Prevalence of *Campylobacter jejuni* and *Campylobacter coli* from Unorganised and Organised Small Scale Poultry Dressing Units of Northern India

R.S. Rajkumar*, A.S. Yadav, R.S. Rathore¹, H.V. Mohan¹ and R.P. Singh
Food microbiology laboratory, Division of Post Harvest Technology, Central Avian Research Institute, Izatnagar-243122 (UP), India.

**ABSTRACT**

A study was conducted to compare the prevalence rate of *Campylobacter jejuni* and *Campylobacter coli* in two different systems of poultry slaughtering units in India. A total of 300 chicken breast skin samples comprising 200 samples from organized and 100 from unorganized on-floor retail poultry dressing units of Bareilly market were screened. Out of 300 chicken breast skin samples, 42 (14%) isolates of thermophilic *Campylobacter* spp. were recovered. Out of these 42 samples, 24 (57.14%) were from organized and 18 (42.85%) from unorganized small scale on-floor retail dressing units. On biochemical characterization of these 42 isolates, 30 (71.42%) were confirmed as *C. jejuni* and remaining 12 (28.57%) as *C. coli*. The prevalence rate of *Campylobacter* spp. from organized small scale poultry processing unit of Bareilly region of Northern India was comparatively lower probably due to the hygienic slaughtering practices followed like on table dressing with automated defeathering, clean water supply, skilled labour and under strict supervision of a trained personnel.

**Keywords:** *Campylobacter jejuni*, *Campylobacter coli*, poultry carcass

**Introduction**

Thermophilic *Campylobacter* spp. particularly *C. jejuni* and *C. coli* have emerged as major cause of diarrhoeal illness globally and are also responsible for the mortality and morbidity, especially among children. *Campylobacter* spp. infections have been linked to poultry in many outbreaks, primarily due to the consumption of raw or undercooked chicken (Tauxe, *et al.* 1988). Campylobacters have been reported to cause severe conditions like Guillain Barre syndrome, Chinese paralytic syndrome, hepatitis, meningitis and reactive arthritis (Allos, 1997; Korman *et al.*, 1997; Tenkate and Stafford, 2001). The incidence of campylobacteriosis in patients with AIDS was 39 times higher than the rate in the general population (Sorvillo *et al.*, 1992), which is the cause for the concern, especially in developing countries like India. The prevalence of *Campylobacter* spp. in raw poultry products vary from 0 to 100% with an average of 62% (Bryan and Doyle, 1995). *Campylobacter* spp. contamination in chicken processing plants comes almost exclusively from the viscera, particularly lower parts of the intestinal tract (ileum, caecum, and colon). Keeping in view the above facts the present study was carried out to determine prevalence of *Campylobacter jejuni* and *Campylobacter coli* in broiler carcasses slaughtered in two different small scale of poultry dressing units i.e., organized and unorganized in Bareilly region of Northern India.

**Material and Methods**

**Sample collection**

A total of 300 chicken breast skin samples comprising 200 from dressed broiler chicken
processed in an organized small scale poultry processing unit of Bareilly region and 100 from unorganized small scale on-floor retail poultry dressing units of Bareilly market were aseptically collected. All the samples were maintained at 4°C temperature and brought to the laboratory for the isolation of *C. jejuni* and *C. coli*.

**Enrichment**

Twenty five grams of breast skin samples were taken and homogenized with 225 ml of buffered peptone water (BPW), and then 1 ml of homogenate was taken for enrichment in 9 ml of Park and Sander’s broth base having 5% sterile defibrinated lysed sheep blood and Park and Sander’s selective supplement I and II, and incubated in McIntosh and Flide’s jar at 42-43°C for 24 h under micro-aerophilic conditions.

**Selective plating**

After incubation, the enriched inoculum was streaked onto selective media (Park and Sander’s broth base) supplemented with 2% agar, 5% sterile defibrinated lysed sheep blood and reconstituted contents of Park and Sander’s selective supplement I and II, and incubated in McIntosh and Flide’s jar assembly for 48 h at 42-43°C under micro-aerophilic conditions.

**Selection of suspected Campylobacter colonies and identification**

Characteristic *Campylobacter* colonies i.e. small, mucoid, non-hemolytic, gray (sometimes tan and watery) and discrete or spreading swarming types were picked up and subjected to presumptive identification like Gram’s staining, motility, oxidase, catalase tests. These isolates were further subjected to other biochemical tests for confirmation and species differentiation.

**Maintenance procedure**

Egg based medium recommended by Nair *et al.* (1984) was used for maintenance of *Campylobacter* isolates. Six freshly laid hen eggs were washed thoroughly with soap solution and wiped with 70% ethanol. The contents of the eggs were poured into a pasteurized wide mouthed flask containing 100 ml of sterile buffer saline (m/40, pH 7.0). The contents of the beaker were thoroughly emulsified by shaking and subsequently filtered through sterile muslin cloth. The emulsified egg (5 ml) was aseptically dispensed into screw cap tubes (16 x 50 mm). Egg slants were prepared by keeping the tube at 80°C for 1 h, for three successive days in an insipissator. After sterility testing, the egg slants were streaked with required cultures and then overlaid with 5 ml of thioglycollate broth containing 0.025% w/v each of sodium pyruvate, sodium metabisulphite and ferrous sulphate (FBP growth supplement).

**Biochemical tests**

The following biochemical tests were carried out for the identification and speciation as described by Skirrow and Benjamin (1980) and Gracia *et al.* (1985) viz., catalase activity, oxidase reaction, growth at 25°C and 42°C, growth in 1% glycine, growth in 3.5% sodium chloride, hydrogen sulphide (H₂S) production on lead acetate strips, hydrogen sulphide (H₂S) production in triple sugar iron (TSI) agar medium. Sensitivity to nalidixic acid and resistance to cephalothin, hippurate hydrolysis test. DNA hydrolysis test. In all groups, two independent replications were performed. The data obtained were analyzed as per the method of Snedecor and Cochran (1980).

