Enrichment Medium for Isolation of Campylobacter jejuni-Campylobacter coli

M. ROGOL, B. SHPAK, D. ROTHMAN, AND I. SECHTER

National Center for Campylobacter, Central Laboratories, Ministry of Health, Jerusalem 91060

Received 15 November 1984/Accepted 26 March 1985

A broth enrichment medium for the improvement of isolation of Campylobacter jejuni-Campylobacter coli from stool samples and other specimens is presented. Of 1,228 samples examined in parallel, positive results were obtained from 81 by direct inoculation of selective media and from 112 after enrichment. Thus, an increase of 27.7% in the isolation rate was obtained by using the enrichment medium. The same medium without antibiotics allows the preservation of isolates of C. jejuni-C. coli for at least 2 months at 4°C.

Several enrichment media for improving the isolation of Campylobacter jejuni-Campylobacter coli from stool samples and foodstuffs have been proposed during the last few years (2, 3, 4, 7, 9). The efficiency of some of them was also studied comparatively (1, 6).

Since 1983 we have used a broth enrichment medium (BEM) developed in the National Center for Campylobacter, Jerusalem, and preliminary results obtained from the examination of meat and meat products have been published (8). In this paper we describe this medium and the results of its use in the isolation of C. jejuni-C. coli from stool samples, foodstuff, and other samples in comparison with direct seeding on selective media.

MATERIALS AND METHODS

Samples. A total of 1,050 stool samples from patients hospitalized with diarrhea, 70 meat samples from a chicken abattoir and from restaurants, 93 swabs taken from the environment in the chicken abattoir, and 15 samples of washings of chicken organs and sewage water in the abattoir were examined.

BEM. BEM contains the following: nutrient broth (no. 2, CM67; Oxoid Ltd., London, England), 1 liter; agar-agar (no. 3, L13; Oxoid), 0.75 g; the air-tolerant supplement of George et al. (5) containing ferrous sulfate, sodium metabisulfite, and sodium pyruvate, 0.5 g each; yeast extract (B127; Difco Laboratories, Detroit, Mich.), 1 g; and bile salts (no. 3, 130; Difco), 1.5 g (final pH 7.4). After sterilization by autoclave (121°C, 15 min), the medium was cooled to 50°C and the following substances were added: 1 vial of Skirrow dried antibiotics (SR69; Oxoid) containing 5 mg of vancomycin, 2.5 mg of trimethoprim lactate, and 1,250 IU of polymyxin B; and 50 ml of defibrinated blood. The medium was distributed in 5-ml volumes to tubes (100 by 10 mm).

Solid selective medium. Solid selective medium contained blood agar base (CM55; Oxoid) supplemented with 5% human defibrinated blood and the dried antibiotic supplement of Butzler (SR85; Oxoid).

Seeding method. Stool samples and anal swabs were each emulsified in 2 ml of phosphate-buffered saline (pH 7.2 to 7.4). The suspension was inoculated onto solid selective medium with a swab, and the swab was then immersed in tubes containing BEM. Both media were incubated at 42°C under microaerophilic conditions (anaerobic jar with GasPak; BBL Microbiology Systems, Cockeysville, Md.) for 48 h.

Meat samples were collected in sterile beakers, transported to the laboratory under cooling conditions, and seeded within 2 to 3 h. Portions (10 g) of meat were added to 90 ml of BEM and homogenized in a Colworth stomacher. 0.2 ml of the homogenate was seeded onto selective plates, and the remainder was incubated under the same conditions as were the stool samples.

Environmental swabs were put in tubes containing BEM and transported to the laboratory under cooling conditions; 0.2-ml portions were seeded on selective plates, and the remainder was incubated as described above.

Water samples were collected in sterile bottles and transported to the laboratory under cooling conditions; 0.2-ml portions were seeded on selective plates, and the same quantity was introduced into BEM tubes for enrichment.

All the seeded plates were examined after 48 h of incubation, and all the enrichment media seeded were dispersed on solid selective plates after 24 h of incubation.

RESULTS

In total, 1,228 samples were examined by direct seeding on solid selective plates and with the enrichment medium. Of 112 positive results, 81 (72.3%) were obtained by direct seeding, and 31 (27.7%) were obtained only after enrichment (Table 1), i.e., more than one-quarter of the positive results would have been missed without the use of the enrichment procedure. The percent improvement of results by enrichment was higher in the examination of stool samples (40.0%) and meat samples (36.6%) and lower in the examination of swabs and water samples.

The same enrichment medium was used for recovery of C. jejuni-C. coli strains from cultures sent from laboratories all over the country to the National Centre in Jerusalem; since part of these cultures may have been lost during transportation, they were seeded in parallel on selective plates and in BEM. Of 774 cultures received, 674 did grow on the direct seeded plates and 50 (6.9%) were recovered only after enrichment.

Conservation of isolates. It is well known that conservation of isolates of C. jejuni-C. coli in the laboratory is a difficult task. BEM without antibiotic supplement was used successfully for this purpose. Twenty different isolates of C. jejuni-C. coli were seeded in tubes (100 by 10 ml) with 5 ml of BEM (without antibiotic supplement), incubated at 42°C for 24 h under microaerophilic conditions, and preserved at 4°C.

* Corresponding author.
TABLE 1. C. jejuni-C. coli isolations from various specimens by direct seeding and after enrichment in BEM

<table>
<thead>
<tr>
<th>Source of samples (no.)</th>
<th>No. of positive results*</th>
<th>% improvement of isolation rate by enrichment*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct +, BEM +</td>
<td>Direct -, BEM -</td>
</tr>
<tr>
<td>Stools (1,050)</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Meat (70)</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Swabs (93)</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Water (15)</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>

* Direct +, Positive by direct seeding; direct -, negative by direct seeding; BEM +, positive after enrichment; BEM -, negative after enrichment.

* Average improvement, 27.7%.

Passages made after 10, 20, 30, 45 and 60 days were positive for all the cultures. Passages made after 70 and 80 days were positive for only 16 and 8 cultures, respectively.

DISCUSSION

The isolation of C. jejuni-C. coli strains from stool samples of acute cases of diarrhea by direct seeding on selective media is actually an easy task. However, their isolation from stool samples of convalescents, from stool samples of people after antibiotic treatment, or from food samples is more difficult. Therefore, enrichment media must be used in these cases.

The semisolid medium proposed by Chan and MacKenzie (3) allowed them to obtain a 6% increase in the isolation rate of Campylobacter jejuni-C. coli from stool samples. The enrichment medium of Doyle and Roman (4) allowed Rothenberg et al. (9) to obtain a 21% increase in the isolation rate. Bolton and Robertson (2), using the medium LEM, obtained a 30% increase. In our examinations, the BEM allowed a 40% increase in the isolation rate from stool samples and a 27.7% increase in the isolation rate from all the samples examined.

These results confirm the usefulness of the BEM as an enrichment medium for C. jejuni-C. coli. The BEM (without antibiotics) is recommended for longer preservation of cultures of C. jejuni-C. coli at 4°C.

LITERATURE CITED