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Prevalence of *recR* gene in *Campylobacter* spp. isolated from domestic animals and water

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ABSTRACT

Although, many genes are involved in pathogenesis of *Campylobacter*, *rac*R gene considered the main gene for *Campylobacter* pathogenicity in humans.The purpose of this study was determined the prevalence of *recR* gene in *Campylobacter* spp. isolated from domestic animals and water**.** To perform the study 392 fecal and water samples were collected from poultry (182), cow (141), sheep (25) and goat (16) and water (28). All samples were subjected for isolation of *Campylobacter* spp. using prêt KB method and the presumptive isolates authenticated by DNA sequencing of 16s*rRNA* genes. Finally, *Campylobacter* isolates assessed for detection of *racR* gene. The results obtained indicated that 50 strains of *Campylobacter* spp. were isolated. High isolate frequency (37/50) was for Poultry and low frequency (2/50) was for sheep and goat. Of All isolates thirty six strains were identified as *Campylobacter jejuni* and the rest (14 isolates) was *Campylobacter coli*. The genome of 30 (83.3%) *C.jejuni* and 14 (100%) *C.coli* were contained *racR* gene. Hence based on foregoing evidence *racR* gene existed approximately in all isolates. Therefore, *Campylobacter* strains isolated from different sources could be considered infectious agent for human. It is because phenotypical character inducted by *racR* gene (colonization and heat tolerance) helps them to survive and cause infection.

1. Introduction

Campylobacters are small (0.2–0.9 μm in length and 0.2–5.0 μm in width), slender, and spiral curved rods. They are gram negative bacteria with a polar flagellum at one or both ends of the cell, which gives them high motility. These microscopic characteristics can be used to recognize them from other food-borne pathogens. *Campylobacters* are urease negative and catalase is oxidase positive. They can catalyse hydrogen peroxide to produce oxygen and water. They grow under microaerobic conditions (Van Vliet and Ketley, 2001; Khoshbakht *et al.*, 2013).

Campylobacter species, especially *Campylobacter jejuni* and *Campylobacter coli*, have been recognized as a major cause of acute bacterial gastroenteritis in humans since 1970 and it has been found that *Campylobacter* spp. is responsible for 400–500 million cases of

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diarrhoea across the world each year (Tam *et al.*, 2003; Gblossi Bernadette *et al.*, 2012).

These bacteria are normal flora in animals gut such as poultry, pigs, and cattle. Many studies have shown that *Campylobacter* infections in different countries were linked to high levels of poultry gut colonization by these microorganisms (Negahdari *et al.*, 2016).

Campylobacter spp. like *C.jejuni* and *C.coli* grow between 37˚C and 42˚C, (optimally at 41.5˚C) but cannot grow below 30˚C. The normal body temperature for a poultry is 42˚C. The *racR*(reduced ability to colonize) gene, a member of the racR-racS system, has a regulatory protein that has an effect on the growth of *Campylobacter* in a temperature-dependent way and on the colonization of the intestinal tract of chickens (Brás *et al.*, 1999; Hamidian *et al.*, 2011).

Therefore, based on foregoing evidence the present study conducted to Comparison study on the presence of *racR* gene in *Campylobacter* spp. isolates from domestic animals and water, in order to achieve maximum information concerning survival of *Campylobacter* in environment as well as animal gut.

2. Materials and Methods

2.1. Sample collection

In all, 392 faecal and water samples were collected from different farms of behbahan city. 364 faecal samples were collected from poultry, cow, sheep and goat and 28 samples were collected from water. The fecal samples were collected using sterile sticks and polyethylene bags (Isun Medical, Tehran, Iran) and transferred to the laboratory of Islamic Azad University, Behbahan branch within one hour of sampling. The water samples were collected in 500 ml sterile falcon tube (Isun Medical, Tehran, Iran) and placed on the ice and transferred to laboratory within 2 hours. The samples were subjected for detection of *Campylobacter* immediately upon arrive to the laboratory.

