Research Note

Comparison of Three Selective Media and Validation of the VIDAS Campylobacter Assay for the Detection of Campylobacter jejuni in Ground Beef and Fresh-Cut Vegetables

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ABSTRACT

In this study, three different selective media, modified cefoperazone charcoal deoxycholate agar (mCCDA), Karmali agar, and Preston agar, were compared for isolating Campylobacter jejuni from artificially contaminated ground beef and fresh-cut vegetables that have different levels of background microflora. Concurrently, an automated enzyme-linked immunosorbent assay method for detecting Campylobacter spp. (VIDAS Campylobacter) was evaluated by comparing it with the culture methods. Food samples inoculated with C. jejuni were enriched in Bolton broth at 42°C for 44 h and then streaked onto the three different selective media, followed by incubation under microaerobic conditions at 42°C for 48 h. The enriched Bolton broth (1 ml) was used in the VIDAS Campylobacter assay. No statistical differences in sensitivities were observed between the three selective media for ground beef and fresh-cut vegetables, but the selectivity of Preston agar was better (P < 0.05) than those of mCCDA and Karmali agar. The VIDAS Campylobacter assay showed a recovery rate similar (P > 0.05) to those of all of the medium combinations in ground beef. However, more positive samples (P < 0.05) were detected with the VIDAS Campylobacter than with the selective agars, except for the combinations of mCCDA plus Preston agar or mCCDA plus Karmali agar plus Preston agar in fresh-cut vegetables.

Campylobacter spp. are microaerophilic bacteria that cause human foodborne illness throughout the world (19, 23). Among the Campylobacter spp., C. jejuni accounts for 90% of foodborne campylobacteriosis (6). Poultry meats have been known to be a primary cause of campylobacteriosis, and other types of food, such as ground beef, water, oysters, eggs, vegetables, and milk, have also been reported as causes of Campylobacter illness (2, 5, 13, 26–28). The detection and identification of Campylobacter spp. are somewhat difficult because of its long incubation period and unique culture requirements, such as microaerobic conditions (5). Moreover, the amount of Campylobacter sp. organisms is often low and the organism is usually stressed in food samples (12, 16, 21).

The Campylobacter selective media being used in current standard culture methods are classified into two groups. One group, which includes Skirrow agar, Blaser agar, Campy-Cefex agar, and Preston agar, uses animal blood as a supplement. On the other hand, there is a blood-free group of media that includes modified cefoperazone charcoal deoxycholate agar (mCCDA), Karmali agar, and cefoperazone amphotericin teicoplanin agar (1, 4, 8, 18, 22). These selective media have been validated in previous studies (15, 16, 22, 25). However, most studies were conducted with feces or poultry samples but not with ground beef or fresh produce. Undercooked ground beef or cross-contaminated fresh produce carrying C. jejuni could be a cause of campylobacteriosis (6, 28).

To overcome the drawbacks of the culture method, various rapid and sensitive detection methods have recently been developed. One of these is the VIDAS apparatus, which uses an automated enzyme-linked immunosorbent assay technique. This automated immunoassay could be an effective screening tool, since it saves time and labor (11, 24). However, the kit has not been fully validated for its efficiency and accuracy in detecting Campylobacter in various food samples, including fresh vegetables.

In this study, three different selective media, one containing blood (Preston agar) and two blood free (mCCDA and Karmali agar), were compared for their sensitivities and selectivities in detecting C. jejuni. Concurrently, the VIDAS Campylobacter was evaluated by comparing it with the culture method using various combinations of the three selective media, to determine whether this assay could be used as an alternative rapid...
screening tool for the presence of Campylobacter in the same food samples. To determine the effect of background microflora on the detection of Campylobacter jejuni, foods which have different levels of background microflora, ground beef and fresh-cut vegetables, were used as food matrices.

MATERIALS AND METHODS

Bacterial strain. One clinical isolate of C. jejuni, kindly provided by the Centers for Disease Control of Korea, was used in this study. The isolate was streaked onto 5% horse blood agar (Oxoid, Basingstoke, Hampshire, UK) for two passages and incubated in Bolton broth (Oxoid) at 42°C for 48 h. The incubated Bolton broth was serially diluted in phosphate-buffered saline (PBS, pH 7.4) (Sigma, St. Louis, MO), and inocula with optimum numbers of cells (25 to 750 CFU/ml) were used for artificial inoculation. Culturable C. jejuni counts were obtained by plating 100 μl of the inocula onto 5% horse blood agar and incubating at 42°C for 24 h under microaerobic condition (5% O2, 10% CO2, and 85% N2). Colonies were counted, and the extent of inoculation was estimated.

