

## Prevalence of *Campylobacter jejuni* and *Campylobacter coli* among broilers in Bareilly region

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### Abstract

**Aim:** To determine the prevalence of *Campylobacter jejuni* and *Campylobacter coli* among broilers at the time of slaughter in and around Bareilly, India.

**Materials and Methods:** A total of 100 chicken caecal samples were screened by conventional plating in modified charcoal cefoperazone deoxycholate agar with incubation at 42°C for 48 h under microaerophilic conditions. The characteristic colonies were confirmed by morphological and biochemical characteristics and multiplex polymerase chain reaction (mPCR) assay targeting *lpxA* gene.

**Results:** Out of 100 chicken caecal samples, 32 yielded isolates with typical phenotypic of *Campylobacter* species. The hippurate hydrolysis test found to be positive for 2 isolates, categorized as *C. jejuni* and negative for 30 isolates. The mPCR assay targeting *lpxA* gene also confirmed 2 (6.25%) isolates as *C. jejuni*, and 30 (93.75%) isolates as *C. coli*.

**Conclusion:** The present study showed broilers to an important source of *Campylobacter* in the region with predominance of *C. coli* than *C. jejuni* indicating a shift in the prevalence of important species of *Campylobacter*. To understand the variation in pattern of occurrence of species with high prevalence of organisms, detail studies on the ecology of campylobacteriosis are suggested.

**Keywords:** *Campylobacter coli*, *Campylobacter jejuni*, multiplex polymerase chain reaction, *lpxA* gene.

### Introduction

Campylobacters are gram-negative, spiral, catalase, oxidase and indoxyl acetate positive bacteria with cork screw motility. The genus *Campylobacter* comprises of 25 species, 8 subspecies and 2 provisional species [1]. Thermophilic campylobacters are known to cause gastroenteritis in human worldwide, with major involvement of *Campylobacter jejuni* and *Campylobacter coli*. Earlier reports suggest that the campylobacteriosis accounts for 5-15% of all illnesses worldwide [2].

It has been reported to occur more frequently than infections caused by *Salmonella* spp., *Shigella* spp., or *Escherichia coli* O157:H7 [3]. In 2012, annual incidence rate of the disease was reported as 4.4-9.3 per 1000 human population [4]. The 14% increase was observed in the incidence rate from 2006 to 2012, and 2011 was declared as the year with the highest incidence for campylobacteriosis [5]. A 4.5% incidence rate of the disease was observed in the southern part of India, with the majority of the infections by *C. jejuni* along with few *C. coli* and mixed infection with both the species [6]. Several workers have reported *Campylobacter* infection in 10.28-13.5% of diarrheic

cases from North India [7,8]. This disease is characterized by diarrhea, fever and abdominal cramps. The disease is generally self-limiting but may sometimes leave behind debilitating sequelae with complications like reactive arthritis and Guillain-Barre' syndrome, a neuropathological disorder characterized by acute ascending bilateral paralysis [9]. Poultry has been recognized as the primary reservoir of *Campylobacter* spp. thus, most of the infections are acquired by the consumption and handling of contaminated poultry.

Keeping this in view, the present study was undertaken to determine the prevalence of *C. jejuni* and *C. coli* among broilers at the time of slaughter in Bareilly, India.

### Materials and Methods

#### Sampling procedures

A total of 100 broiler caecal samples were collected from retail meat shops (37) and slaughter houses (63) in sterile containers and transported immediately to the laboratory under cold conditions. The samples were processed without any delay to ensure that the organisms remain viable and culturable.

#### Isolation and identification of *Campylobacter*

The caecal contents were scraped with the help of sterile inoculation loop and streaked directly onto modified charcoal cefoperazone deoxycholate agar (mCCDA) plates and incubated under microaerophilic conditions (BD GasPak<sup>TM</sup> EZ, USA) at 42°C for

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48 h. The plates were observed for the development of characteristic colonies and one characteristic colony from each plate was sub cultured and examined for phenotypic characteristics by Gram's staining, motility, oxidase, catalase and hippurate hydrolysis tests.

