping method. *Arcobacter* isolates (15) from domestic geese were evaluated for RAPD, wherein 7 *A. cryaerophilus*, 2 *A. butzleri* and 6 *A. skirrowii* isolates produced 6, 2 and 3 distinct profiles, respectively. These showed high heterogeneity among *Arcobacter* spp. supporting previous study. From the same flocks, the isolates showed same patterns (Atabay *et al.*, 2008). A wide variation of *Arcobacter* isolate has been reported from poultry (Houf *et al.*, 2002). Nine isolates of *A. butzleri* obtained from diarrheal patients have been reported to show different ERIC-PCR profiles (Kayman *et al.*, 2012).

In dendogram analysis, clustering together of one of the *A. butzleri* isolate of sheep feces and human stool in the same group indicated similarity in phylogeny and possibility of zoonotic nature of *Arcobacters*. The presence of multiple parent genotypes for the three important *Arcobacter* spp. (*A. butzleri*, *A. cryaerophilus* and *A. skirrowii*) and high genetic recombinations between the progeny of parent genotypes may be reasons for huge amount of heterogeneity in *Arcobacters*, this is indicative of a multiple source contamination events to be happening (Houf *et al.*, 2002; Aydin *et al.*, 2007). *Arcobacter butzleri* isolates (92) from different sources gave 13 distinct DNA profiles and some of the isolates originated from different sources contributed the same DNA profiles (Aydin *et al.*, 2007). The possible explanations for the large amount of heterogeneity include multiple sources of contamination, the presence of multiple parent genotypes for all the three species in a single animal and a high degree of genomic recombination among the progeny of parent genotypes (Houf *et al.*, 2002; Collado and Figueras, 2011). The present study reporting for the first time the genotyping and diversity of *Arcobacter* spp. recovered from different sources (chicken meat, pork, poultry intestines, sheep feces, goat feces and human stools) from India adds to the heterogeneity reports among *Arcobacter* species worldwide, supporting diversity among same species.

CONCLUSION

The present study reports for the first time the significant rate of genetic diversity among *Arcobacter* spp. recovered from different sources from India using RAPD-PCR, which is a very rapid tool for such studies. The results revealed that both the primers used (HLWL85, OPA-11) yielded a satisfactory typeability and discriminatory power and the high genetic diversity was observed among the *Arcobacter* species. The analysis of *Arcobacter* isolates showed that single host may harbour not only more than one species but also multiple genotypes. *Arcobacters* showing close clustering between human and animal origin are indicative of zoonotic and public health concerns, for which further explorative studies are needed to reveal more information about the organism as such and to combat any adversary effect of *Arcobacters* in future.

REFERENCES

Atabay, H.I., A. Unver, S. Otlu and A.T. Kalaycioglu, 2008. Genotyping of various *Arcobacter* species isolated from domestic geese by Randomly Amplified Polymorphic DNA (RAPD) analysis. Archiv Lebensmittelhygiene, 59: 84-88.

Atabay, H.I., J.E.L. Corry and S.L.W. On, 1998. Diversity and prevalence of *Arcobacter* spp. in broiler chickens. J. Applied Microbiol., 84: 1007-1016.

Atabay, H.I., M. Waino and M. Madsen, 2006. Detection and diversity of various *Arcobacter* species in Danish poultry. Int. J. Food Microbiol., 109: 139-145.

Aydin, F., K.S. Gumussoy, H.I. Atabay, T. Ica and S. Abay, 2007. Prevalence and distribution of *Arcobacter* species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR. J. Applied Microbiol., 103: 27-35.

Bagalakote, P.S., R.S. Rathore, T.P. Ramees, H.V. Mohan and M. Sumankumar *et al*., 2013. Study on prevalence of *Arcobacter* spp. in human and foods of animal origin. J. Vet. Public Health, 11: 105-109.

Collado, L. and M.J. Figueras, 2011. Taxonomy, epidemiology and clinical relevance of the genus *Arcobacter*. Clin. Microbiol. Rev., 24: 174-192.

Collado, L., G. Kasimir, U. Perez, A. Bosch and R. Pinto *et al*., 2010. Occurrence and diversity of *Arcobacter* spp. along the Llobregat River catchment, at sewage effluents and in a drinking water treatment plant. Water Res., 44: 3696-3702.

