REPETITIVE EXTRAGENIC PALINDROMIC-PCR (REP-PCR) TYPING OF *CAMPYLOBACTER JEJUNI* ISOLATED FROM POULTRY

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Revised Received on: 12.07.2017
Accepted on: 09.10.2017

ABSTRACT

Repetitive extragenic palindromic-PCR (REP-PCR) is polymerase chain reaction (PCR) amplification of noncoding, multi-locus, interspersed repetitive DNA elements by a set of defined primers in prokaryotic genomes. In the present study, typing of 43 *Campylobacter jejuni* isolates from poultry was carried out by REP-PCR. It showed 100% type ability amongst the isolates with production of 12 different REP patterns (REP1–REP12) on the basis of different arrangements of band sizes ranging from 80 to 700 bp. The discriminatory power of REP-PCR method was recorded as 0.9181. The REP8 pattern comprised maximum number of 7 isolates whereas REP4 and REP9 patterns had a single isolate only. The number of bands produced in all REP patterns ranged from one to four where maximum numbers of four bands were produced in REP11 and REP12 with different band sizes. Single amplicon of different sizes were produced by REP1, REP2 and REP3 patterns. In the present study REP-PCR was found to be an appropriate method for *C. jejuni* typing.

Key words: *Campylobacter* species, *C. jejuni*, poultry, genotyping, REP-PCR

Introduction

Campylobacter jejuni is considered to be one of the most common causes of food borne gastroenteritis in animals and humans worldwide (Silva *et al.,* 2011). It can colonize intestinal tract of various animal species such as cattle, buffalo, sheep, goat and pigs but poultry is considered to be its major reservoir (Coward *et al.,* 2008). It is also a major concern for public health due to its zoonotic potential through consumption of poultry products which is an important risk factor for human Campylobacteriosis (Epps *et al.,* 2013). Therefore, typing of the field isolates is important for differentiating and identifying various strains molecularly (Khoshbakht *et al.,* 2013).

Several genetic typing methods have been used to differentiate *C. jejuni* strains. The currently available methods for typing of *C. jejuni* are generally classified as gel based methods, sequence based typing schemes, microarrays, genotyping by real-time PCR analysis and high resolution melt analysis (Wassenaar and Newell, 2000; Boxrud, 2010; Guerin *et al.,* 2010 and Sheppard *et al.,* 2009). Multiple copies of conserved repetitive DNA sequences get continuously dispersed in naturally occurring bacterial genomes of various species. These sequences could have a role in genome evolution (Gilson *et al.,* 1984). Such sequences are targeted by a defined set of primers in REP-PCR which enables amplification of different sized DNA fragments consisting of sequences lying between these elements. Multiple amplicons of different sizes can be separated by gel electrophoresis resulting in generation of specific fingerprinting pattern for individual bacterial clones (Versalovic *et al.,* 1994). Three families of repetitive sequences: the 35-40-bp repetitive extragenic palindromic (REP) sequence, the 124-127-bp enterobacterial repetitive intergenic consensus (ERIC) sequence, and the 154-bp BOX element sequences (Stern *et al.,* 1984; Lupski and Weinstock, 1992) have frequently been used for DNA finger printing. Repetitive extragenic palindromic-PCR (REP-PCR) is an amplification-based method that targets known, conserved, repetitive DNA sequences that are usually present in bacterial genomes as multiple copies (Versalovic *et al.* 1991; Lupski and Weinstock 1992; Hiett *et al.,* 2006). REP sequences are around 75% shorter than ERIC sequences, often several REP sequences are found in one area of a genome, while ERIC sequences only occur individually (Koeuth *et al.,* 1995).

Repetitive extragenic palindromic PCR (REP-PCR) can be optimized for typing of *C. jejuni* from various sources including all food-producing animals, pets, poultry products and humans. The REP-PCR divide isolates into spatially and temporally relevant epidemiological groups (Hiett *et al.*, 2006). Therefore, the objective of this study was to investigate the utility of repetitive extragenic palindromic PCR (REP-PCR) for genetic relatedness and strain differentiation among *C. jejuni* isolates from poultry in Rajasthan.

Materials and Methods

DNA from confirmed 43 *Campylobacter jejuni* isolates was extracted as per the protocol of Ertas *et al.* (2004) and subjected to REP-PCR using single pair of primers (REP1-5'-IIIICG ICGICATCIGGC-3' and REP2- 5'-ICGITTATCIGGCCTAC-3') targeting noncoding conserved sequence with multiple number of repeats in *C. jejuni* genome according to the method

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described by Versalovic *et al.* (1994).

The PCR amplified products were electrophoresed on 1.5% agarose gels and visualized under UV trans-illuminator (Bio Doc-It Imaging System). The images were analyzed with Pyelph application (Pavel and Vasile, 2012) and the resultant binary matrix was subjected to one of the agglomerative hierarchial clustering (UPGMA) method along with (Dice) similarity coefficient, used for comparing the similarity of samples using Dice + UPGMA tool (Bikandi *et al.,* 2004). Further the Discriminatory index of REP-PCR amplified products were calculated using Discriminator power calculator tool (Bikandi *et al.,* 2004).

