Research Note

Comparison of Methods for the Isolation of Thermotolerant Campylobacter from Poultry

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ABSTRACT

Human campylobacteriosis has become the major cause of foodborne gastroenteritis in industrialized countries. Although there have been numerous studies investigating the prevalence of Campylobacter in animals and raw meat, sensitive and low-cost detection methods are needed to implement effective control measures during primary production and to use as tools in risk assessment studies. Thermophilic Campylobacter spp. in naturally contaminated (n = 64) and inoculated (n = 16) broiler samples were detected using two International Organization for Standardization (ISO)-approved methods. Both enrichment broths (those of Preston and of Park and Sanders) were inoculated with (i) Campylobacter jejuni ATCC35921, (ii) boneless breast from broilers, (iii) boneless breast rinse solution, (iv) boneless breast rinse solution inoculated with C. jejuni ATCC35921 before centrifugation, and (v) boneless breast rinse solution inoculated with C. jejuni ATCC35921 after centrifugation. The results indicated that the Park and Sanders broth was superior to the Preston broth for recovery of Campylobacter spp., and no significant differences (P > 0.05) were found between ISO (meat pieces) and modified ISO (centrifuged chicken rinse solution) methods for the detection of Campylobacter spp.

Campylobacter spp. are currently regarded as the leading cause of bacterial gastroenteritis in humans worldwide. Campylobacter jejuni is zoonotic and is a normal inhabitant of the small intestines of numerous animals (e.g., poultry, pigs, and cattle). C. jejuni is now recognized as a common cause of acute enterocolitis in humans (3). In developing countries, Campylobacter spp. are an important cause of childhood morbidity from diarrheal illness. In industrialized countries, Campylobacter infections cause diarrheal disease 2 to 7 times more frequently than do Salmonella, Shigella, or Escherichia coli O157:H7 (2).

The single most important route of Campylobacter infections in the United States and other industrialized countries remains the consumption and handling of chicken (9). Nachamkin et al. (13) reported that in industrialized countries 80 to 90% of Campylobacter infections are probably due to C. jejuni and 5 to 10% are due to Campylobacter coli. In Spain, C. jejuni was isolated from about 50% of retail chicken meat, and this prevalence rate was greater than that of any other pathogen (4). In Northern Ireland, Campylobacter spp. were isolated from 94% of fresh poultry carcasses and 77% of frozen poultry carcasses. Carcasses were contaminated with C. jejuni, C. coli, and Campylobacter lari, accounting for 69, 30, and 1% of the contaminants, respectively (12). In 1989, in a case-control study of endemic diarrheal disease among 1,230 Thai children less than 5 years of age Campylobacter was detected in 13% (C. jejuni in 11.5% and C. coli in 1.7%), Shigella was detected in 13%, Salmonella was detected in 12%, and Escherichia coli (enterohemorrhagic form) was detected in 9% of cases (6). Similarly, in 1993, C. jejuni was identified in 25%, E. coli was identified in 13%, and nontyphoidal Salmonella spp. were identified in 8% of 24 persons who submitted diarrheal specimens (5).

The infective dose for C. jejuni is very small; as few as 500 cells can cause human illness (11), and severe complications, including reactive arthritis and Guillain-Barré syndrome, can occur after the infection has resolved (1). Thus, even very small numbers of C. jejuni cells in food or water may pose a potential health hazard. Sensitive methods are needed to detect C. jejuni cells in food and water samples. Standard methods for the detection of C. jejuni, including those described in the Bacteriological Analytical Manual (8) and by the International Organization for Standardization (ISO) (10), require an enrichment step because the cells of this organism may also be sublethally injured by starvation and physical stress. However, both enrichment methods are time-consuming and relatively expensive because they require large amounts of medium and antibiotic supplements. Modifications of the enrichment step have been described. For example, sample suspensions have been concentrated by centrifugation to decrease the volume of medium and antibiotics needed (15, 17).