De Smet, S., L. de Zutter, J. van Hende and K. Houf, 2010. *Arcobacter* contamination on pre- and post-chilled bovine carcasses and in minced beef at retail. J. Applied Microbiol., 108: 299-305.

Dhama, K., S. Rajagunalan, S. Chakraborty, A.K. Verma, A. Kumar, R. Tiwari and S. Kapoor, 2013. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: A review. Pak. J. Biol. Sci., 16: 1076-1085.

Douidah, L., L.D. Zutter, P. Vandamme and K. Houf, 2010. Identification of five human and mammal associated *Arcobacter* species by a novel multiplex-PCR assay. J. Microbiol. Methods, 80: 281-286.

Ellis, W.A., S.D. Neill, J.J. O'Brien and J. Hanna, 1978. Isolation of spirillum-like organisms from pig fetuses. Vet. Rec., 102: 106-106.

Ellis, W.A., S.D. Neill, J.J. O'Brien, H.W. Ferguson and J. Hanna, 1977. Isolation of spirillum/vibrio-like organisms from bovine fetuses. Vet. Rec., 100: 451-452.

Engberg, J., S.L. On, C.S. Harrington and P. Gerner-Smidt, 2000. Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter* and *Sutterella* spp. in human fecal samples as estimated by a reevaluation of isolation methods for Campylobacters. J. Clin. Microbiol., 38: 286-291.

Ferreira, S., M.J. Fraqueza, J.A. Queiroz, F.C. Domingues and M. Oleastro, 2013. Genetic diversity, antibiotic resistance and biofilm-forming ability of *Arcobacter butzleri* isolated from poultry and environment from a Portuguese slaughterhouse. Int. J. Food Microbiol., 162: 82-88.

Figueras, M.J., A. Levican and L. Collado, 2012. Updated 16S rRNA-RFLP method for the identification of all currently characterised *Arcobacter* spp. BMC Microbiol., Vol. 12. 10.1186/1471-2180-12-292

Figueras, M.J., L. Collado and J. Guarro, 2008. A new 16S rDNA-RFLP method for the discrimination of the accepted species of *Arcobacter*. Diagn. Microbiol. Infect. Dis., 62: 11-15.

Hernandez, J., A. Fayos, M.A. Ferrus and R.J. Owen, 1995. Random amplified polymorphic DNA fingerprinting of *Campylobacter jejuni* and *C. Coli* isolated from human faeces, seawater and poultry products. Res. Microbiol., 146: 685-696.

Houf, K., A. Tutenel, L. De Zutter, J. van Hoof and P. Vandamme, 2000. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Immunol. Med. Microbiol., 49: 337-345.

Houf, K., L. De Zutter, J. Van Hoof and P. Vandamme, 2002. Assessment of the genetic diversity among *Acobacters* isolated from poultry products by using two PCR-based typing methods. Applied Environ. Microbiol., 68: 2172-2178.

Hume, M.E., R.B. Harvey, L.H. Stanker, R.E. Droleskey, T.E. Poole and H.B. Zhang, 2001. Genotypic variation among *Arcobacter* isolates from a farrow-to-finish swine facility. J. Food Prot., 64: 645-651.

Hunter, P.R. and M.A. Gaston, 1988. Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. J. Clin. Microbiol., 26: 2465-2466.

Kayman, T., S. Abay, H. Hizlisoy, H.I. Atabay, K.S. Diker and F. Aydin, 2012. Emerging pathogen *Arcobacter* spp. in acute gastroenteritis: Molecular identification, antibiotic susceptibilities and genotyping of the isolated *Acobacters*. J. Med. Microbiol., 61: 1439-1444.

Lehner, A., T. Tasara and R. Stephan, 2005. Relevant aspects of *Arcobacter* spp. as potential foodborne pathogen. Int. J. Food Microbiol., 102: 127-135.

Levican, A. and M.J. Figueras, 2013. Performance of five molecular methods for monitoring *Arcobacter* spp. BMC Microbiol., Vol. 13. 10.1186/1471-2180-13-220

Mazurier, S., V. de Giessen, K. Heuvelman and K. Wernars, 1992. RAPD analysis of Campylobacter isolates: DNA fingerprinting without the need to purify DNA. Lett. Applied Microbiol., 14: 260-262.

