Comparison of Selective Enrichment Broths for Isolation of Campylobacter jejuni/coli from Freshly Deboned Market Chicken

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(Received for publication March 4, 1991)

ABSTRACT

The efficiency of Brucella broth supplemented with vancomycin-trimethoprim-polymyxin B (VTP) at the same concentration as Skirrow agar and bacitracin-colistin-cephalothin-cycloheximide-novobiocin (BCN) at the same concentration as Butzler agar was evaluated in the recovery of Campylobacter jejuni/coli from fresh retail chicken. Ninety-two samples were analyzed by enrichment in VTP and BCN broths, each streaked on Skirrow and Butzler agars. Thirty-three (36%) of the samples were positive by any route of analysis. The recovery of Campylobacter jejuni/coli was enhanced significantly. Campylobacter was two enrichment broths the ability to recover from chicken, and that by simultaneously using an agent for use in an enrichment-plating procedure to recover vancomycin-trimethoprim-polymyxin B mixture was not a suitable mixture. In the case of Salmonella (4), in the technique for isolation of Campylobacter spp. from certain foods.

The purpose of this work is to evaluate the efficiency of Skirrow and Butzler (18) antibiotics added to enrichment and plating media, and combinations of these broths and agars for recovery of C. jejuni/coli from naturally contaminated fresh retail chicken.

MATERIALS AND METHODS

Media

Double strength vancomycin-trimethoprim-polymyxin B (VTP) and bacitracin-colistin-cephalothin-cycloheximide-novobiocin (BCN) broths were prepared as follows: 25 ml of double strength brucella broth (Bioxon, Becton Dickinson, Mexico) was distributed in 100-ml screw-capped flasks. The sterile broth was cooled to room temperature prior to supplementation with filter-sterilized FBP aerotolerant solution composed of 0.025% each of sodium metabisulfite (J. T. Baker, Phillipsburg, NJ), ferrous sulfate (J. T. Baker) and sodium pyruvate (Merck, Rahway, NJ) (11). One of the flasks was also supplemented with vancomycin (20 µg/ml), trimethoprim (10 µg/ml), and polymyxin B sulfate (5 IU/ml), and another flask with bacitracin (50 IU/ml), colistin (20 µg/ml), vancomycin (20 µg/ml), trimethoprim (10 µg/ml), and polymyxin B sulfate (5 IU/ml) was prepared as the VTP broth.
IU/ml), cephalothin (30 µg/ml), novobiocin (10 µg/ml), and cycloheximide (10 µg/ml). The first mixture was named VTP broth and the last BCN broth.

The plating media were brucella agar FBP supplemented with 7% lysed defibrinated horse blood and those antibiotics of Skirrow agar (24) and the same basis supplemented with the antibiotics included in Butzler agar (8). For biochemical and growth tests, brucella agar FBP was used, and triple sugar iron agar (TSI) with lead acetate strip was used to test for H2S production.

**Samples**

A total of 92 samples of freshly deboned chicken was obtained from retail markets in Guadalajara. All samples were from the thigh of the bird and were not skinned. Portions weighing about 200 g were tested. The samples were individually placed in nonsterile plastic bags and were transported to the laboratory without refrigeration. Bacterial and fungal counts from the plastic bags were conducted before use, which yielded most probable number values lower than 0.03 microorganisms per bag. Upon arrival at the laboratory, the samples were held at 4-5°C until analysis. The time between collection and analysis did not exceed 2 h.

**Isolation procedure**

One hundred ml of 0.1% peptone water was added to the contents of each plastic bag. Samples were massaged and shaken vigorously for two minutes. From the resultant liquid, 25 ml were added to a flask with double-strength VTP broth and another 25 ml to double-strength BCN broth. The components of the broths were then at proper concentration. The flasks containing inoculated VTP and BCN broths were placed with loosened caps inside an anaerobic jar with valves (Difco, Detroit, MI) which was evacuated and refilled twice with a gas mixture of 5% O2, 10% CO2, and 85% N2 (Linde Div., Union Carbide, Danbury, CT). Samples were incubated at 42°C for 48 h. After incubation, three loopfuls from each enrichment were streaked onto Skirrow and Butzler agar plates. The plates were incubated as described above. One to three typical Campylobacter colonies were streaked onto brucella agar FBP and identification from these isolates was made by the following tests: Gram reaction, typical cell morphology, catalase and oxidase reactions, motility, H2S production in TSI slants and in lead acetate strips, sensitivity to 30 µg of nalidixic acid, resistance to 30 µg of cephalothin, growth at 42°C but not at 25°C, failure to grow under aerobic conditions and hippurate hydrolysis.

**Statistical analysis**

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

**RESULTS**

Thirty-three (36%) of 92 samples obtained from retail markets in Guadalajara tested positive for C. jejuni/coli in at least one of the enrichment broths examined. The results of the comparison among recovery rates using VTP and BCN broths are given in Table 1. By simultaneously using BCN and VTP, percent recovery increased significantly compared to that obtained with VTP only (p<0.05).

The frequency of recovery by only one of the two enrichment broths in the 33 positive samples was studied (Table 2). Results indicate that a number of samples containing Campylobacter gave negative results when only one broth was utilized. In other words, the use of only one broth resulted in a greater number of false negatives than when both broths were utilized. Thirty percent were positive in VTP but negative in BCN, and 45% of samples were positive in BCN but negative in VTP. Apparently the use of VTP led to a greater number of false negatives than BCN; however, these differences were not significant (see Table 2). Thus, the latter broth was not found to be sufficiently reliable in itself to recover Campylobacter. There appeared to be a heterogeneous distribution between the positive samples in both broths studied, since there were more positives in only one broth (10 in VTP and 15 in BCN) than in both media together (8 samples).

