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RESEARCH ARTICLE
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# Biotyping of thermophilic *Campylobacter* spp. isolated from poultry in and around Anand city, Gujarat, India

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## Abstract

**Aim:** To study the prevalence of different biotypes of thermophilic *Campylobacter* spp. in the study area.

**Materials and Methods:** A total of 150 samples comprising 90 chicken and 60 caecal content were collected from retail meat market and processed for isolation of *Campylobacter* spp. 52 *Campylobacter* isolates obtained from raw poultry meat (6) and caecal content (46) were subjected to biotyping using Lior's biotyping scheme.

**Results:** Among the 52 *Campylobacter* isolates studied, 60.46 % isolates were identified as *Campylobacter jejuni* Biotype I and 39.53% were *C. jejuni* Biotype II, whereas 83.33 % were *C. coli* Biotype I and 16.66 % *C. coli* Biotype II. No other biotypes were identified.

**Conclusions:** The present study revealed that *C. jejuni* Biotype I was more prevalent than Biotype II whereas in case of *C. coli*, Biotype I was more prevalent than Biotype II providing basis for further epidemiological study.

**Keywords**: biotype, *Campylobacter*, poultry, thermophilic.

## Introduction

Food borne pathogens have been estimated to cause roughly 1.5 billion annual episodes of diarrhea and 3 million problems in death under the age of five worldwide [1]. In the last five decades, *Campylobacter* spp. have emerged as the most common cause food borne bacterial gastroenteritis in humans throughout the world with the number of cases caused by the *Campylobacter* has exceeded those caused by *Salmonella* [2]. A recent population based surveillance study conducted in United Kingdom concluded that *Campylobacter* caused the greatest impact on the healthcare sector with 15918 hospitalizations [3]. In 2007, *Campylobacter* infections were the most frequently reported zoonotic disease in humans across the European Union with 2,00,507 cases compared to 1, 75,561 in the previous year [4].

The association of zoonotic *Campylobacters* with chronic and potentially life threatening complications has increased the public health concern worldwide. As a consequence of *C. jejuni* infection a small number of individuals develop a secondary condition such as reactive arthritis or Guillain-Barré syndrome [5].

Poultry was identified as risk factors for *Campylobacter* infection as early as 1977. Ever since, numerous reports have implicated poultry in different forms; handling poultry [6], consumption of poultry [7], consumption of raw or undercooked poultry [8], consumption of liver [9] and consumption of poultry at a restaurant or commercial establishment [10]. In a retail survey conducted between 2005–2008 in New Zealand 72.7% of poultry carcasses were found contaminated with *C. jejuni* [11]. A baseline survey carried out in the EU in 2008 revealed that 75.8% of broiler carcasses sampled were contaminated with *Campylobacter* spp. [12]. The vast majority (97%) of sporadic *Campylobacter* infections in the UK could be attributed to animals that are farmed for meat and poultry [13].

Several epidemiological studies have demonstrated high prevalence of different biotypes of *Campylobacter* spp. in chickens, ducks and turkeys, ranging from 40% to 100% [14, 15] but their presence in poultry meat marketed has not been extensively investigated. So, present study was undertaken to study the occurrence of *Campylobacter* spp. prevalent in the area.

## Materials and Methods

**Samples:** A total of 150 samples comprising 90 poultry meat and 60 caecal content were collected aseptically from retail meat market in and around Anand city, Gujarat.

**Isolation:** All the collected samples were processed to isolate *Campylobacter* spp. as per the method described by FDA BAM [16] with little modification. Approximately, 25 gram of meat sample and 1 gram of caecal contents were enriched in 100 ml and 10 ml of selective enrichment broth (Park and Sanders

**Table-1:** Biotyping scheme of *Campylobacter* spp.

| Test | *C. jejuni* | | | | *C. coli* | | *C. lari* | |
|---|---|---|---|---|---|---|---|---|
| | Biotype-I | Biotype-II | Biotype-III | Biotype-IV | Biotype-I | Biotype-II | Biotype-I | Biotype-II |
| Hippurate hydrolysis test | + | + | + | + | - | - | - | - |
| Rapid $H_2S$ test | - | - | + | + | - | - | + | + |
| DNAse test | - | + | - | + | - | + | - | + |

**Table-2:** Biotyping of *Campylobacter* isolates from poultry

| Species | No. of isolates | Biotype-I | Biotype-II | Biotype-III | Biotype-IV |
|---|---|---|---|---|---|
| *C. jejuni* | 43 | 26 (60.46%) | 17 (39.53%) | - | - |
| *C. coli* | 6 | 5 (83.33%) | 1 (16.66%) | - | - |
| Unidentified *Campylobacter* spp. | 3 | - | - | - | - |

