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DETECTION OF VIRULENCE FACTOR ASSOCIATED GENES OF *CAMPYLOBACTER* SPP. FROM CATTLE IN ANAND, GUJARAT, INDIA

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ABSTRACT:

The aim of this study was to detect the virulence factor associated genes of *Campylobacter* spp. from cattle faecal samples in Anand city, Gujarat, India. *Campylobacter* isolates (n=8) used in this study were isolated from 200 faecal samples collected from the Anand city, Polymerase chain reaction technique was used for detection of five genes, namely *fla*A, *cad*F, *iam*A, *flg*R and *cdt*B genes of *Campylobacter* spp. Out of eight isolates, all the eight were positive for *cad*F, *cdt*B and *flg*R (100 percent) while six isolates were positive for *fla*A gene and four isolates were positive for *iam*A gene of *Campylobacter* spp. Presence of *cad*F, *cdt*B and *flg*R gene in all isolates shows that they are the specific targets for *Campylobacter* identification and are capable of producing gastroenteric illness to humans.

KEY WORDS: *Campylobacter, Cattle, Gastroenteritis, Virulence genes.*

INTRODUCTION:

Foodborne infection is an important public health problem in world. Among the food borne pathogens, public health burden of *Campylobacter* has risen substantially all over world during the past 30 years. *Campylobacter* has

become recognized as the major cause of gastroenteritis in industrialized and developed countries (Dabiri *et al*., 2014).

Campylobacter species are common components of animal faecal droppings, which are frequent contaminants of raw milk during the milking process (Humphrey *et al*., 2007). The importance of cattle in Campylobacteriosis is not just restricted to the contamination of milk at the farm and the carcass at slaughter, but they are also responsible for environmental and water contamination because of the disposal of abattoir effluents and slurries to land (Stanley and Jones, 2003).

Campylobacteriosis is an acute gastrointestinal infection with severe abdominal pain, fever, nausea, headache, muscle pain and diarrhoea. The length of the incubation period is 3-5 days with symptoms lasting for 5-7 days. A serious public health concern regarding Campylobacteriosis is the occurrence of severe sequelae. One out of every one thousand cases of Campylobacteriosis develops serious autoimmune sequelae 1-3 weeks after infection. These include Guillain-Barre Syndrome (GBS), associated with acute flaccid paralysis, weakness of limbs and respiratory muscles and reactive arthritis (CDC, 2011).

The putative virulence factor for adhesion and invasion of epithelial cells, toxin production and flagellar motility are thought to be important in virulence mechanisms (Van Vliet and Ketley, 2001). *C. jejuni* flagella and flagellar motility are vital to many aspects of *C. jejuni* biology, including host colonization, secretion and host cells invasion. Consequently, the regulation of flagellar biogenesis and motility is an active area of research (Young *et al*., 2007). The Cell distending toxin in *C. jejuni* is encoded by a three-gene operon (*cdt*ABC) in which *cdt*B is the active subunit (Poly and Guerry, 2008). CDT activity causes certain cell types, such as intestinal epithelial cells, to become slowly distended which progresses into cell death (Van Vliet and Ketley, 2001).

The purpose of this study was to evaluate the potential virulence of *Campylobacter* isolates from cattle faeces by detecting the presence of the virulence genes *fla*A, *cad*F, *iam*A, *flg*R and *cdt*B using the polymerase chain reaction (PCR).

METHODS AND METHODS:

Approximately, a total 200 faecal samples were collected aseptically and transported to the laboratory in an icebox for microbiological analysis. Samples were processed in laboratory immediately within two hour.

Our study used *Campylobacter* isolates (n=8) recovered from cattle faecal samples collected from Anand city, Gujarat, India. The confirmed isolates of *Campylobacter* species were characterized for *in vitro* detection of virulence genes by PCR for five well-known virulence gene encoding flagellin gene (*fla*A)

(Crushell *et al*., 2004), *campylobacter* adherence gene (*cad*F) (Cunningham *et al*., 2010), invasion associated marker, *iam*A (Carvalho *et al*., 2001), flagellar synthesis and modification, *flg*R (Wilson *et al*., 2010) and cytolethal distending toxin subunit B gene (*cdt*B) (Asakura *et al*., 2008). The details of primers for target virulence genes and PCR conditions are described in Table-1 and 2, respectively. The DNA of virulence gene positive control strains available in our department was used in PCR for detection of virulence genes while for negative control DNA template was replaced with nuclease-free distilled water. The positive control PCR revealed PCR product of appropriate size and in a negative control, no product was amplified. The reaction was standardized in thin walled PCR tubes in 25 µl reaction volume with different concentration of reactants under different annealing temperatures and cycling conditions. Finally, the reaction mixture was optimized to contain 12.5 µl 2 X PCR master mix, 10 pmole of each forward and reverse primer, 7.5 µl nuclease free distilled water and 3 µl DNA template.