The objective of this study was to investigate modifications to the ISO method for Campylobacter detection and
to compare the Preston and the Park and Sanders enrichment broths for *Campylobacter* detection in fresh broiler chicken meat. The ISO procedure in which the sample was enriched in either Preston broth or Park and Sanders broth was compared with a modified ISO method in which the sample was first rinsed in diluent, the diluent was then centrifuged, and the cell pellet was inoculated into both enrichment broths.

**MATERIALS AND METHODS**

**Test strain.** *C. jejuni* ATCC 35921 was obtained from the Department of Medical Sciences (Ministry of Public Health, Thailand). After 48 h of incubation on Columbia blood agar (Merck, Ltd., Bangkok, Thailand) at 42°C in a vacuum desiccator (MVD300, Nikko Company, Matte City, Japan) under a microaerobic atmosphere (5% O₂, 10% CO₂, and 85% N₂), *Campylobacter* colonies were transferred to 0.1% peptone water with added growth supplement containing 0.025% ferrous sulfate, sodium pyruvate, and sodium metabisulfite (SR084, Oxoid Ltd., Basingstoke, UK). Cell numbers were adjusted to an optical density of 0.5 on the McFarland scale (equivalent to 1.5 × 10^8 CFU/ml), and serial dilutions were made to achieve an initial bacterial count of 2 log CFU/ml. The bacterial concentrations were confirmed by plating on Columbia blood agar plates.

**Selective plating media.** Karmali agar (CM935, Oxoid) containing Karmali selective supplement (SR205, Oxoid) and *Campylobacter* blood-free selective agar base (CM739, Oxoid) containing charcoal cefoperazone deoxycholate agar selective supplement (SR155, Oxoid) were used for recovery of *Campylobacter*. The media were prepared according to the manufacturer’s instructions and sterilized by autoclaving at 121°C for 15 min. The molten agars were tempered to 50°C, and then selective supplements were added. The plates were poured, kept at 4°C, and dried at room temperature overnight in the dark before inoculation. Samples from all enrichments were plated onto both selective media.

**Collection of broiler chicken samples.** Chicken samples were purchased from a local market in Hatayai district, Songkhla Province, Thailand. The samples, consisting of fresh boneless breast (BB) with skin, were cut into pieces of approximately 150 to 200 g. All samples were held in an ice box (4°C) for less than 2 h and analyzed on the day of purchase. The experiment was repeated eight times with duplicate samples taken at each time for a total of 16 samples for each treatment.

**Modified enrichment method from inoculated samples.** Sodium citrate (1 g/liter) (Sigma-Aldrich, Singapore), 0.25 g/liter sodium pyruvate (Sigma-Aldrich), 5% laked horse blood (SR048, Oxoid), growth supplement (SR084, Oxoid), and selective supplement (SR204, Oxoid) were added to Preston broth (PB) and Park and Sanders broth (PS) (10) that contained 40 g/liter *Brucella* broth (Becton Dickinson Ltd., Bangkok, Thailand). The selective supplement included polymyxin B (5,000 IU/liter), rifampin (0.010 g/liter), trimethoprim lactate (0.010 g/liter), and amphotericin B (0.010 g/liter).

For the recovery methods, PB and PS were both inoculated with 1 ml of *C. jejuni* ATCC35921 suspension (an initial bacterial count of 2 log CFU/ml) (solution A), BB from broilers analyzed using the ISO method (10) (solution B), BB rinse solution (solution C), BB rinse solution inoculated with *C. jejuni* ATCC35921 before centrifugation (solution D), and BB rinse solution inoculated with *C. jejuni* ATCC35921 after centrifugation (solution E). BB rinse solution was prepared according to the methods of Stern and Line (17) and Petersen et al. (15). Twenty-five-gram pieces of meat from BB were transferred to stomacher bags containing 225 ml of peptone water saline with added 5% laked horse blood (PWS). The stomacher bags were vigorously mixed by hand for 2 min, and the suspensions were filtered through a sieve (mesh 35, 500-μm pore size). This BB rinse solution was separated into three parts (C, D, and E). For solution C, 1 ml of BB was used. For solution D, 100 ml of BB rinse solution was inoculated and centrifuged at 10,000 × g for 10 min. The pellets were resuspended in 5 ml of PWS. For solution E, 100 ml of BB rinse solution was centrifuged at 10,000 × g for 10 min and resuspended in 5 ml of PWS prior to inoculation with 0.1 ml of solution A. The preparation of the sample treatments is shown schematically in Figure 1.

