ORIGINAL

Genotyping of *Campylobacter jejuni* isolates from raw meat of animal species

Genotipado de aislados de Campylobacter jejuni procedentes de carne cruda de especies animales

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Abstract

Background: *Campylobacter jejuni* strains are important causes of foodborne diseases globally, the present survey was done to assess the prevalence and genotypic profile of *C. jejuni* strains isolated from raw meat samples.
Methods: Two-hundred raw meat samples were collected and analysis. Culture and biochemical tests were used for identification of *C. jejuni*. Isolates were also confirmed using the PCR test. PCR was also used to assess the distribution of virulence genes.
Results: 13.50% of samples were contaminated with *C. jejuni* strains. Raw cattle meat had the highest (25%), while raw goat meat (7.14%) had the lowest prevalence of *C. jejuni*. Significant difference was found amid the type of samples and prevalence of *C. jejuni* ($P< 0.05$). cafF (55.55%), cdtA (51.85%), and ciaB (44.44%) were the most commonly detected genes. Amongst the combined genotyping patterns, cadF+cdtA (25.92%) and ciaB+cdtA (18.51%) were the most commonly detected genetic properties. Additionally, cadF+ciaB+cdtA was identified amongst the 11.11% of isolates.
Conclusion: Role of raw meat, particularly raw cattle meat as a reservoir of *C. jejuni* strains was determined in the survey.

Key words: *Campylobacter jejuni*, prevalence, raw meat, epidemiology, virulence characters.

Resumen

Antecedentes: Las cepas de *Campylobacter jejuni* son importantes causas de enfermedades transmitidas por los alimentos a nivel mundial, el presente estudio se realizó para evaluar la prevalencia y el perfil genotípico de las cepas de *C. jejuni* aisladas de muestras de carne cruda.
Métodos: Se recogieron y analizaron 200 muestras de carne cruda. Se utilizaron pruebas de cultivo y bioquímicas para la identificación de *C. jejuni*. Los aislamientos también se confirmaron mediante la prueba PCR. También se utilizó la PCR para evaluar la distribución de los genes de virulencia.
Resultados: El 13,50% de las muestras estaban contaminadas con cepas de *C. jejuni*. La carne cruda de bovino fue la más alta (25%), mientras que la carne cruda de caprino (7,14%) tuvo la menor prevalencia de *C. jejuni*. Se encontró una diferencia significativa entre el tipo de muestras y la prevalencia de *C. jejuni* ($P< 0,05$). cafF (55,55%), cdtA (51,85%) y ciaB (44,44%) fueron los genes más comúnmente detectados. Entre los patrones de genotipado combinados, cadF+cdtA (25,92%) y ciaB+cdtA (18,51%) fueron las propiedades genéticas más comúnmente detectadas. Además, cadF+ciaB+cdtA se identificó en el 11,11% de los aislados.
Conclusión: En el estudio se determinó el papel de la carne cruda, en particular de la carne cruda de vacuno, como reservorio de cepas de *C. jejuni*.

Palabras clave: *Campylobacter jejuni*, Prevalencia, Carne cruda, Epidemiología, Caracteres de virulencia.

Introduction

Foods with animal origins are determined as causative agent of different types of zoonotic diseases[1-5]. In this regard, Campylobacter species are the predominant cause of acute bacterial enteritis in both developing and developed countries[6]. It has been estimated that 32,086 cases were notified in Australia with an incidence rate of 130 cases per 100,000 population[7]. Among species, Campylobacter jejuni is considered for more than 80% of cases of campylobacteriosis characterized by fever, diarrhea, and abdominal cramps[8].

During slaughter and processing, cross-contamination and further spread of *C. jejuni* can occur. Even after chilling and cutting of meat products, high contamination rates with *C. jejuni* are possible[9].

Researches revealed that some virulence factors are responsible for the pathogenesis of Campylobacter infections. Among them, Campylobacter adhesin to Fn (*cadF*), campylobacter invasion antigen B (*ciaB*), cytolethal distending toxin genes (*cdtA*), and Phospholipase A1 (*pldA*) are responsible for adhesion and invasion to host cells[10].

Rendering the high importance of the *C. jejuni*, as a causative agent of campylobacteriosis and Guillain-Barré syndrome (GBS)[11], it is essential to assess its epidemiology and routes of transmission into the human population. Thus, the present survey was done to assess the distribution and virulence characters of the *C. jejuni* strains isolated from raw meat samples of sheep, goat and cattle species.

Materials and methods

Samples

200 raw meat samples of sheep (n= 70), goat (n= 70) and cattle (n= 60) species were collected and examined from January 2019 to January 2020. Thigh muscle was collected fro this purpose. In this regard, 100 g of meat samples were collected separately in sterile plastic bags and transferred to laboratory.