2.2. Sample processing and isolation

Campylobacter detection in the present study was pre-treatment-Kapandis Baseri (prêt-KB) technique and the medium was blood and antibiotic free Kapandis Baseri (KB) medium (*HiMedia*, Mumbai, India). To perform the method faecal samples were added (10% (w/v)) in sterile phosphate-buffered saline (0.1 mol l^{-1}, pH 7) (Merck, Germany) to obtained 10% suspension. The suspension was centrifuged at 8500 rpm for 10 min, then it was placed at room temperature. Afterward 10–15 min, 0.1 ml supernatant from the tube was plated on the KB medium (*HiMedia*, Mumbai, India). The tubes containing water samples were centrifuged in 4000 rpm within 10 minutes; then, 0.1 to 0.2ml of supernatant from each tube was plated on the KB medium (Baserisalehi *et al.*, 2004).

The plates were incubated at 37˚C for 48 h in microaerophilic conditions and tested daily for 5 days.

2.3. Identification and authentication of Campylobacter spp.

All suspected colonies grew on the KB medium confirmed by typical morphology, darting motility, gram staining, oxidase, and catalase tests. The isolates with presumptive *Campylobacter* character were subjected to standard *Campylobacter* phenotypic identification tests recommended by Atabay and Corry (Atabay and Corry, 1997). These tests included H_2S by lead acetate strip, nitrate reduction, growth in 1% glycine and 3.5% NaCl, growth at temperatures 25˚C, 37˚C, and 42˚C, hippurate hydrolysis, indoxyl acetate hydrolysis, urease production, and resistance to Nalidixic acid (30 µg) and Cephalothin (30 µg). Additional tests for identification of *Campylobacters* were alkaline phosphatase production, oxidative-fermentative test (OF Test), and glucose fermentation. All items used in the phenotypic identification tests were purchased from Parsalab (Tehran, Iran).

At the end, the PCR method was carried out in order to confirm the phenotyping results and detection of *racR* gene.

2.4. DNA extraction, PCR method and detection of racR gene

The PCR assay was done for authentication of *Campylobacter* and detection of *racR* gene. DNA was extracted from suspected colony using phenol chloroform method. The PCR was performed in 25 ml of the reaction mixture with a final concentration of 1×PCR reaction mix, 10pg–1µg concentration of template

deoxynucleotide triphosphate (DNA), 0.1–1 µmol L^{-1} concentrations of each primer (Macrogen, Inc, Seoul, Korea), 3.2 m mol L^{-1} $MgCl_2$ solution, and 1.25–2.5 U/50 µl of GoTaq DNA polymerase. All items used in the PCR were purchased from Yekta Tajhiz Azma (Tehran, Iran) and the experiment was performed by thermal cycler (Bio-Red, Singapore). A 100-bp DNA ladder (Yekta Tajhiz Azma, Tehran, Iran) was used as a DNA molecular ladder. PCR products were electrophorized using 1% agarose gel (Yekta Tajhiz Azma, Tehran, Iran) at 80 V for 60 minutes. In addition detection of *racR* gene from each DNA extract was carried out and all amplified DNAs were visualized with UV transilluminator (*Heidolph, Germany*). Table 1 illutrated primer sequences and their thermal conditions used in the present study.

Table 1. Primer sequences

Primers	Sequence (5′→ 3')	Target gene	References
27F 1492R	AGAGTTTGATCMTGGCTCAG CGGTTACCTTGTTACGACTT	*16SrRNA*	(Lane, 1991)
racRF-25 *racRR*-593	GATGATCCTGACTTTG TCTCCTATTTTTACCC	*racR*	(Datta *et al.*, 2003)

Target gene	PCR conditions					Amplicon (bp)
	Primary denaturation	Denaturation	Annealing	Extension	Final extension	
16SrRNA	1m، °C 94	94°C,20s	55°C,30s	72°C,40s	72°C,2m	1490bp
racR	1m، °C 94	94°C,20s	45°C,30s	72°C,30s	72°C,2m	584bp

3. Results

Sampling from the domestic animals and water was conducted between March 2016 and August 2016 in Behbahan City, Khuzestan Province, iran.

