Genetic Diversity of *Arcobacter* Species of Animal and Human Origin in Andhra Pradesh, India

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Abstract *Arcobacter* is an emerging foodborne pathogen having zoonotic significance. Enterobacterial repetitive intergenic consensus (ERIC) PCR and repetitive sequence-based PCR (rep-PCR) analysis of a total of 41 *Arcobacter* isolates revealed a greater degree of genetic diversity. ERIC-PCR genotyping distinguished 14, 13 and 12 genotypes among 16, 13 and 12 isolates of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, respectively. Rep-PCR genotyping distinguished 15, 12 and 11 genotypes among 16, 13 and 12 isolates of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, respectively. The discriminatory power for ERIC and rep-PCR was found to be 0.997 and 0.996, respectively. Close clustering between isolates of animal and human origin are indicative of probable zoonotic significance.

Keywords *Arcobacter* · Discriminatory power · ERIC-PCR · Genetic diversity · rep-PCR

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Arcobacter is an emerging food borne pathogen, of which *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* have been frequently isolated from different livestock species, foods of animal origin as well as humans [1]. PCR based genotyping techniques such as enterobacterial repetitive intergenic consensus (ERIC)-PCR and repetitive sequence-based PCR (rep-PCR) have been successfully applied in various epidemiological studies for the characterization of genetic diversity of *Arcobacters* below species level [2–4]. The present study was carried out to assess the genetic diversity of 41 *Arcobacter* isolates (16 *A. butzleri*, 13 *A. cryaerophilus* and 12 *A. skirrowii*) recovered from diverse sources like faecal swabs of livestock (21), raw foods of animal origin (13) and diarrhoeic humans (7) in Andhra Pradesh (India). The reference strain of *A. butzleri* (ATCC 49616) as well as positive DNA standards of *A. cryaerophilus* and *A. skirrowii* used in the present study were obtained from Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, India.

ERIC-PCR fingerprinting was carried out using the primer pair 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') in 25 µl optimized reaction mixture under the following standardized thermal cycling conditions: initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 94 °C for 1 min, annealing at 25 °C for 1 min, elongation at 72 °C for 2 min, final elongation at 72 °C for 10 min [4]. Rep-PCR fingerprinting was carried out using single oligonucleotide primer (GTG)₅ (5'-GTG GTG GTG GTG GTG-3') in 25 µl optimized reaction mixture under the following standardized thermal cycling conditions: initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 94 °C for 45 s, annealing at 40 °C for 1 min, elongation at 65 °C for 10 min, final elongation at 65 °C for 20 min [2]. Gel electrophoresis was carried out

and banding patterns were visualized using BIO-RAD Gel Documentation system (USA). Presence of a particular band in a strain was coded as 1 and the absence of that particular band was coded as 0 in a binary matrix. The binary data was analysed using dollop programme of phylip version 3.6 software and dendrograms were constructed. Numerical index of discrimination was calculated by Simpson's index of diversity [5].

Of the 16 *A. butzleri* isolates analyzed, 14 ERIC-PCR patterns (E₁ to E₁₄) and 15 rep-PCR patterns (R₁ to R₁₅) were obtained. A pair of *A. butzleri* isolates that had identical ERIC-PCR pattern (E₃) also had indistinguishable rep-PCR pattern (R₃). However, a pair of *A. butzleri* isolates that had identical ERIC-PCR pattern (E₇) were distinguished by rep-PCR into two different genotypes (R₇ and R₈). The two *A. butzleri* isolates sharing identical ERIC-PCR and rep-PCR pattern (E₃ and R₃) were recovered from chicken meat samples (CM 13 and CM 18) of different birds slaughtered in the same chicken retail shop, indicating the possibility of cross-contamination of carcasses during slaughter process. Cluster analysis of ERIC and rep-PCR profiles differentiated *A. butzleri* isolates from different sources into two clusters (cluster I, with isolates of human origin and cluster II, with isolates of

animal origin) (Supplementary Fig. 1 and 2). However, under rep-PCR cluster analysis, an isolate recovered from cattle faeces clustered into human group and isolates from a pig farm worker and a diarrhoeic human clustered into animal group (Supplementary Fig. 2), indicating the possibility of zoonotic nature of *Arcobacters*.

