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

**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™EZ, USA) at 42°C for

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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 H₂S 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,
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.

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