Merga, J.Y., A.J.H. Leatherbarrow, C. Winstanley, M. Bennett, C.A. Hart, W.G. Miller and N.J. Williams, 2011. Comparison of *Arcobacter* isolation methods and diversity of *Arcobacter* spp. in Cheshire, United Kingdom. Applied Environ. Microbiol., 77: 1646-1650.

Merga, J.Y., N.J. Williams, W.G. Miller, A.J. Leatherbarrow and M. Bennett *et al*., 2013. Exploring the diversity of *Arcobacter butzleri* from cattle in the UK using MLST and whole genome sequencing. PLoS ONE, Vol. 8. 10.1371/journal.pone.0055240

Mohan, H.V., R.S. Rathore, K. Dhama, T.P. Ramees and A. Patyal *et al*., 2014. Prevalence of *Arcobacter* spp. in humans, animals and foods of animal origin in India based on cultural isolation, antibiogram, PCR and multiplex PCR detection. Asian J. Anim. Vet. Adv., 9: 452-466.

On, S.L., C.S. Harrington and H.I. Atabay, 2003. Differentiation of *Arcobacter* species by numerical analysis of AFLP profiles and description of a novel *Arcobacter* from pig abortions and turkey faeces. J. Applied Microbiol., 95: 1096-1105.

On, S.L., H.I. Atabay, K.O. Amisu, A.O. Coker and C.S. Harrington, 2004. Genotyping and genetic diversity of *Arcobacter butzleri* by Amplified Fragment Length Polymorphism (AFLP) analysis. Lett. Applied Microbiol., 39: 347-352.

Patyal, A., R.S. Rathore, H.V. Mohan, K. Dhama and A. Kumar, 2011. Prevalence of *Arcobacter* spp. in humans, animals and foods of animal origin including sea food from India. Transboundary Emerg. Dis., 58: 402-410.

Power, E.G.M., 1996. RAPD typing in microbiology: A technical review. J. Hosp. Infec., 34: 247-265.

Quinones, B., C.T. Parker, J.M. Janda Jr., W.G. Miller and R.E. Mandrell, 2007. Detection and genotyping of *Arcobacter* and *Campylobacter* isolates from retail chicken samples by use of DNA oligonucleotide arrays. Applied Environ. Microbiol., 73: 3645-3655.

Ramees, T.P., R.S. Rathore, P.S. Bagalkot, H.V. Mohan, A. Kumar and K. Dhama, 2014a. Multiplex PCR detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in skin of poultry. J. Pure Applied Microbiol., 8: 1755-1758.

Ramees, T.P., R.S. Rathore, P.S. Bagalkot, H.V. Mohan, A. Kumar and K. Dhama, 2014b. Detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in clinical samples of humans and foods of animal origin by cultural and multiplex PCR based methods. Asian J. Anim. Vet. Adv., 9: 243-252.

Ramees, T.P., R.S. Rathore, P.S. Bagalkot, G.V.P.P.S Ravi Kumar and H.V. Mohan *et al*., 2014c. Real-time PCR detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in chicken meat samples. J. Pure Applied Microbiol., Vol. 8, No. 4.

Rivas, L., N. Fegan and P. Vanderlinde, 2004. Isolation and characterisation of *Arcobacter butzleri* from meat. Int. J. Food Microbiol., 91: 31-41.

Shah, A.H., A.A. Saleha, Z. Zunita, Y.K. Cheah, M. Murugaiyah and N.A. Korejo, 2012. Genetic characterization of *Arcobacter* isolates from various sources. Vet. Microbiol., 160: 355-361.

Suelam, I.I.A., 2012. Isolation and identification of *Arcobacter* species recovered from rabbits in Zagazig, Egypt. Int. J. Microbiol. Res., 3: 87-92.

Tenover, F.C., R.D. Arbeit, R.V. Goering, P.A. Mickelsen, B.E. Murray, D.H. Persing and B. Swaminathan, 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J. Clin. Microbiol., 33: 2233-2239.

Van de Peer, Y. and R. de Wachter, 1994. TREECON for Windows: A software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Applied Biol. Sci., 10: 569-570.

Vandamme, P. and J. De Ley, 1991. Proposal for a new family, *Campylobacteraceae*. Int. J. Syst. Bacteriol., 41: 451-455.