Isolation from different sample types. Samples of fresh broiler chicken were obtained from BB, thigh, liver, and intestine. These were collected in a manner similar to that for the BB with skin and processed by the modified ISO method according to Stern and Line (17) and Petersen et al. (15), which involved centrifugation of the rinse solution (100 ml) at 10,000 × g for 10 min followed by resuspension of the pellet in 5 ml of PWS. Total plate count was determined using plate count agar (Merck). All rinses were prepared in the same manner and enriched with PBS containing 5% laked horse blood, growth supplement, and selective supplement. All samples were incubated in a vacuum desiccator under a microaerobic atmosphere at 42°C for 48 h.

Isolation procedure. The ISO isolation procedure (10) was followed. Campylobacter was isolated by the streak plate technique on selective agar plates, which were incubated under microaerobic atmosphere at 42°C for 48 h. Five different presumptive Campylobacter colonies per plate were Gram stained and characterized. Campylobacter is usually a gram-negative, slender, curved to S-shaped rod that exhibits corkscrew motility. It commonly produces gray, flat, irregular, spreading colonies, particularly on freshly prepared medium. To confirm the identity of Campylobacter, biochemical tests were performed: oxidase reaction, catalase reaction, indoxyl acetate hydrolysis, growth at 25°C, triple sugar iron reaction, hippurate reaction, and susceptibility to nalixidic acid. Campylobacter jejuni ATCC35921, susceptibility to nalixidic acid was determined. Campylobacter spp. are oxidase and catalase positive, Campylobacter fetus is the only species able to grow at 25°C, and Campylobacter spatorum is the only species able to produce H₂S during growth in triple sugar iron medium. Campylobacter jejuni and C. coli are able to hydrolyze indoxyl acetate but C. fetus and Campylobacter lari cannot utilize this substrate. Campylobacter jejuni and C. coli are both susceptible to cephalotin, and this characteristic distinguishes them from other species. Campylobacter jejuni is the only species able to hydrolyze hippurate.

Statistical analysis. The chi-square method and nonparametric analysis were used to evaluate the media and methods for their ability to detect Campylobacter spp. using treatments A, B, C, D, and E with the fresh samples. A two-way analysis of variance was used to evaluate the sample types for their total plate count and Campylobacter contamination. The least significant difference test was used to quantify significant differences (P < 0.05) between culture count means, which were transformed to log CFU per milliliter before analysis.

RESULTS AND DISCUSSION

The efficacy of two enrichment media for the recovery of C. jejuni ATCC 35921, C. jejuni from BB, and C. coli using treatments A, B, C, D, or E in combination with the enrichment medium PB or PS was determined (Table 1). No significant differences (P > 0.05) were found between PB and PS for recovery of C. jejuni ATCC 35921 and C. coli. However, C. jejuni was recovered from more samples with PS than with PB (P < 0.05). Characteristic colonies of Campylobacter on both Karmali agar and charcoal cefoperazone deoxycholate agar were more easily identifiable after enrichment in PS rather than PB (data not shown). Thus, PS was considered more suitable than PB for isolation of Campylobacter. This difference may be due to the improved recovery of sublethally injured cells in PS, which consists of a rich basal medium and a combination of yeast extract, sodium citrate, and oxygen-quenching ingredients such as sodium pyruvate (7).