As shown in table 2 and Fig. 1 lowest and highest numbers of *Campylobacter* isolated in the various samples are 2 and 37 respectively. In addition numbers of *Campylobacter* isolated from cow faeses and water samples are 8 and 3 respectively. After conducting of the phenotyping methods and their confirmation with molecular technique, 36 strains of *C.jejuni* and 14 strains of *C.coli* were identified.

With regard to the obtained results, prevalence of *C.jejuni* and *C.coli* in the domestic animals faeces and water samples of the Behbahan city was evaluated as 72 and 28 percent respectively. As shown in table 3 and table 4 lowest and highest numbers of *C.jejuni* isolated in the various samples are 1 and 29 respectively and lowest and highest numbers of *C.coli* isolated in the various samples are 1 and 8 respectively. In addition numbers of *C.jejuni* isolated from cow and water samples are 4 and 2 respectively and numbers of *C.coli* isolated from cow is 4.

With regard to the obtained results, prevalence of *racR* gene in *C.jejuni* and *C.coli* in the domestic animals faeces and water samples of the Behbahan city was evaluated as 83.3 and 100 percent respectively. As shown in table 5 lowest prevalence of *racR* gene detected in the poultry faeces samples is 79.3% and highest prevalence of *racR* gene in the cow faeces, sheep and goat faeces and water samples are 100%.

4. Discussion

Campylobacter colonizes the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human usage. *Campylobacter* species are found in birds, cattle, dairy cows, sheep, and swine. Birds are the most common hosts for *Campylobacter* because of their higher body temperature. Food production animals are considered the primary source of *Campylobacter* infections in humans in developed countries.

Table 2. Frequency of occurrence of *Campylobacter* spp. isolated from domestic animals faeces and water

Samples	No. of samples	No. of *Campylobacter* spp. isolated	Occurrence of *Campylobacter* spp. isolated(%)
Poultry faeces	182	37	20.3
Cow faeses	141	8	5.7
Sheep faeces	25	1	4
goat faeces	16	1	6.25
Water	28	3	10.7
Total	392	50	12.8

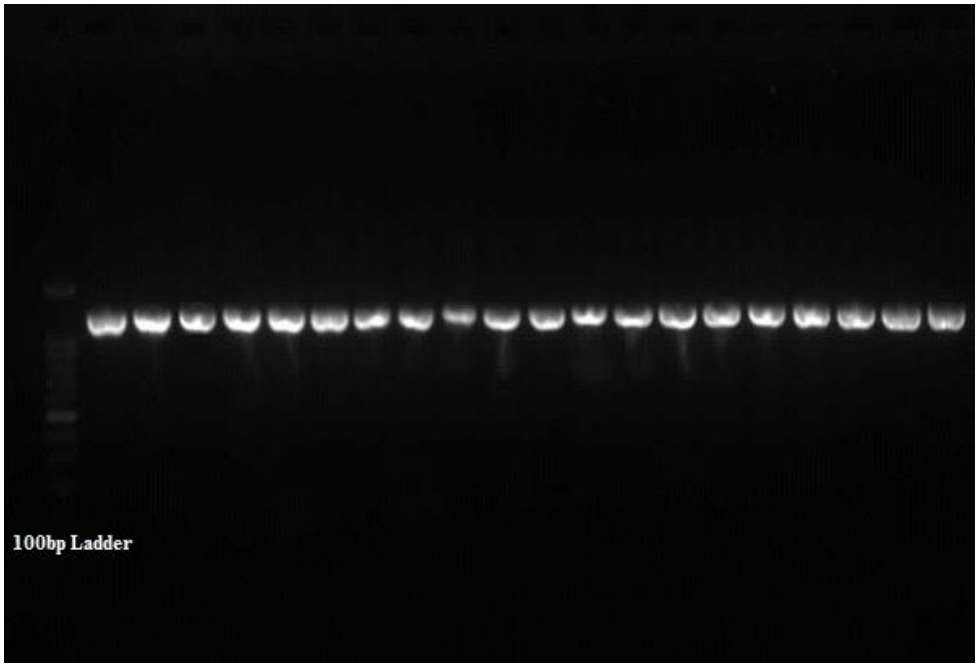

Figure 1. PCR products of 16SrRNA gene

Table 3. Frequency of occurrence of *C.jejuni* and *C.coli* isolated from domestic animals faeces and water samples