Isolation and identification of *C. jejuni*

25 g of each raw meat sample was homogenized and transferred to 225 mL of Preston enrichment broth base containing Campylobacter-selective supplement IV (HiMedia, India) with 5% defibrinated sheep blood. After inoculation at 42 C for 24 h in a microaerophilic condition (85% N2, 10% CO2, 5% O2), 0.1 mL of the enrichment was then streaked onto the Campylobacterselective agar base (HiMedia, India) supplemented with an antibiotic supplement for the selective isolation of Campylobacter spp. (HiMedia, India) and 5% defibrinated sheep blood and incubated at 42 C for 48 h under the same condition. One presumptive Campylobacter colony from each selective agar plate was subcultured, and identification of presumptive Campylobacter spp. was performed using standard microbiological and biochemical procedures including Gram staining, hippurate hydrolysis, production of catalase, urease activity, oxidase test, indoxyl acetate hydrolysis, and susceptibility to cephalotin.

DNA extraction and analysis

DNA was extracted from *C. jejuni* isolates using DNA extraction kit (Cinnagene, Iran)[12-15]. Purity and quality of extracted DNA were then checked using the method described previously[16-20]. *C. jejuni* strains were identified another time using the PCR (**Table I**)[21].

Identified DNA, were subjected to several PCR procedures to obtain the profile of genetic properties as shown in **table II**[22].

Electrophoresis was done using the procedure reported previously[23-25].

Statistical analysis

Chi-square test was used for data analysis in a SPSS software. *P*< 0.05 was considered significant[26-30].

Results

Figure 1 shows the PCR electrophoresis of *C. jejuni* identification.

Table III shows the distribution of *C. jejuni* amongst raw meat samples of animal species. Total prevalence of *C. jejuni* was 13.50%. Raw cattle meat had the highest (25%), while raw goat meat (7.14%) had the lowest prevalence of *C. jejuni*. Significant difference was found amid the type of samples and prevalence of *C. jejuni* (*P*< 0.05).

Table I: PCR for detection of *C. jejuni*.

PCR programs	PCR Volume (50μL)	PCR product (bp)	Primer sequence (5'-3')	Target gene
1 cycle: 94 ᵒC ------------ 1 min 35 cycle: 94 ᵒC ------------ 30 s 60 ᵒC ------------ 30 s 72 ᵒC ------------ 40 s 1 cycle: 72 ᵒC ------------ 3 min	5 μL PCR buffer 10X 2 mM Mgcl$_2$ 150 μM dNTP (Fermentas) 0.75 μM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 μL DNA template	589	F: CTATTTTATTTTTGAGTGCTTGTG R: GCTTTATTTGCCATTTGTTTTATTA	MapA (*C. jejuni*)

Table II: PCR for determination of *C. jejuni* genetic properties.

PCR programs	PCR Volume (50µL)	PCR product (bp)	Primer sequence (5'-3')	Target gene
1 cycle: 94 °C ----------- 2 min. 35 cycle: 94 °C ----------- 40 s 43 °C ----------- 50 s 72 °C ----------- 4600 s 1 cycle: 72 °C ----------- 8 min	5 µL PCR buffer 10X 2 mM Mgcl$_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template	400	F: TTGAAGGTAATTTAGATATG R: CTAATACCTAAAGTTGAAAC	*cadF*
1 cycle: 94 °C ----------- 1 min. 35 cycle: 94 °C ----------- 60 s 54 °C ----------- 60 s 72 °C ----------- 60 s 1 cycle: 72 °C ----------- 10 min	5 µL PCR buffer 10X 2 mM Mgcl$_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template	527	F: TGCGAGATTTTTCGAGAATG R: TGCCCGCCTTAGAACTTACA	*ciaB*
1 cycle: 95 °C ----------- 2 min. 35 cycle: 94 °C ----------- 50 s 49 °C ----------- 60 s 72 °C ----------- 40 s 1 cycle: 72 °C ----------- 7 min	5 µL PCR buffer 10X 2 mM Mgcl$_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template	370	F: CCTTGTGATGCAAGCAATC R: ACACTCCATTTGCTTTCTG	*cdtA*
1 cycle: 94 °C ----------- 1 min. 35 cycle: 95 °C ----------- 60 s 46 °C ----------- 60 s 72 °C ----------- 60 s 1 cycle: 72 °C ----------- 8 min	5 µL PCR buffer 10X 2 mM Mgcl$_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template	385	F: AAGAGTGAGGCGAAATTCCA R: GCAAGATGGCAGGATTATCA	

Figure 1: PCR electrophoresis of *C. jejuni* identification. PC: Positive control, NC: Negative control, M: Marker (100 bp). Others: Positive samples.
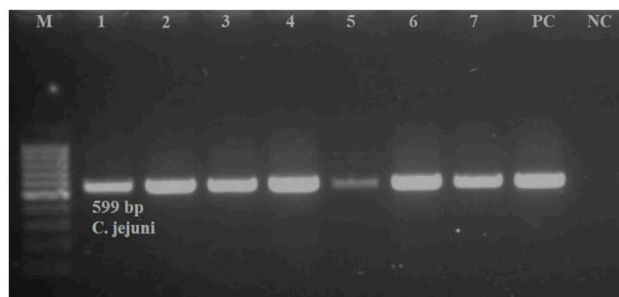

Table IV shows the genetic properties of *C. jejuni* strains isolated from raw meat samples. According to this table, *cafF* (55.55%), *cdtA* (51.85%), and *ciaB* (44.44%) were the most commonly detected genes. Amongst the combined genotyping patterns, *cadF+cdtA* (25.92%) and *ciaB+cdtA* (18.51%) were the most commonly detected genetic properties. Additionally, *cadF+ciaB+cdtA* was identified amongst the 11.11% of isolates. Significant difference was found amid the type of samples and distribution of *C. jejuni* genetic properties (*P*< 0.05).

