Research Note

Campylobacter jejuni/coli: Methodology of Isolation and Possible Interfering Factors in Primary Culture

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ABSTRACT

The prevalence of Campylobacter jejuni and Campylobacter coli was investigated in 64 samples of fresh retail chicken purchased from commercial slaughterhouses located in Brazil. Campylobacter spp. were isolated from 40 (62.5%) of 64 analyzed samples. The strains biotyped according to Lior were classified as C. jejuni biotypes I and II, and C. coli biotypes I and II.

The efficiency of different procedures for recovering Campylobacter spp. from chicken carcasses was tested. The enrichment procedure was significantly less effective than direct plating (P < 0.05), detecting 19 of 40 (47.5%) as opposed to 38 of 40 (95%) positive samples. Using direct plating the efficiency of Blaser's selective supplement was significantly more effective (P < 0.05) than Skirrow's selective supplement. To verify which factors could be affecting Campylobacter spp. growth in enrichment broth, the pH was measured after incubation for 48 h at 42°C and lactobacilli, coliforms, and enterococci were enumerated.

Most of the Campylobacter-negative samples presented high levels of indicator microorganisms, which may have hindered the recovery of Campylobacter spp. during the enrichment procedure.

Key words: Campylobacter spp., isolation, interfering factors

For the last twenty years, Campylobacter jejuni has been increasingly associated with human gastroenteritis. This organism is carried in the intestinal tract of a wide variety of animals (3). It has been shown that 30 to 100% of poultry carry C. jejuni as a commensal inhabitant of the intestinal tract. Poultry typically excrete about 10⁴ to 10⁸ cells of this bacteria per gram of feces (21). Campylobacter spp. have also been detected in more than 30% of retail poultry at levels of 10² to 10⁴ colony-forming units (CFU)/g (18).

Different methodologies for C. jejuni isolation were assayed, since this organism is often difficult to isolate as it requires a microaerobic atmosphere and an enriched media for growth and also as it is sensitive to dry conditions. Products from animal sources have a varied microbiota which inhibits the growth of Campylobacter spp. by antagonism and competition. Hence, the procedures used for the isolation of Campylobacter spp. from clinical specimens, in which they may be present in large numbers, are not always suitable for food samples. Different selective enrichment media have been described, depending on the type of food to be analyzed. Moreover, the need for enrichment has been questioned (6).

The purpose of this study was (i) to evaluate several methods for isolating Campylobacter spp. from chicken, (ii) to investigate possible undergrowth of Campylobacter caused by inherent microflora (lactobacilli, enterococci, total, and fecal coliforms) during enrichment and (iii) to examine the relationship between acid production and detection of Campylobacter during enrichment.

MATERIALS AND METHODS

Sampling

A total of 64 samples of freshly processed retail chicken purchased from four commercial slaughterhouses was examined. Thirty grams of chicken sample (breast and thigh) were added to 270 ml of 0.1% buffered peptone water and homogenized in a Waring blender for 1 min. Serial dilutions were performed using the same diluent.

Isolation of Campylobacter spp.

Direct selective plating. Three loopfuls of the homogenate were streaked onto plates of Campylobacter agar base (Oxoid) containing defibrinated sheep blood (7%), ferrous sulfate (0.025%), sodium metabisulfite (0.025%) and sodium pyruvate (0.025%) (7) supplemented with either Blaser's (1) selective agents (vancomycin, trimethoprim, polymyxin B, amphotericin B and cefalotin) or Skirrow's (22) selective agents (vancomycin, trimethoprim and polymyxin B). The plates were incubated (42°C, 48 h) in a microaerophilic atmosphere obtained by copper passivation (15).

Selective-enrichment-plating procedure. Fifteen milliliters of the sample was transferred into 50 ml of Doyle and Roman broth (5) modified by the addition of Blaser's selective supplement (1).
After incubation (42°C, 48 h) under microaerobic conditions, i.e., with loosely capped bottles, a loopful of culture was streaked onto plates of media supplemented with either Blaser’s or Skirrow’s selective agents, followed by incubation under the same conditions. The pH of the enrichment broth of 54 samples was measured with a pH meter (Metrohm combination electrode) after incubation for 48 h.

Identification and biotyping

Two to four typical colonies were selected from each agar plate for presumptive Gram stain identification and stored in semisolid Brucella medium for Campylobacter conventional identification tests (25) and biotyping according to Lior (14).

Lactobacilli, enterococci, total, and fecal coliforms enumeration

The media used for most probable number determinations and the presumptively positive tubes confirmation for lactobacilli, enterococci, total, and fecal coliforms were according to Vedamuthu et al. (26), Hartman et al. (10), and Hitchins et al. (11), respectively. The most probable number counts were according to Obinger and Koburger (17).

Statistical evaluation

Differences among recovery rates were analyzed for statistical significance by McNemar’s chi-square test for correlated proportions at a significance level of 0.05 (20).

RESULTS AND DISCUSSION

Campylobacter spp. isolation and biotyping

Campylobacter spp. were isolated from 40 (62.5%) samples, and the incidence varied according to the source of samples (Table 1). Among the 94 Campylobacter spp. isolates, 81 (86.2%) were C. jejuni and 13 (13.8%) C. coli. Most of the C. jejuni and C. coli isolates belonged to Lior biotype I (Table 2). The isolation rates (62.5%) obtained in this work were similar to those described by several authors (5, 16, 19). However, rates ranging from 1.8 to 100% have been reported (12, 19, 24). Such discrepancies may result from some interfering factors such as sample collecting and transport methods, isolation methodology, procedures used at slaughter, hygienic sanitary conditions and poultry infection rates at breeding.