Samples	No. of *Campylobacter* spp. isolated	No. of *C.jejuni* isolated(%)	No. of *C.coli* isolated (%)
Poultry feces	37	29(78.4)	8(21.6)
Cow feces	8	4(50)	4(50)
Sheep and goat faeces	2	1(50)	1(50)
Sheep faeces	1	1(100)	-
goat faeces	1	-	1(100)
Water	3	2(66.67)	1(33.34)
Total	50	36(72)	14(28)

Table 4. Sources and accession numbers of *campylobacter* spp. isolated

Source*	*Campylobacter* spp. isolated	GenBank accession no.
P1	*Campylobacter jejuni*	CP020776.1
P2	*Campylobacter jejuni*	CP020776.1
P3	*Campylobacter jejuni*	CP007193.1
P4	*Campylobacter jejuni*	CP020776.1
P5	*Campylobacter jejuni*	CP020776.1
P6	*Campylobacter jejuni*	CP017862.1
P7	*Campylobacter jejuni*	CP017456.1
P8	*Campylobacter Coli*	CP007181.1
P9	*Campylobacter Coli*	CP007181.1
P10	*Campylobacter Coli*	CP007181.1
P11	*Campylobacter Coli*	CP007181.1
P12	*Campylobacter jejuni*	CP020776.1
P13	*Campylobacter jejuni*	CP017862.1
P14	*Campylobacter jejuni*	CP020776.1
P15	*Campylobacter jejuni*	CP020776.1
P16	*Campylobacter jejuni*	CP020776.1
P17	*Campylobacter jejuni*	CP020776.1
P18	*Campylobacter jejuni*	CP007193.1
P19	*Campylobacter jejuni*	CP020776.1
P20	*Campylobacter jejuni*	CP020776.1
P21	*Campylobacter Coli*	CP019977.1
P22	*Campylobacter jejuni*	CP007191.1
P23	*Campylobacter jejuni*	CP020776.1
P24	*Campylobacter jejuni*	CP007193.1
P25	*Campylobacter jejuni*	CP020776.1
P26	*Campylobacter jejuni*	CP020776.1
P27	*Campylobacter jejuni*	CP020776.1
P28	*Campylobacter jejuni*	CP020776.1
P29	*Campylobacter jejuni*	CP020776.1
P30	*Campylobacter Coli*	CP007183.1
P31	*Campylobacter Coli*	CP007183.1
P32	*Campylobacter jejuni*	CP007191.1
P33	*Campylobacter Coli*	CP019977.1
P34	*Campylobacter jejuni*	CP020045.1
P35	*Campylobacter jejuni*	CP007188.1
P36	*Campylobacter jejuni*	CP020776.1
P37	*Campylobacter jejuni*	CP007193.1
W1	*Campylobacter Coli*	CP007183.1
C1	*Campylobacter Coli*	CP006702.1
W2	*Campylobacter jejuni*	CP007188.1
W3	*Campylobacter jejuni*	CP017862.1
C2	*Campylobacter Coli*	CP007183.1
C3	*Campylobacter Coli*	CP019977.1
C4	*Campylobacter jejuni*	CP017862.1
C5	*Campylobacter jejuni*	CP020776.1
C6	*Campylobacter Coli*	CP007183.1
G	*Campylobacter Coli*	CP019977.1
C7	*Campylobacter jejuni*	CP007193.1
C8	*Campylobacter jejuni*	CP007192.1
S	*Campylobacter jejuni*	CP007193.1

***Source** : P: poultry, C: cow, S: sheep, G: goat, W: water

Table 5. Occurrence of *racR* gene results of *C.jejuni* and *C.coli* isolated from domestic animals faeces and water samples.