RESULTS AND DISCUSSION:

In the present study, the recovered isolates of *Campylobacter* spp. were examined for the presence of five virulence gene encoding for viz., Flagellin gene (*fla*A), Campylobacter adherence gene (*cad*F), Invasion associated marker (*iam*A) gene, Flagellar synthesis and modification (*flg*R) and Cytolethal distending toxin Subunit B gene (*cdt*B) were detected by polymerase chain reaction (PCR) using specific primers and cyclic condition previously described by different author with certain modification. The results indicated that out of eight isolates, all the eight were positive for *cad*F, *cdt*B and *flg*R (100 percent) while six isolates were positive for *fla*A gene and four isolates were positive for *iam*A gene.

All the eight *Campylobacter* isolates were positive for the presence of *cad*F gene and yielded the DNA fragment of 400bp (Figure 1). The result of present study was concurrent with the study of Bang *et al*. (2003), Datta *et al*. (2003), Ripabelli *et al.* (2010), Biswas *et al.* (2011) and Chatur (2014) who examined and found the 100 percent prevalence of *cad*F gene in *Campylobacter* isolates which was almost similar with our result.

In the present study, 6 isolates (75 percent) out of 8 were positive for *fla*A gene and yielded the DNA fragment of 1725bp (Figure 2). In contrast to our results, Bang *et al*. (2003), Datta *et al*. (2003), Ripabelli *et al.* (2010) and Acik *et al*. (2013) detected 100 percent presence of *fla*A gene.

Among eight isolates studied, four isolates (50 percent) were found positive for the presence of *iam*A gene and yielded the DNA fragment of 518bp(Figure 3). Chatur (2014) detected 100 percent prevalence of *iam*A gene in *Campylobacter* isolates which was much higher in compare with our study. Sanad *et al.* (2011) studied on occurrence of the invasion associated marker (*iam*) in *Campylobacter jejuni* isolated

from cattle faeces. Among 129 *C. jejuni*, he examined that 6.8 percent of the cattle isolates were *iam*-positive, which was much lower in compare with our results i.e. 50 percent.

All the eight *Campylobacter* isolates were positive for the presence of *flg*R gene and yielded the DNA fragment of 390bp (Figure 4). Our result is concurrent with the study of Chatur (2014) who examined and found 100 percent prevalence of *flg*R gene from *Campylobacter* isolates.

All the eight *Campylobacter* isolates were positive for the presence of *cdt*B gene and yielded the DNA fragment of 495bp (Figure 5). The result of present study was concurrent with the study of Bang *et al*. (2003), Datta *et al*. (2003), Ripabelli *et al.* (2010) and Gisela *et al*. (2013) who examined and found the 100 percent prevalence of *cdt*B gene in *Campylobacter* isolates. In contrast to present study, Acik *et al*. (2013) investigated 70.9 percent prevalence of *cdt*B gene in 210 *Campylobacter* strain isolated from cattle which was lower in comparison to present study.

CONCLUSION:

Presence of *cad*F, *cdt*B and *flg*R gene in all isolates shows that they are the specific targets for *Campylobacter* identification and are capable of producing gastroenteric illness to humans. Application of good hygiene procedures during milking, personal hygiene and health education will be necessary to protect the consumer from this zoonotic pathogen.

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Table 1: Oligonucleotide sequence of virulence genes:

Sr. No	Target gene	Primer	Primer Sequence (5'→3')	Amplicon size(bp)	Reference
1.	Flagellin gene	fla 1	GGATTTCGTATTAACACAAATGGTGC	1725	Nachamkin *et al*., 1993
		fla 2	CTGTAGTAATCTTAAAACATTTTG		
2.	Campylobacter adherence gene	cad F	TTGAAGGTAATTTAGATATG	400	Konkel *et al*., 1999
		cad R	CTAATACCTAAAGTTGAAAC		
3.	Invasion associated Marker	iam F	GCGCAAAATATTATCACCC	518	Carvalho *et al*., 2001
		iam R	TTCACGACTACTATGCGG		
4.	Flagellar synthesis and modification, flgR	JL 1225	GAGCGTTTAGAATGGGTGTG	390	Wilson *et al*., 2010
		JL 1226	GCCAGGAATTGATGGCATAG		
5.	Cytolethal distending toxin SubunitB gene	CdtB-F	GTTGGCACTTGGAATTTGCAAGGC	495	Bang *et al*., 2003
		CdtB-R	GTTAAAATCCCCTGCTATCAACCA		