No significant differences were found between treatments A, D, and E for recovery of C. jejuni ATCC 35921 that had been inoculated into the samples; the organism was recovered from all the samples (Table 1). As expected, C. jejuni ATCC 35921 was not recovered from the uninoculated samples (treatments B and C) with either PB or PS. C. coli was recovered from 50% of samples subjected to treatments B, C, D, and E and enriched in PB or PS. However, when PB was used as the enrichment medium, C. jejuni was recovered at an efficiency of 25% by treatment C but was not recovered by treatments B, D, or E. Incubation periods were longer for treatment C than for treatment B (48 and 18 h, respectively), which might explain the poor results obtained (7). However, C. jejuni was not recovered by treatments D or E despite the fact that the samples were inoculated with C. jejuni ATCC 35921. Thus, the chance of C. jejuni isolation might decrease if this procedure were used. When PS was used for enrichment, C. jejuni was recovered from 50% of samples subjected to treatments B, C, or E and from 25% of samples subjected to treatment D. No significant difference (P > 0.05) was found between the ISO (meat pieces) and the modified ISO.
TABLE 2. Approximate cost and time comparisons of Campylobacter enrichment methods as performed in this study

<table>
<thead>
<tr>
<th>Enrichment broth</th>
<th>Method</th>
<th>Cost ($/sample)</th>
<th>Technician time (min/sample)</th>
<th>Test duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Media</td>
<td>Labor</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Preston</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISO</td>
<td>12.58</td>
<td>0.25</td>
<td>12.83</td>
<td>15</td>
</tr>
<tr>
<td>Modified ISO</td>
<td>3.60</td>
<td>0.48</td>
<td>4.08</td>
<td>29</td>
</tr>
<tr>
<td>Park and Sanders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISO</td>
<td>12.11</td>
<td>0.25</td>
<td>12.36</td>
<td>15</td>
</tr>
<tr>
<td>Modified ISO</td>
<td>3.57</td>
<td>0.48</td>
<td>4.05</td>
<td>29</td>
</tr>
</tbody>
</table>

*Mean volume of enrichment media was 225 ml per sample for the ISO method and 9 ml per sample for the modified ISO method.

(centrifuged meat solution) methods for Campylobacter spp. detection.

Several advantages of the modified ISO method using centrifuged meat solution were observed in this study. A simple cost analysis of the two methods was performed. The cost of materials for the tests (not including selective media and biochemical testing) was calculated based on estimated labor costs at about $1.00/h (typical pay rate in Thailand). Labor costs were approximately doubled when the modified ISO method was performed, but when all associated costs were taken into consideration, the modified ISO method was estimated to be over three times cheaper than the ISO method (Table 2). Fewer materials were required for the modified ISO method because of the lower volume of enrichment medium and lower concentration of antibiotics used, although more technician time was needed to perform the modified ISO method.

Recovery of Campylobacter spp. from the different fresh poultry samples analyzed by the modified ISO method revealed that 75% of BB, thigh, and intestine samples were contaminated with C. jejuni, and C. coli was isolated from 50% of samples of these products. Thus, Campylobacter spp. were recovered from 100% of samples (Table 3). In contrast, 25% of chicken liver samples were contaminated with either C. jejuni or C. coli, with an overall contamination rate of 50% for Campylobacter spp. (Table 3).

No significant difference (P > 0.05) was found for total plate count from samples of BB, thigh, and intestine (7.50, 7.54, and 7.21 log CFU/ml, respectively), but counts were significantly lower for liver samples (P < 0.05; 6.04 log CFU/ml). Broiler chickens are often intestinal carriers of C. jejuni, and a chicken colonized with this organism usually excretes large numbers of bacteria (16), leading to contamination of broiler carcasses during slaughter (14, 18), whereas livers might be cleaned with water before their sale.

PS was as effective as PB for isolation of C. jejuni ATCC 35921 and C. coli but was better than PB for isolation of C. jejuni from BB samples. The modified ISO method was as effective as the ISO protocol for the isolation of C. jejuni ATCC 35921 and of C. jejuni and C. coli from poultry samples. Therefore, enrichment in PS combined with the modified ISO method is an effective and inexpensive procedure for the isolation of Campylobacter spp. from poultry samples.

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**REFERENCES**