Samples	No. of *C.jejuni* isolated	No. of positive *C.jejuni* isolates for *racR* gene(%)	No. of *C.coli* isolated	No. of positive *C.coli* isolates for *racR* gene(%)	occurrence of *racR* gene in *Campylobacter* spp. (%)
Poultry feces	29	23(79.3)	8	8(100)	31/37(83.8)
Cow feses	4	4(100)	4	4(100)	8/8(100)
Sheep feces	1	1(100)	1	1(100)	1/1(100)
goat feces	-	-	1	1(100)	1/1(100)
Water	2	2(100)	1	1(100)	3/3(100)
Total	36	30(83.3)	14	14(100)	44/50(88)

Isolation and identification of *Campylobacter* spp. from the domestic animals and water in behbahan city was not reported. The results obtained from present study achieved during March 2016 and August 2016 from 392 samples were collected of which 50 strain of *Campylobacter* spp. isolated. Of the 50 strains, 36 *C.jejuni* and 14 *C.coli* were isolated. This is evidently could concluded that there exist *C.jejuni* and *C.coli* in domestic animals and water in behbahan city**.** A variation in the prevalence of *Campylobacter* in poultry, cow, sheep and goat and water was found in different studies in various parts of the world (Guessoum *et al.*, 2016).

In our current work, we noted 12.8% positivity rates for *Campylobacter* spp.. This result is consistent with some studies (Desmonts *et al.*, 2004; Igimi *et al.*, 2008; Bae *et al.*, 2005; Garcia *et al.*, 2010). But it is different from others (Dadi and Asrat, 2016; Salihu *et al.*, 2009; Messad *et al.*, 2014). As the authors report, the difference in *Campylobacter* isolation frequency observed between different studies would depend on a number of factors such as the season (Hannon *et al.*, 2009), geographic area (Berrang *et al.*, 2000), and the sample size (Jeffrey *et al.*, 2001). The choice of research method and use of enrichment, along with selective and specific media can also play an important role. For example, Garcia *et al*,. (2010) and Hakkinen *et al*,. (2007) showed an improvement of %15 to 30% respectively due to the use of a medium enrichment. The reasons for the variations are unknown and could be related to differences in the type of animal, production practices, storage conditions, rearing conditions, and the environment (Guessoum *et al.*, 2016; Chen *et al.*, 2010).

The *racR* gene of *Campylobacter* spp. has been reported to be important in the ability to colonize chickens and support optimal growth rates at 42°C, suggesting that the *racR* gene regulates genes important for in vivo colonization in a temperature-dependent manner.

In our research, the prevalence rate of *racR* gene was 88%. The results, similar to our research, have been reported where in the prevalence rate of *racR* is high (Koolman *et al.*, 2015; Zhong *et al.*, 2016; Frazao *et al.*, 2017). In other research, low *racR* rate has also been reported (Khoshbakht *et al.*, 2013; Rizal *et al.*, 2010). In Conclusion: These high proportions of positive samples in poultry indicate that poultry in general can be an important vehicle for *Campylobacter* infections in humans. The high prevalence of *Campylobacter* in poultry is because there is the *racR* gene.The present study clearly demonstrated the significance of domestic animals and water as wide reservoirs of *Campylobacters*. Present findings shows that frequency of occurrence of *Campylobacter* was high in the areas of investigation. In addition, presence of *C.jejuni* and *C.coli* suggested that the domestic animals and water harbor a variety of the pathogenic *Campylobacter* spp.

Baserisalehi *et al.*, (2007) stated that incidence of *Campylobacter* in the environment of each geographical area is depended on its climate conditions. It means frequency of existence of *Campylobacter* in dry climate conditions compared to humid climate conditions is low. These data also concerning to the level of occurrence of *Campylobacter* in the environment supported their report. It is because, climate conditions in Khuzestan provinces in Iran are dry and temperature range is 0 to 50°C, and the climatic conditions and temperatures in south iran totally differ from the north, so accordingly frequency of incidence of *Campylobacter* in the south of iran was relatively low.

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