Table 2 PCR cyclic condition of virulence genes

Gene	Initial denaturation	35 cycles			Final extension
		Denaturation	**Annealing**	**Extension**	
Flagellin gene	94°C 5 min	94°C 45 s	52°C 45 s	72°C 1 min	72°C 10 min
***Campylobacter* adherence gene**	94°C 5 min	94°C 1 min	45°C 45 s	72°C 1 min	72°C 8 min
Invasion associated marker	94°C 5 min	94°C 1 min	52°C 1 min	72°C 1 min	72°C 5mim
Flagellar synthesis and modification, *flg*R	95°C 5 min	95°C 1 min	54°C 1 min	72°C 1 min	72°C 5min
Cytolethal distending toxin subunit B gene	95°C 5 min	95°C 30 s	57 °C 30 s	72°C 30 s	72°C 10 min

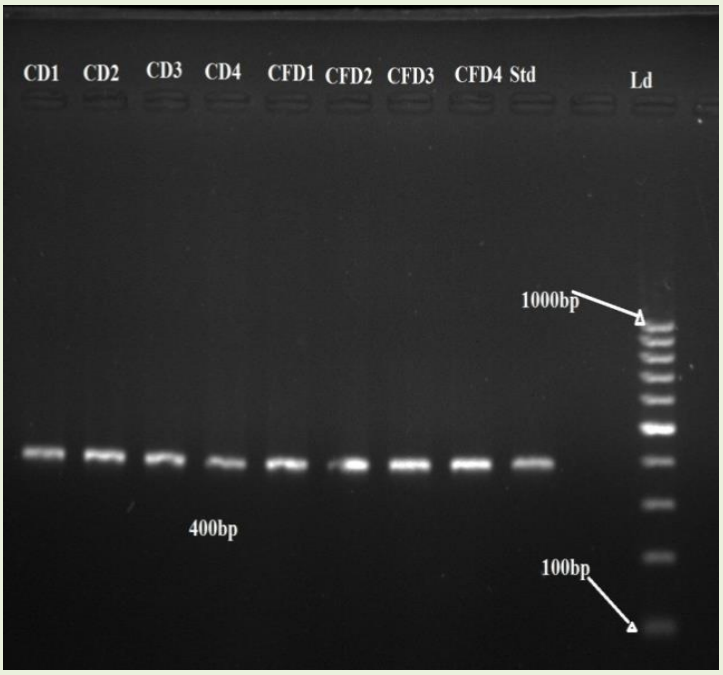
Figure-1: Amplification of PCR products for the detection of *cad*F genes