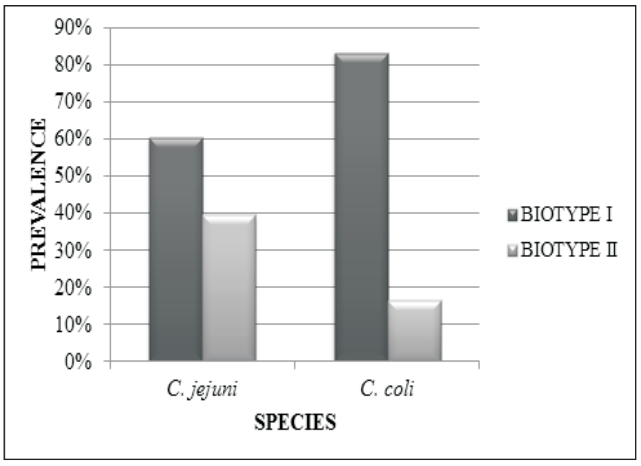


**Figure-1:** Prevalence of *Campylobacter* biotypes in poultry

enrichment broth supplemented with 5 % sterile, lysed sheep blood) respectively. Both the samples were then incubated at 42 °C for 24 hr under microaerophilic condition using $CO_2$ incubator (AutoFlow NU-4750, USA). Following enrichment a loop full of inoculum was then plated on selective agar (Park and Sanders agar) plates and the plates were then incubated at 42° C for 24 hr under microaerophilic condition as above. The presumptive *Campylobcters* showing typical, translucent, dew drop like colonies were picked up and subcultured on the nonselective blood agar for further identification.

A total of 52 *Campylobacter* isolates comprising 43 *C. jejuni*, 6 *C. coli* and 3 unidentified species were confirmed by polymerase chain reaction and subjected to biotyping by using Lior's scheme [17]. According to the Lior's scheme of biotyping, *C. jejuni, C. coli, C. lari* were divided into seven biotypes based on the three phenotypic tests viz., hippurate hydrolysis test, rapid $H_2S$ production and deoxyribonuclease enzyme production (DNase) test (Table-1).

Hydrolysis of hippurate is indicated by colour change due to the release of glycine. A loopfull of 48 hr test culture from blood agar was picked up and then rubbed on commercially available hippurate hydrolysis disc and observed for colour change. The positive reaction was indicated by presence of violet-purple colour. No color or a faint trace of purple is negative test.

For rapid $H_2S$ production test a large size ball like inoculums of 24-48 hr test culture of *Campylobacter* spp. from non selective blood agar plate was picked up and inoculated in rapid $H_2S$ test medium just below the surface. The tube is then kept in water bath at 37° C for 6 hrs and observed for colour change. The positive reaction was indicated by blackening of the test medium around the inoculums of test culture.

The production of nucleases by various bacteria has been demonstrated by growing the organism on DNA-conataining media with methyl green (MG) or toluidine blue O as an indicators. A loopfull of 48 hr old culture was used to inoculate heavily about 5 mm in diameter on DNAse agar plate and incubated at 43 ° C under microaerophilic condition. Positive reactions were indicated by appearance of large, pinkish, clear zones of hydrolysis on TB-DNAse agar after 24 to 48 hrs of incubation.

## Results

In present study, *C. jejuni* and *C. coli* were the only confirmed isolated species. Among the 52 *Campylobacter* isolates studied, 43 and 6 isolates were identified as *C. jejuni* and *C. coli* respectively and submitted for biotyping. Three among the 52 *Campylobacter* isolates were unidentified *Campylobacter* species and hence were not biotyped.

The study revealed that, *C. jejuni* Biotype I (60.46%) was more prevalent than *C. jejuni* Biotype II (39.53 %) whereas in case of *C. coli*, *C. coli* Biotype I (83.33 %) was more prevalent than *C. coli* Biotype II (16.66 %) (Table-2 and Figure-1). No other biotypes

were detected other than Biotype I and II for both *Campylobacter* spp.

## Discussion

*Campylobacters* are often present in the intestinal tract of domestic as well as of wild animals including poultry and are shed in the faeces of these animals [18, 19]. These are transmitted to humans via the faecal-oral route, predominantly through the consumption of contaminated food or water [20]. In order to ascertain the likely sources of *Campylobacter* in the human infections it is necessary to characterize strains commonly isolated from food chain and environment. The combination of the different typing scheme, including biotyping provided additional epidemiological markers further differentiating the organism by species and biotypes towards the investigation of *Campylobacter* related outbreaks. In this context the present study was conducted to study the existence and the frequency of occurrence of *Campylobacter* from the poultry in the study area.

The results in the present study are in accordance with Salihu et al [21] who noted 63.9 % and 25% occurrence of *C. jejuni* biotype I and biotype II respectively but contradict with Shelly et al [22] who reported 85 % and 14 % occurrence which is on extreme side compared to present study.

However, *C. coli* biotypes in the present study shows similar pattern of occurrence with Shelly et al [22] who recorded 84.3 % and 15.7 % but contradicts with Salihu et al [21] who observed 57 % and 43 % of occurrence for biotype I and biotype II.

In another study by Baserilsalehi *et al.* [23], isolation rate of 20% and 0% was recorded for *C. jejuni* biotype I and *C. jejuni* biotype II among poultry which contradicts with present study. Also, frequency of occurrence of 10 % each for both *C. coli* biotype I and II were recorded which is far from the results in the present study.

However, the frequency with which birds are associated with these organisms suggests their role in the dissemination of infection and needed to be investigated extensively.

## Conclusions

Overall, poultry in the area of investigation were found to be carrier of *Campylobacter* and certain biotypes have been identified as demonstrated in the study. The presence of biotypes other than those identified cannot be ruled out considering the smaller sample size and duration of the present study.

## Authors' contributions

RST and MNB have prepared and finalized the research plan. RST have carried out the research work. MNB have guided RST to carry out the work, draft and revised the manuscript as well. Both 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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