#### Molecular confirmation

A multiplex polymerase chain reaction (mPCR) assay targeting the lipid gene '*lpxA*' was used for confirmation of *Campylobacter* at species level as per the method described by Klena *et al.* [10] with slight modifications. In this modified mPCR assay two species of thermophilic campylobacters *viz.* *C. jejuni* and *C. coli* were targeted. The mPCR was performed in a total reaction volume of 25 µl containing 2.5 µl of 10X dream *Taq* buffer, 2.5 µl of 2 mM of each dNTP, 15 pmol of each primer (Table-1), 1 U dream *Taq* polymerase, 2 µl of bacterial DNA template extracted using DNeasy blood and tissue kit (Qiagen, Germany) and nuclease-free water up to 25 µl. The mPCR amplification was performed in a thermal cycler (Eppendorf, Germany) with initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min. Final extension was carried out at 72°C for 10 min. The amplified products were electrophoresed in 2.0% agarose gel (SRL, India) stained with ethidium bromide (0.5 µg/ml) and image was documented (Figure-1) in a gel documentation system (UVP, USA).

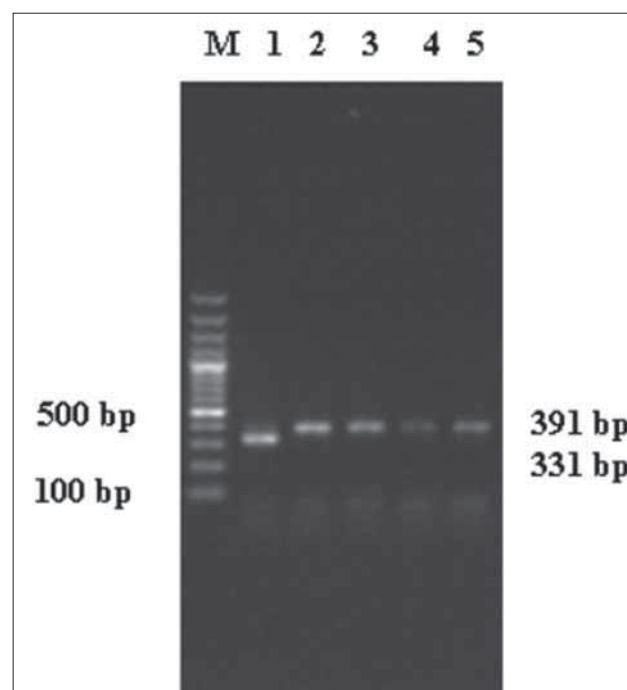
#### Results and Discussion

Out of 100 broiler caecal samples screened for the presence of *Campylobacter* using mCCDA plating, and identified by morphological and biochemical tests, *viz.*: oxidase, catalase and indoxyl acetate hydrolysis tests and H<sub>2</sub>S production in triple sugar iron slant, 32 samples were found positive. Further, all the isolates were subjected to hippurate hydrolysis test to identify *C. jejuni* (hippurate hydrolysis positive), in which only two isolates were identified as *C. jejuni* while the remaining 30 isolates negative for hippurate hydrolysis were considered as other *Campylobacter* species.

The isolates were then subjected to mPCR assay in which out of 32 isolates, two were identified as *C. jejuni* and the remaining 30 isolates were identified as *C. coli* based on the production of species specific amplicons 331 bp and 391 bp respectively. These findings revealed that the mPCR based assay was in accordance with hippurate hydrolysis test for species level identification of campylobacters.

The finding of the present study showed 32% overall prevalence of thermophilic campylobacters among broilers, which is in accordance with the previous report from Bareilly regions [11]. However, earlier workers from this region have reported a comparatively lower prevalence of *Campylobacter* in poultry, *viz.* 14.28% [12], 15.48% [13], 18% [14], 20% [15], 21.8% [16] and in contrast to these reports a much higher prevalence (65%) of *Campylobacter* in chicken caecum was also reported [17].