**TABLE 1. Recovery of Campylobacter jejuni/coli from 92 samples of freshly deboned chicken by enrichment in VTP and BCN broths.**

<table>
<thead>
<tr>
<th>Enrichment broth</th>
<th>Positive samples</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTP</td>
<td>18</td>
<td>19.6a</td>
</tr>
<tr>
<td>BCN</td>
<td>23</td>
<td>25.0</td>
</tr>
<tr>
<td>VTP + BCN</td>
<td>33</td>
<td>35.9b</td>
</tr>
</tbody>
</table>

b Difference among marked values is statistically significant (p<0.05).

**False-negative results were influenced by plating media.** Among samples in which the presence of Campylobacter was confirmed, on both Skirrow and Butzler agars there were samples that gave negative results after these two media were streaked with the two enriched broths (Table 3). Differences between false-negative rates in both broths were nonsignificant when only Butzler agar was used, but significant when only Skirrow agar was employed (see Table 3). Moreover, Table 4 shows that, while isolation on Butzler agar exhibited a similar distribution between positives derived from VTP and BCN broths, the efficiency of Skirrow agar inoculated from VTP broth was significantly lower than that obtained when its inoculum originated from BCN broth (p<0.001). It is clear that using
only Skirrow antibiotics in an enrichment-plating procedure leads to poor results, at least in the environment in which this study was conducted.

TABLE 3. Distribution of 33 Campylobacter-positive chicken samples by enrichment broth isolating on Butzler and Skirrow agars.

<table>
<thead>
<tr>
<th>Distribution of positive samples</th>
<th>VTP+</th>
<th>VTP</th>
<th>VTP+</th>
<th>VTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butzler agar</td>
<td>7</td>
<td>11</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>% positive</td>
<td>21.2</td>
<td>33.3</td>
<td>27.3</td>
<td>18.2</td>
</tr>
<tr>
<td>p&lt;0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skirrow agar</td>
<td>1</td>
<td>1</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>% positive</td>
<td>3.0</td>
<td>3.0</td>
<td>57.6</td>
<td>36.4</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td></td>
<td></td>
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</tbody>
</table>


TABLE 4. Efficiency of enrichment-plating procedures in the recovery of Campylobacter jejuni/coli from 92 samples of freshly deboned chicken.

<table>
<thead>
<tr>
<th>Enrichment media</th>
<th>VTP</th>
<th>BCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butzler Agar</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Skirrow Agar</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Percent of recovery</td>
<td>17.4</td>
<td>16.3</td>
</tr>
</tbody>
</table>


DISCUSSION

Results suggest that Skirrow antibiotics are not suitable agents for incorporation into enrichment broths for isolation of C. jejuni/coli from raw chicken. Chan et al. (5) reported that by increasing the concentration of polymyxin B in media containing vancomycin, trimethoprim, and polymyxin B, the selectivity of these media increased. Using this principle, Park et al. (17) proposed an enrichment medium for isolation of Campylobacter from chicken. However, Wesley et al. (27) reported that Park broth showed poor results in the isolation of C. jejuni from poultry products when compared with a broth in which rifampicin, cefsulodin, and polymyxin B were added as selective agents.

Using a VTP broth plus cycloheximide, Doyle and Roman (6) and Hunt et al. (13) reported good recovery in raw milk and hamburger samples but poor results for samples of chicken skin. Hence, our report is not the first to state that the use of VTP antibiotics applied in an enrichment-plating technique is not an efficient procedure for isolating C. jejuni/coli from poultry, although these antibiotics have been used by several workers in poultry (15,17,19,23,26).

Antimicrobials proposed by Butzler (BCN) are not commonly applied to Campylobacter research in foods, and no enrichment broths have been used with this supplement. Moreover, in some authoritative handbooks of methods for the microbiological analysis of foods (18), there are no comments contemplating the possibility that Butzler antimicrobials could be used in an enrichment broth. Perhaps this is due to the recognized greater inhibition of the associated flora and even of the Campylobacter organisms in Butzler agar (8); however, results from this study have shown their superiority over VTP broth.

From the results of this study, it cannot be stated that BCN broth is the best medium for analysis of Campylobacter in poultry samples. In other studies (2,22) in which comparisons between enrichment media have been made, isolation rates of C. jejuni are higher than those reported in the present study, especially when other steps have been included to enhance incubation conditions (14,22). Nevertheless, the usefulness of this medium should be evaluated in greater detail.

Another finding of interest was the increase in positive samples when two enrichment broths were utilized. In the isolation of pathogens such as Salmonella, better results are obtained in some foods by using selenite cysteine broth, and in other foods better results are obtained by using tetrathionate broth (4,7). Hence, in the case of Salmonella the use of both media is recommended. Nevertheless, in the isolation of Campylobacter the general tendency is to use only one recommended enrichment broth. In this sense it is interesting to observe that perhaps there exists no medium which does not miss an important number of positive samples, giving false-negative results, and that it may be necessary to use two enrichment broths simultaneously.

In conclusion, we can state that by using two enrichment broths simultaneously, the ability to recover C. jejuni/coli in freshly deboned retail chicken was enhanced significantly, and also that vancomycin, trimethoprim, and polymyxin B (VTP) mixture is apparently inadequate for use in an enrichment-plating procedure for the recovery of Campylobacter from poultry.

REFERENCES

algunos factores en la recuperación de *Salmonella* a partir de alimentos y otras fuentes. Abstracts. XVII National Meeting of Microbiology, Puebla, Mexico.