**Results and Discussion**

Campylobacters are now getting increasing recognition as important human and animal pathogens. Food of animal origin, especially poultry have been considered as most important sources of infection to human beings. Hence an attempt was made to study the prevalence of *C. jejuni* and *C. coli* from unorganized and organised small scale poultry dressing units in Northern India.

Predominance of glossy or watery and spreading type of colonies were observed in fresh culture plates, whereas smooth, convex and discrete colonies were invariably seen on dried and refrigerated plates. Occasionally both types
of colonies were seen on the same plate in some cases. However, no clear cut correlation between colony type and species of *Campylobacter* could be observed in the present study. These colonies were found non-haemolytic, circular and convex. Similar observations have been reported by other workers also (Skirrow and Benazin, 1980).

Typical Gram's negative seagull wing shaped to spiral or comma shaped organisms with tapering ends were observed in Gram's staining from 42 h old culture. Hanging drop preparation of 48 h old culture from *Campylobacter* selective agar plates showed typical cork screw darting type motility. After 72 h of incubation, few long spiral forms were observed and majority of organisms got converted into coccobacilli form.

Suspected samples (243) colonies of organisms which gave Gram's negative stain with spiral or vibrioid morphology and showing cork screw or darting motility were tested for oxidase and catalase reaction. Out of these, 135 were positive for both oxidase and catalase tests, which

Table 1: **Number of Campylobacter** spp. suspected (phenotypically) samples subjected to commonly used biochemical tests

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Place of collection</th>
<th>No. of samples</th>
<th>Catalase (+ve)</th>
<th>Oxidase (+ve)</th>
<th>Growth @ 42°C (+ve)</th>
<th>Growth @ 25°C (-ve)</th>
<th>H₂S on TSI (+ve)</th>
<th>Glycine (+ve)</th>
<th>NaCl (-ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organized small scale poultry processing unit of Bareilly region</td>
<td>200</td>
<td>167</td>
<td>82</td>
<td>28</td>
<td>37</td>
<td>150</td>
<td>24</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>Unorganized small scale on-floor retail poultry dressing units of Bareilly market</td>
<td>100</td>
<td>76</td>
<td>53</td>
<td>17</td>
<td>14</td>
<td>91</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>300</strong></td>
<td><strong>243</strong></td>
<td><strong>135</strong></td>
<td><strong>45</strong></td>
<td><strong>51</strong></td>
<td><strong>241</strong></td>
<td><strong>42</strong></td>
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Table 2: Hippurate hydrolysis, rapid H₂S test, DNAse hydrolysis test, nalidixic acid sensitivity and cephalothin resistance produced by isolates.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Place of collection</th>
<th>Total isolates</th>
<th>Hippurate hydrolysis*</th>
<th>DNA hydrolysis*</th>
<th>Nalidixic acid sensitive*</th>
<th>Cephalothin resistant*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organized small scale poultry processing unit of Bareilly region</td>
<td>24</td>
<td>18 (75.0%)</td>
<td>18 (75.0%)</td>
<td>18 (75.0%)</td>
<td>18 (75.0%)</td>
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<tr>
<td>2</td>
<td>Unorganized small scale on-floor retail poultry dressing units of Bareilly market</td>
<td>18</td>
<td>12 (66.66%)</td>
<td>12 (66.66%)</td>
<td>12 (66.66%)</td>
<td>12 (66.66%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>42</strong></td>
<td><strong>30 (71.40%)</strong></td>
<td><strong>30 (71.40%)</strong></td>
<td><strong>30 (71.40%)</strong></td>
<td><strong>30 (71.40%)</strong></td>
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were further subjected for the remaining biochemical tests (Table 1). A total of 42 (14%) isolates were confirmed as *Campylobacter* spp. Out of which, 30 (10%) were positive for *C. jejuni* and remaining 12 (4%) were positive for the *C. coli*. (Table 2 and 3)

A total of 42 (14%) isolates of thermophilic *Campylobacter* spp. were recovered. The prevalence rate of *Campylobacter* spp. from the organized small scale poultry processing unit was low (12%) as compared to the unorganized small scale on-floor retail poultry dressing units (18%). Out of the 42 isolates of *Campylobacter* spp., 30 (71.42%) isolates were *C. jejuni* and 12 (28.57%) were *C. coli*.

Chicken skin samples were taken in the study because they have been shown to harbor and support the survival of *C. jejuni* (Lee et al., 1998). Fecal contamination of feathers and skin during transport to the slaughter facility, leakage of fecal content from the cloaca, intestinal breakage and contact with contaminated equipment, water, or other carcasses have been cited as probable sources of *Campylobacter* on poultry products (Jacobs-Rietsma, 2000). Similar studies have been conducted by Khan and Khanna (1992) who reported 20% isolation rate from chicken samples from Bareilly region. Likewise, Khurana and Kumar (1996) have reported the same prevalence rate (20%) from Hissar region of Haryana. Lately, Garbyal (2000) has shown 37.41% prevalence from chicken samples from Bareilly region.

The lower prevalence rate in organized small scale poultry processing unit than unorganized small scale on-floor retail poultry dressing units of Bareilly market may probably be attributed to the hygienic slaughtering procedures practiced at organized small scale poultry processing unit like on table slaughtering, automated defeathering, clean water supply, skilled labour and slaughtering under strict supervision of a trained personnel.

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References


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