This study showed that *C. coli* were prevalent in 93.75% (30/32) and *C. jejuni* in 6.25% (2/32) among broilers slaughtered at chicken shop which was in concordance with the finding of other workers at Pantnagar and Bareilly regions [13,18]. Similar findings were also reported by other workers [19-22]. In contrast to the findings of the present study, many of the workers reported a higher prevalence of *C. jejuni* in poultry than *C. coli* [6,23-27]. The reason for this difference in prevalence rates of *C. jejuni* and *C. coli* among poultry is unknown; however, impact of differences in the isolation procedures and geographic differences has been suggested [21]. Modifications in poultry breeding conditions, use of growth promoters,



**Figure-1:** Electrophoresis of *lpxA* gene mPCR products on 2.0% agarose. Lane M: 100 bp DNA ladder, Lane 1 and 2: Positive controls, Lane 3-5: Samples with positive amplicon. [http://apps.who.int/iris/bitstream/10665/80751/1/9789241564601\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/80751/1/9789241564601_eng.pdf). 14 % increase <http://www.cdc.gov/foodnet/PDFs/ncezid-trends-factsheet-508c.pdf>.

**Table-1:** Details of oligonucleotide primers used for mPCR.

Primers	Sequence (5'-3')	Product size	Target gene	References
mP-Cj	F: ACAACTTGGTGACGATGTTGTA	331 bp	<i>lpxA</i>	[9]
mP-Cc	F: AGACAAATAAGAGAGAATCAG	391 bp		
mP-R	R: CAATCATGDGCDATATGASAATAHGCCAT			

mPCR= Multiplex polymerase chain reaction

and increased antimicrobial resistance in *C. coli* can be some of the reasons [19].

### Conclusion

It may be concluded from the present study that thermophilic campylobacters are highly prevalent in the broilers slaughtered in Bareilly region. The high level of presence of *C. coli* than *C. jejuni* may be due to laboratory practice and ecological discrepancies including variation in antibiotic resistance, changes in breeding and feeding practices of poultry. However, it has again been observed that the broilers may serve as a prime source of human campylobacteriosis, presently with predominance of *C. coli* infection; therefore, the work on epidemiology of campylobacteriosis is suggested to be continued.

### Authors' Contributions

HM, AK and SR designed the work plan; HM conducted experiments under supervision of AK and SR. Anjay, JLK, SS and SR helped in laboratory processing of samples. All authors contributed in drafting and revision of the manuscript. All authors read and approved the final manuscript.

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### Competing Interests

The authors declare that they have no competing interests.

