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Isolation, Epidemiological and Molecular Characterization of *Campylobacter* from Meat

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Abstract:
Study conducted to know epidemiology and accomplish molecular characterization of Campylobacter isolated from meat of different species and human stool samples. A total of 759 samples, consisting of human stool (50) and meat of poultry (251), chevon (183), pork (127), fish (106) as well as carabeef (42) were processed, 50 samples showed characteristic colonies on mCCDA plates. All the 50 isolates were subjected to various biochemical tests and Latex agglutination test for confirmation of the genus Campylobacter. All the isolates were further analysed for molecular confirmation and PCR based genus specific amplification of 16S rRNA gene which yielded product of 816 bp in all the isolates. Multiplex PCR was performed for genus as well as species-level identification; all 50 isolates revealed 857 bp amplicon of 16S rRNA gene specific for genus Campylobacter. Thirty-five isolates exhibited 589 mapA gene amplicon specific for C. jejuni and 16 isolates gave 462 amplification product of ceuE gene specific for C. coli. Overall prevalence was 6.58%. The highest prevalence rate of 13.54% was recorded in poultry meat, followed by 7.6% in chevon, 0.78% in pork and 2% from human stool samples. None of the isolates were recovered from beef and fish meat samples. Most of the obtained isolates were classified as C. jejuni (35 strains, 70%), whereas C. coli was identified in 15 (30%) samples, indicating that the C. jejuni was the most commonly found species.

1. Introduction

Zoonotic *Campylobacter* spp. are the leading cause of human food borne bacterial gastroenteritis worldwide. *Campylobacter* is frequently found in the environment and are ubiquitous in nature (Humphrey *et al.,* 2007). According to latest reports by Center for Disease Control and Prevention, campylobacters are the 4th major cause of food-borne illness (9%), 3rd major cause of hospitalization (15%) and 5th main cause of human deaths (6%) due to food-borne infections annually in USA alone (Scallan *et al*., 2011). Data regarding its prevalence in India is lacking so an attempt has been made through study underhand to estimate the epidemiology in food animals and human.

2. Materials & Methods

A total of 759 samples, consisting of human stool (50) and meat of poultry (251), chevon (183), pork (127), fish (106) as well as carabeef (42) were collected for the isolation of *Campylobacter* spp. from various towns' areas of Nainital and Udham Singh Nagar districts of Uttarakhand. The isolation and identification of *Campylobacter* spp. was carried out as per the procedures outlined by OIE terrestrial manual (2008) with necessary modification. Morphological, biochemical and serological characterization of the *Campylobacter* genus was done by methods of Prasanna (2013). For identification and confirmation of genus of the isolates, 16SrRNA gene fragment was amplified following the method described by Linton *et al*., (1996). The primer used for the amplification of 16SrRNA gene fragment is given in Table 1.

Primer Name	Primer Sequence (5'-3')	Gene Targeted	Product Size	Primer specific for
MD16S1 MD16S2	ATCTAATGGCTTAACCATTAAAC GGACGGTAACTAGTTTAGTATT	16S rRNA	816	Genus *Campylobacter*

Table 1: Primer profile used in genus identification of the isolates

For identification of the genus and species of the isolates, multiplex PCR was carried out. Simultaneous amplification of 16SrRNA gene fragment (genus-specific), mapA gene (for *C. jejuni*) and ceuE gene (for *C. coli*) was carried using primers and protocol as described by Denis *et al.,* (2001) with suitable modifications in cycling conditions. The details of primers are given in Table 2.

Set	Primer Name	Primer Sequence (5'-3')	Gene Targeted	Product Size	Primer specific for
1	C412F C1228R	GGATGACACTTTTCGGAGC CATTGTAGCACGTGTGTC	16SrRNA	857	*Campylobacter spp.*
2	mapA 1 mapA 2	CTATTTTATTTTTGAGTGATTGTG GCTTTATTTGCCATTTGTTTTATTA	mapA	589	*C. jejuni*
3	ceuE 1 ceuE 2	AATTGAAAATTGCTCCAACTATG TGATTTTATTATTTGTAGCAGCG	ceuE	462	*C. coli*

Table 2: Details of primers used in mPCR assay for genus and species confirmation

3. Results and Discussion

Prevalence of campylobacter in various samples were depicted in table 3 and different species identified through molecular means in table 4. A total of 251 processed chicken meat samples were 34 (13.54%) found to be Campylobacter, in concomitant to Rajkumar *et al.,* (2010) observation of 18% from unorganized and 12% from organized farms in Uttar Pradesh. Analysis of 34 *Campylobacter* species identified in the present study revealed that most of them were *C. jejuni* (24) while *C. coli* (10) was detected in the remaining poultry meat samples in agreement with Zhao *et al.* (2010). Among 42 carabeef samples, none could recover the target bacterium in accordance with Wieczorek *et al.,* (2012). Of 127 pork samples, only 1 (0.78%) revealed the presence of *Campylobacter* spp. similar to Little *et al.,* 2008). Out of 106 fish meat samples processed, none could show presence of Campylobacter, as eating fish has never been found a risk factor (Loewenherz-Lüning *et al.,* 1996). A total of 50 human stool samples elucidated only 1 (2%) *Campylobacter* and identified to be *C. jejuni* concomitant to Pant (2011). Out of 183 chevon samples 14 (7.6%) *Campylobacter* could be isolated, of which 10 were identified to be *C. jejuni* and 4 as *C. coli* through molecular means (table 4), similar to the findings of Rahimi (2010), who reported a prevalence rate of 6.4% in chevon purchased from retail outlets in Iran.

S. No.	Sample	Total no. of samples	Positive samples	Prevalence rate
1.	Chicken Meat	251	34	13.54%
2.	Chevon	183	14	7.6%
3.	Pork	127	01	0.78%
4.	Fish Meat	106	01	2%
5.	Carabeef	42	00	0%
6.	Human Stool	50	00	0%

Table 3: Prevalence rate of Campylobacter from different samples

S. No.	Sample	Positive samples	*C. jejuni* (%)	*C. coli* (%)
1.	Chicken Meat	34	70.58	29.41
2.	Chevon	14	71.42	28.57
3.	Pork	01	0	100
4.	Human Stool	01	100	0

Table 4: Isolation rates of different Campylobacter spp. from different samples

4. Conclusion

This study provides information on the prevalence of thermophilic campylobacters in Nainital and Udham Singh Nagar of Uttarakhand and highlights the widespread presence of this important food borne pathogen in chicken meat. Although the thermophilic species of *Campylobacter* are recognized as emergent human pathogens, the epidemiological studies for these microorganisms are recent. However, since the presence of this pathogen has a great impact on public health and on meat trading, it is likely that the microbiological standards for *Campylobacter* spp. in meat need to be defined similar to those for pathogens such as *Salmonella* spp. and *Listeria monocytogenes*, which require the absence of pathogens in 25-gm sample.

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