Of the 13 *A. cryaerophilus* isolates analyzed, 13 ERIC-PCR patterns (E₁ to E₁₃) and 12 rep-PCR patterns (R₁ to R₁₂) were obtained. A pair of *A. cryaerophilus* isolates that had same rep-PCR pattern (R₆) were distinguished by ERIC-PCR into two different genotypes (E₆ to E₇). Cluster analysis of ERIC and rep-PCR profiles differentiated *A. cryaerophilus* isolates from different sources into two clusters (cluster I, with isolates of foods of animal origin and cluster II, with isolates of animal faecal origin) (Supplementary Fig. 3 and 4). Of the 12 *A. skirrowii* isolates analyzed, 12 ERIC-PCR patterns (E₁ to E₁₂) and 11 rep-PCR patterns (R₁ to R₁₁) were obtained. A pair of *A. skirrowii* isolates that had same rep-PCR pattern (R₇) were distinguished by ERIC-PCR into two different genotypes (E₇ to E₈). Cluster analysis of ERIC and rep-PCR profiles differentiated *A. skirrowii* isolates from different sources into three clusters (cluster I with isolates of sheep faecal origin and mutton samples, cluster II with isolates of pig

Table 1 Genetic diversity of *Arcobacter* isolates from different sources

Species	Source	Number of isolates	Number of ERIC-types	Number of rep-types
<i>Arcobacter butzleri</i> (n = 16)	Poultry faeces	2	2	2
	Pig faeces	2	1	2
	Cattle faeces	1	1	1
	Chicken meat	2	1	1
	Pork	1	1	1
	Milk	1	1	1
	Veterinary students	2	2	2
	Farm workers	3	3	3
	Diarrhoeic humans	2	2	2
	Total	16	14	15
<i>Arcobacter cryaerophilus</i> (n = 13)	Poultry faeces	2	2	2
	Pig faeces	3	3	2
	Cattle faeces	1	1	1
	Chicken meat	3	3	3
	Pork	3	3	3
	Milk	1	1	1
	Total	13	13	12
<i>Arcobacter skirrowii</i> (n = 12)	Poultry faeces	5	5	5
	Pig faeces	3	3	2
	Sheep faeces	2	2	2
	Mutton	2	2	2
	Total	12	12	11
Grand total		41	39	38

faecal origin and cluster III with isolates of poultry faecal origin) (Supplementary Fig. 5 and 6).

A greater degree of heterogeneity was observed among 41 *Arcobacter* isolates from different sources as revealed by the presence of 39 and 38 different genotypes under ERIC-PCR and rep-PCR analysis, respectively (Table 1). In a study from Belgium using ERIC-PCR, 3, 81 and 4 genotypes were distinguished among 5, 164 and 13 isolates of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*, respectively [6]. Other studies reported 248 genotypes out of 275 *A. butzleri* isolates and 60 genotypes out of 63 *A. cryaerophilus* isolates [7] and 9 genotypes out of 10 *Arcobacter* isolates [8]. In the present study, the discriminatory power of the two typing methods was found almost identical i.e. 0.997 and 0.996, for ERIC-PCR and rep-PCR, respectively. This was in accordance with earlier studies [2, 3] and discriminatory powers above 0.90 are considered highly significant [5]. The present study reporting the genotyping and genetic diversity of *Arcobacter* species adds to the heterogeneity reports among *Arcobacter* species worldwide. The rep-PCR dendrogram analysis also indicated the genetic similarity among *A. butzleri* isolates recovered from livestock farm workers, diarrhoeic humans and healthy animals, revealing the possibility of zoonotic transmission.

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