### References

- Man, S.M. (2011) The clinical importance of emerging *Campylobacter* species. *Nat. Rev. Gastroenterol. Hepatol.*, 8: 669-685.
- Adak, G.K., Meakins, S.M., Yip, H., Lopman, B.A. and O'Brien, S.J. (2005) Disease risks from foods, England and Wales, 1996-2000. *Emerg. Infect. Dis.*, 11: 3.
- CDC. (2008) Division of Food-Borne, Bacterial and Mycotic Diseases (DFBMD) Listing. Centers for Disease Control and Prevention, United States.
- World Health Organization. (2012) The global view of campylobacteriosis: report of expert consultation, Utrecht, Netherlands, 9 to 11 July 2012. World Health Organization, Geneva, Switzerland.
- CDC. (2012) Available from: <http://www.foodsafetynews.com/2013/04/campylobacter-and-vibrio-rates-rose-in-2012-cdc-progress-report/#.UpYMqcEhhk>. Last accessed on 25-11-2013.
- Rajendran, S., Babji, P., George, A.T., Rajan, D.P., Kang, G. and Ajjampur, S.S. (2012) Detection and species identification of *Campylobacter* in stool samples of children and animals from Vellore, south India. *Indian J. Med. Microbiol.*, 30: 85-88.
- Ghosh, R., Uppal, B., Aggarwal, P., Chakravarti, A. and Jha, A.K. (2013) Increasing antimicrobial resistance of *Campylobacter jejuni* isolated from paediatric diarrhea cases in a tertiary care hospital of New Delhi, India. *J. Clin. Diagn. Res.*, 7: 247.
- Jain, D., Sinha, S., Prasad, K.N. and Pandey, C.M. (2005) *Campylobacter* species and drug resistance in a North Indian rural community. *Trans. R. Soc. Trop. Med. Hyg.*, 99: 207-214.
- Yuki, N., Susuki, K., Koga, M., Nishimoto, Y., Odaka, M., Hirata, K. and Yamada, M. (2004) Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barré syndrome. *Proc. Natl. Acad. Sci.*, 101: 11404-11409.
- Klena, J.D., Parker, C.T., Knibb, K., Ibbitt, J.C., Devane, P.M.L., Horn, S.T., Miller, W.G. and Konkel, M.E. (2004) Differentiation of *Campylobacter coli*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpxA*. *J. Clin. Microbiol.*, 42: 5549-5557.
- Garbaly, S.S. (2000) Studies on thermophilic campylobacters isolated from man and foods of animals origin. MVSc Thesis Submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, India.
- Singh, H.D. (2006) Isolation and PCR-based detection of *Campylobacter jejuni* and *Campylobacter fetus* in man, animals and foods of animal origin. MVSc Thesis Submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, India.
- Rajagunalan, S. (2010) Isolation, PCR based detection and FLA typing of thermophilic *Campylobacters*. MVSc Thesis Submitted to G.B.P.U.A&T., Pantnagar, India.
- Kumar, S.M. (2011) Studies on molecular heterogeneity of thermophilic *Campylobacter* isolates from man and animals. MVSc Thesis Submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, India.
- Khan, I.A. and Khanna, P.N. (1992) Occurrence of thermophilic *Campylobacter* of public health importance in animals, birds and food. Proceedings of 3<sup>rd</sup> World Congress. Foodborne Infection and Intoxication, Berlin. p462-467.
- Sharma, A.K. (2004) Detection of zoonotic *Campylobacter* species in animals, human beings and foods. MVSc Thesis Submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, India.
- Khan, I.A. (1989) Occurrence of thermophilic *Campylobacter* of public health importance in animals, birds and food. MVSc Thesis Submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, India.
- Prasanna, K.V. (2013) Isolation and molecular characterization of *Campylobacter jejuni* and *Campylobacter coli* from human and poultry caeca as well as meat. MVSc Thesis Submitted to G.B.P.U.A &T., Pantnagar, India.
- Desmonts, M.H., Dufour-Gesbert, F., Avrain, L. and Kempf, I. (2004) Antimicrobial resistance in *Campylobacter* strains isolated from French broilers before and after antimicrobial growth promoter bans. *J. Antimicrob. Chemother.*, 54: 1025-1030.
- Padungtod, P. and Kaneene J.B. (2005) *Campylobacter* in food animals and humans in northern Thailand. *J. Food Prot.*, 68: 2519-2526.
- Zorman, T., Heyndrickx M., Uzunovic-Kamberovic S. and Mozina S. (2006) Genotyping of *Campylobacter coli* and *C. jejuni* from retail chicken meat and humans with campylobacteriosis in Slovenia and Bosnia and Herzegovina. *Int. J. Food Microbiol.*, 110: 24-33.
- Henry, I., Reichardt, J., Denis, M. and Cardinale, E. (2011) Prevalence and risk factors for *Campylobacter* spp. in chicken broiler flocks in reunion Island (Indian Ocean). *Prev. Vet. Med.*, 100: 64-70.
- Chowdhary, S., Balakrish, N.G. and Pal, S.C. (1984) Occurrence of *Campylobacter jejuni* in country chicken in Calcutta. *Indian J. Med. Res.*, 79: 171-173.
- Harvey, R.B., Young, C.R., Ziprin, R.L., Hume, M.E., Genovese, K.J., Anderson, R.C., Droleskey, R.E., Stanker, L.H. and Nisbet, D.J. (1999) Prevalence of

- Campylobacter* species isolated from the intestinal tract of pigs raised in an integrated swine production system. *J. Am. Vet. Med. Assoc.*, 215: 1601-1604.
25. Saleha, A.A. (2002) Isolation and characterization of *Campylobacter jejuni* from broiler chickens in Malaysia. *Int. J. Poult. Sci.*, 1: 94-97.
26. Mena, C., Rodrigues, D., Silva, J., Gibbs, P. and Teixeira, P. (2008) Occurrence, identification, and characterization of *Campylobacter* species isolated from Portuguese poultry samples collected from retail establishments. *Poult. Sci.*, 87: 187-190.
27. Deckert, A., Valdivieso-Garcia, A., Reid-Smith, R., Tamblyn, S., Seliske, P., Irwin, R., Dewey, C., Boerlin, P. and McEwen, S.A. (2010) Prevalence and antimicrobial resistance in *Campylobacter* spp. isolated from retail chicken in two health units in Ontario. *J. Food Prot.*, 73: 1317-24.

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