Therefore, in the present study, different isolation rates of Campylobacter spp. were found from each slaughterhouse investigated. The high frequency of Campylobacter spp. in the chicken samples purchased in our city must be considered a potential risk for the consumers, since the consumption of chicken and chicken products has been notably implicated in campylobacteriosis (3). In addition, it was reported that approximately 48.2% of human campylobacteriosis has been associated with consumption of undercooked chicken (23) or by cross-contamination of ready-to-eat products (16). All of the C. jejuni isolates belonged to Lior biotypes I and II (14), which are present in chickens (8) and have also been associated with human enteritis (9).

The enrichment procedure was less effective than direct plating (P < 0.05), detecting 19 of 40 (47.5%) as opposed to 38 of 40 (95%) positive samples (Table 3). The enrichment procedure has been developed to selectively recover small numbers of thermophilic campylobacters from foods. However, in the current study direct plating was more efficient in isolating C. jejuni/coli from chicken samples. A high level of microorganisms, inhabitants of poultry intestinal tracts which remain on the chicken carcasses after slaughter, may have contributed to this. In fact, a high level of lactobacilli, coliforms, and enterococci was present on the carcasses collected from the four slaughterhouses. The lactobacilli counts found in 95.2% of the samples were >1.1 × 10^7 MPN/g. The total and fecal coliforms counts ranged from 9 × 10^3 to >1.1 × 10^7 MPN/g. Counts >1.1 × 10^7 MPN/g were found for total and fecal coliforms in 48.8% and 39% of the samples, respectively. The enterococci count found in 61% of the samples analyzed ranged from 4 × 10^3 to 5 × 10^6 MPN/g and in the rest was >5 × 10^6 MPN/g. All of the Campylobacter-negative samples in enrichment broth presented lactobacilli counts of >1.1 × 10^7 MPN/g, and 77% presented total and fecal coliforms counts of >1.1 × 10^7 MPN/g. However, enterococci counts were <5 × 10^6 MPN/g in most of them.

The pH values found in the enrichment broth ranged from 4.7 to 6.4. The average pH was 5.7 and the pH of the enrichment broths in 1 (1.9%), 4 (7.4%), 25 (46.3%), and 24 (44.4%) of the samples ranged from ≥4.5 to <5, from ≥5.0 to <5.5, from 5.5 to 6.0, and ≥6, respectively. Recovery of Campylobacter was obtained from 52% and 48% of the samples with pH 5.5 to 6 and ≥6 respectively. Campylobacter could not be isolated in broth with pH lower than 5.5. The optimum pH for Campylobacter spp. growth ranges from 6.5 to 7.5 (4). However Campylobacter was recovered in the enrichment broth presenting pH lower than 6.5.

The interval for the incubation of enrichment broth probably reduced the efficiency of this procedure, resulting in undergrowth of Campylobacter caused by inherent micro-

<table>
<thead>
<tr>
<th>TABLE 1. Recovery of Campylobacter jejuni/coli from 64 samples of fresh retail chicken: distribution as a function of the slaughterhouses investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains</td>
</tr>
<tr>
<td>C. jejuni</td>
</tr>
<tr>
<td>C. jejuni + C. coli</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
TABLE 2. Distribution of the 94 Campylobacter spp. isolates as a function of the biotypes and slaughterhouses investigated

<table>
<thead>
<tr>
<th>Slaughterhouses</th>
<th>I</th>
<th>II</th>
<th>C. jejuni</th>
<th>C. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>02</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>05</td>
<td>---</td>
<td>01</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>07</td>
<td>04</td>
<td>02</td>
<td>04</td>
</tr>
<tr>
<td>4</td>
<td>01</td>
<td>04</td>
<td>02</td>
<td>01</td>
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</tbody>
</table>

TABLE 3. Efficiency of direct plating and enrichment procedures in the recovery of Campylobacter jejuni/coli from chicken samples

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Positive index no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct plating (D)</td>
<td>21 (52.50)</td>
</tr>
<tr>
<td>Enrichment (E)</td>
<td>02 (5.00)</td>
</tr>
<tr>
<td>D + E</td>
<td>17 (42.50)</td>
</tr>
<tr>
<td>Total</td>
<td>40 (100.00)</td>
</tr>
</tbody>
</table>

a Difference between D and E is statistically significant (P < 0.05).

TABLE 4. Distribution of Campylobacter-positive chicken samples using selective plating

<table>
<thead>
<tr>
<th>Medium</th>
<th>Direct plating</th>
<th>Enrichment selective plating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive (%)</td>
<td></td>
</tr>
<tr>
<td>Medium A</td>
<td>17 (44.70) 05 (26.30)</td>
<td></td>
</tr>
<tr>
<td>Medium B</td>
<td>02 (5.30) 03 (15.80)</td>
<td></td>
</tr>
<tr>
<td>A + B</td>
<td>19 (50.00) 11 (29.40)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>38 (100.00) 11 (29.40)</td>
<td></td>
</tr>
</tbody>
</table>

a Supplemented with vancomycin, trimethoprim, polymyxin B, and amphotericin B and cefalotin (I).
b Supplemented with vancomycin, trimethoprim, and polymyxin B (22).
c Difference between medium A and B is statistically significant (P < 0.05).
d Difference between medium A and B is not statistically significant (P > 0.05).

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REFERENCES