Results and Discussion

All the 43 *C. jejuni* strains could be typed through REP-PCR with amplicons ranging from 80-700bp. On typing 12 different REP patterns (REP1–REP12) were obtained (Fig. 1). The discriminatory power of REP-PCR method to discriminate *C. jejuni* strains on the basis of number of band pattern generated was calculated as 0.9181. The Discriminatory power of a tool giving discrimination above 0.5 considered as good method to discriminate isolates. The REP8 pattern was most common in 7 isolates (C1,C9,C12,C17,C28,C38,C43) followed by REP5 (C5,C20,C32,C36,C40), REP10 (C3,C13,C19,C26,C41) and REP12 (C8,C15,C18,C29,C35) patterns with five isolates each then REP3 (C16,C24,C34,C39), REP6 (C2,C11,C22,C31) and REP7 (C23,C25,C27,C37) patterns with four isolates each and other remaining REP patterns comprising with less than four isolates. REP4 (C14) and REP9 (C7) patterns have single isolate only.

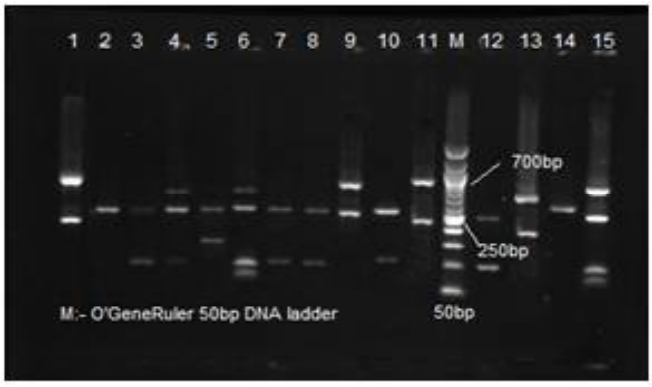

Fig. 1: REP-PCR patterns of *C. jejuni* isolates from poultry

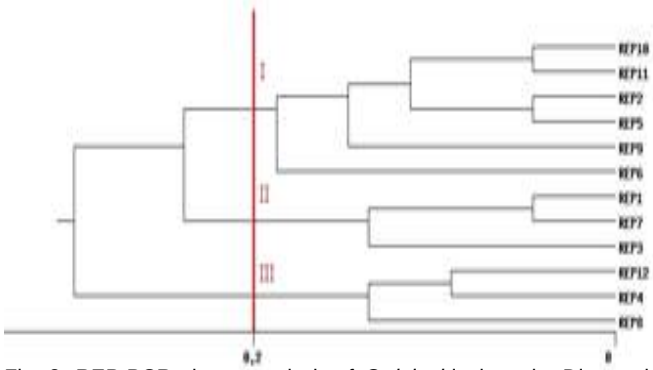

Fig. 2: REP-PCR cluster analysis of *C. jejuni* isolates by Dice and UPGMA method

The maximum number of four bands were produced by REP11 (80bp, 100bp, 300bp and 600bp) and REP12 (100bp, 250bp, 300 bp and 700bp) followed by three bands in REP10 (100bp, 300bp, 600bp) as shown in Table 1.

During phylogenetic cluster analysis of *C. jejuni* REP patterns in the present study, all 12 REP-patterns could be divided into three clusters on the basis of 80% genetic similarity (Fig. 2). First cluster comprised maximum number of 20 isolates and six REP-patterns (REP2, REP5, REP6, REP9, REP10 and REP12), while second cluster comprised ten isolates and three REP patterns (REP1, REP3 and REP7) and third cluster included 13 isolates and three REP-patterns (REP4, REP8, and REP12). The cluster analysis showed that isolates from different phase of sampling were randomly distributed, thus indicated persistence of same *C. jejuni* strains in environment over a long period of time.

Similar to our observation, Hiett *et al.* (2006) obtained four to eight bands size ranging from 400 to 5000 bp among 48 *Campylobacter* spp. isolates with discriminatory index (DI) of 0.8364 at 90% similarity values from various sources and geographical areas. They found that isolates recovered from fecal samples during production were closely related (>90% similarity) to isolates recovered from the processed carcasses. Likewise, Prapas *et al.* (2012) also calculated similar discriminatory power of 0.8917 from 9 REP-PCR finger printing patterns among 16 *C. jejuni* isolates. Behringer *et al.* (2011) detected 100% type ability among 100 *Campylobacter* isolates (*C. jejuni* and *C. coli*) and produced 29 distinct REP profiles, with five of the profiles identified in both species. Twelve REP types contained only one isolate, while three types (h, k and m) included more than 10 isolates each with random distribution of the isolates irrespective of strain, source and area. Therefore, it has been unquestionable now that REP-PCR can be utilized to reliably subtype *Campylobacter* spp.

Recently REP-PCR technology has advanced to the point of eliminating agarose slab gels by employing microfluidic devices for resolution of fluorescently labeled amplicons. This method eliminates gel-to-gel variation, thereby minimizing lab-to-lab variation and maximizing reproducibility. Additionally, it also permits online data collection for analyses, thus allowing for pattern input into databases and bio informatics (Healy *et al.,* 2005).

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Table 1: REP-PCR typing of *C. jejuni* isolates from poultry

S. No	REP pattern	Band size	Isolate number	Number of isolates
1	REP1	200	C30,C42	2
2	REP2	300	C4,C6,C33	3
3	REP3	400	C16,C24,C34,C39	4
4	REP4	100,250	C14	1
5	REP5	100,300	C5,C20,C32,C36,C40	5
6	REP6	150,300	C2,C11,C22,C31	4
7	REP7	200,500	C23,C25,C27,C37	4
8	REP8	250,700	C1,C9,C12,C17,C28,C38,C43	7
9	REP9	300,600	C7	1
10	REP10	100,300,600	C3,C13,C19,C26,C41	5
11	REP11	80,100,300,600	C10,C21	2
12	REP12	100,250,300,700	C8,C15,C18,C29,C35	5
Number of strains: 43 Number of types: 12 Discriminatory index: 0.9181				

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