Discussion

Medical sciences have been developed in recent years[31-36]. However, some diseases remain complicated[37-40]. Campylobacteriosis is one of the most dangerous diseases transmitted from animal

Table III: *C. jejuni* distribution amongst raw meat samples of animal species.

Raw meat samples	N. collected	N. positive (%)
Sheep	70	7 (10)
Goat	70	5 (7.14)
Cattle	60	15 (25)
Total	200	27 (13.50)

Table IV: *C. jejuni* genetic properties.

Raw meat samples (N. positive)	N. isolates harbored each gene (%)												
	cadF	*ciaB*	*cdtA*	*pldA*	*cadF +ciaB*	*cadF +cdtA*	*cadF +pldA*	*ciaB +cdtA*	*ciaB +pldA*	*cdtA +pldA*	*cadF +ciaB +cdtA*	*cadF +cdtA +plda*	*ciaB +cdtA +pldA*
Sheep (7)	3 (42.85)	3 (42.85)	3 (42.85)	2 (28.57)	1 (14.28)	2 (28.57)	1 (14.28)	2 (28.57)	1 (14.28)	1 (14.28)	1 (14.28)	-	-
Goat (5)	3 (60)	1 (20)	2 (40)	1 (20)	1 (20)	-	1 (20)	-	-	-	-	-	-
Cattle (15)	9 (60)	8 (53.33)	9 (60)	5 (33.33)	4 (26.66)	5 (33.33)	2 (13.33)	3 (20)	1 (6.66)	2 (13.33)	2 (13.33)	1 (6.66)	1 (6.66)
Total (27)	15 (55.55)	12 (44.44)	14 (51.85)	8 (29.62)	6 (22.22)	7 (25.92)	4 (14.81)	5 (18.51)	2 (7.40)	3 (11.11)	3 (11.11)	1 (3.70)	1 (3.70)

to human owing to the consumption of raw and undercooked products[41].

This survey showed that 13.50% of examined raw meat samples were contaminated with *C. jejuni*. Alike studies have been performed in this field. Hussain et al.[42] showed that among meat samples, the highest prevalence (48%) of Campylobacter was recorded in raw chicken meat followed by raw beef (10.9%) and raw mutton (5.1%). Korsak et al.[43] showed that Campylobacter species were detected in 690 (49.3%) of 1,400 poultry samples collected from retail trade. Strains were isolated from 50.2 and 41.1% of raw chicken and turkey meat samples, respectively, and from 50.1 and 42.6% of raw chicken and turkey giblets. The incidence of Campylobacter spp. on pork (10.6%) and beef (10.1%) was significantly lower than on poultry. *C. jejuni* was the most prevalent Campylobacter species in chicken (46.6%), pork (68.6%), and beef (66.7%), and Campylobacter coli was the most frequently isolated Campylobacter species in turkey meat (71.2%).

Whole incidence of *C. jejuni* amongst the poultry samples collected from Iraq[44], Pakistan[45], India[46], Korea[47], and China[48] was 10%, 40%, 26.30%, 36.30%, and 1.82% to 56.00%, respectively.

In the present survey high distribution of combined virulence genes was detected amongst the *C. jejuni* isolates. These factors are responsible for adhesion of bacteria into the epithelial cells of the gastric mucosa and invasion to them. In this regard, *cadF*, *ciaB*, *cdtA*, and *pldA* were detected in 55.55%, 44.44%, 51.85%, and 29.62% of isolates. Zheng et al.[49] revealed that All Campylobacter isolates possessed *flaA*, *cadF*, *pldA*, *cdtA*, *cdtB*, and *cdtC*, and most (91%) also contained the *ciaB* gene. However, the *virB11* gene, carried by virulence plasmid *pVir*, was absent in almost all the Campylobacter isolates. Melo et al.[50] reported that The genes *flaA*, *pldA*, *cadF*, and *ciaB* and the CDT complex were detected in 41/55 (74.5%), 35/55 (63.6%), 37/55 (67.3%), 37/55 (67.3%) and 36/55 (65.5%) strains respectively, and transcripts for the *ciaB* and *dnaJ* genes evaluated in 46 strains were detected in 60.9%.

Conclusion

Findings of this survey showed that Raw meat samples of sheep, goat and cattle species are reservoirs for transmission of virulent strains of *C. jejuni* into the human population. Proper cooking of meat before consumption can diminish the risk of Campylobacteriosis in human population. Cattle meat had the higher attitude for *C. jejuni* transmission.

Conflict of interest
The authors declare that there is no conflict of interest.

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