CD= Cattle diarrhea

CFD= Calf diarrhea

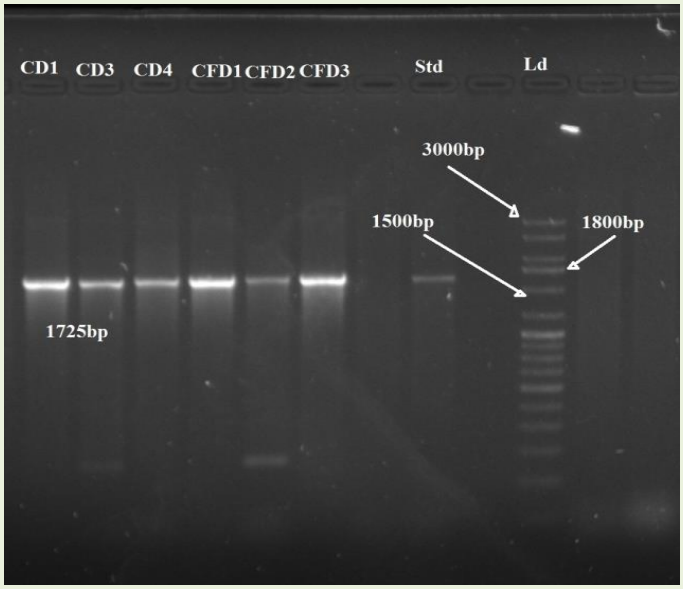
Std= Positive control

Ld= Marker



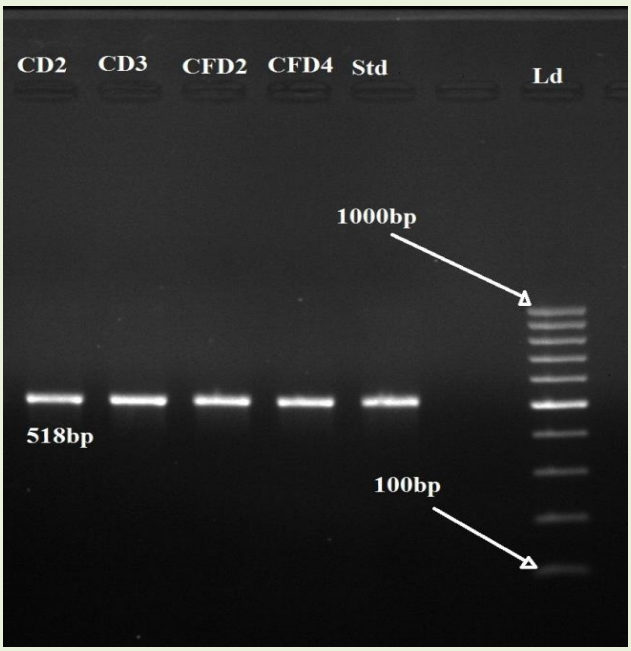
Campylobacter adherence (*cad*F) gene (400 bp)

Figure-2: Amplification of PCR products for the detection of *fla*A genes



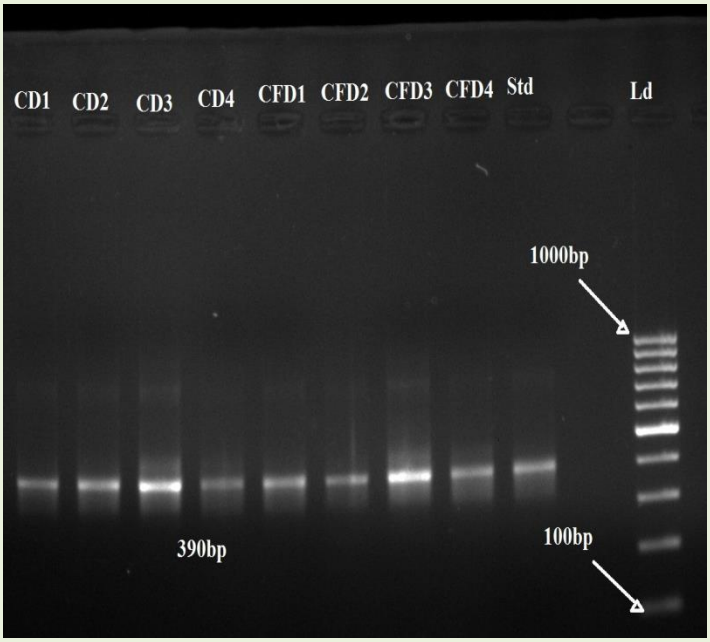
Flagellin (*fla*A) gene (1725 bp)

Figure-3: Amplification of PCR products for the detection of *iam*A genes



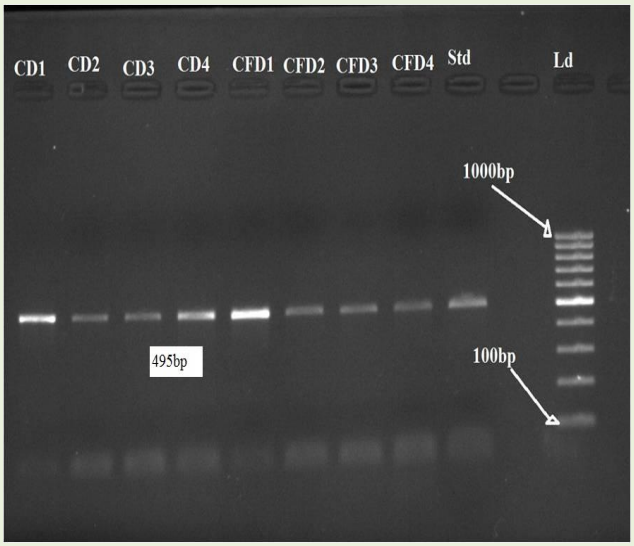
Invasion associated marker (*iam*A) (518 bp)

Figure-4: Amplification of PCR products for the detection of *flg*R genes



Flagellar synthesis and modification (*flg*R) (390 bp)

Figure-5: Amplification of PCR products for the detection of *cdt*B genes



Cytolethal distending toxin (*cdt* B) gene (495 bp)