Food samples and artificial inoculation. Ground beef and a mix of fresh-cut vegetables composed of cabbage, cucumber, carrot, and lettuce were purchased from a local retail market in Seoul, South Korea. One-milliliter amounts of the inocula were spiked into 500 g of bulk samples by pipetting to generate partial positives and partial negatives for statistical comparison when divided into 20 subsamples. The inoculated bulk sample was stored at 4°C for 24 h to assimilate a contamination natural in refrigerated foods and subsequently divided into 20 samples of 25 g each. An additional 25 g of uninoculated food sample was used as a negative control. Three independent experiments were conducted with different inoculum levels: 91, 25, and 42 cells per 500 g of ground beef and 750, 635, and 690 cells per 500 g of fresh-cut vegetables.

Enumeration of background microflora in food samples. A mesophilic aerobic plate count was performed on uninoculated food samples. Twenty-five grams of ground beef and fresh-cut vegetables were homogenized with 225 ml of buffered peptone water (Difco, BD, Sparks, MD). The homogenates were serially diluted with PBS, and each dilution was inoculated into nutrient agar (Difco, BD). After overnight incubation at 37°C, the colonies on the agar plates were enumerated.

Detection of C. jejuni by culture method using three selective agar media. Each 25-g sample was put in a sterilized stomacher bag with 100 ml of Bolton broth. After stomaching for 30 s, homogenated samples were preenriched in Bolton broth at 37°C for 4 h and then enriched at 42°C for 44 h. A loopful of enrichment broth was streaked onto three selective media, mCCDA, Karmali agar, and Preston agar, and microaerobically incubated at 42°C for 48 h. For discrimination of C. jejuni and competing flora based on colony morphology, a pure culture of the inoculated strain was streaked in parallel onto the three selective media. A maximum of three suspected colonies (small, round, gray, and shiny) were removed and subcultured on 5% horse blood agar. Colonies on blood agar were screened with the catalase test, oxidase test, and aerobic-growth test. Presumptive isolates were finally confirmed with real-time colony PCR targeting the mapA gene. The specific primers and probe in Best et al. (3) were used.

Detection of Campylobacter by the VIDAS Campylobacter assay. The VIDAS assay (BioMérieux, Inc., Marcy l’Etoile, France) for detection of Campylobacter was assessed in parallel to the culture method. This assay was carried out according to the manufacturer’s recommendations. The VIDAS apparatus was warmed up for 30 min and calibrated for accurate detection prior to the actual analysis. Samples (1 ml each) obtained from the enriched Bolton broth were heated at 100°C for 15 min. The strip and solid-phase receptacle of the VIDAS Campylobacter kit were inserted into the VIDAS apparatus, and then 0.5 ml of the heated sample was added to the strip. The antibody in the solid-phase receptacle reacts with the inserted sample, causing a fluorescence reaction. Samples were analyzed in the VIDAS apparatus for 70 min. Test values above 0.05 as calculated by the following formula were considered positive: test value = sample RFV/standard RFV, where RFV is relative fluorescence value.

Data analysis. With reference to Peterz’s study (25), the numbers of plates positive for C. jejuni and competing organisms were compared for sensitivity and selectivity, respectively. The number of positives was compared in pairs by Fisher’s exact test using GraphPad Instat software (GraphPad Software, Inc., San Diego, CA), and statistical differences are shown. There was considered to be a significant difference if the P value was less than 0.05.

RESULTS AND DISCUSSION

Background levels of microflora in food samples and inoculation levels. The number of background microflora was much higher in fresh-cut vegetables than in ground beef. As determined by aerobic plate counts, the numbers of background microflora were 5.26 log CFU/g and 6.88 log CFU/g in ground beef and fresh-cut vegetables, respectively. On the other hand, 7 to 30 times higher inoculum levels were required in fresh vegetables (635 to 750 CFU/500 g) than in ground beef (25 to 91 CFU/500 g) to generate at least one positive result among 20 subsamples. This indicates that the amounts of background microflora in different samples significantly affect the detection limits of the assays (Table 1, Table 2). Hyeon et al. (11) reported that enrichment of Salmonella spp. was inhibited in fresh vegetables, so that a larger number of bacteria was required for generating partial positives and partial negatives in fresh vegetables than in meats. They attributed this enrichment inhibition to a difference in the number of background microflora, because the number of background microflora was much higher in fresh vegetables than meat. Our data were consistent with their study.

Comparison of three selective media. C. jejuni was not detected in all uninoculated food samples using both the culture method and the VIDAS assay. Therefore, it was concluded that the samples used in the experiments were not naturally contaminated by C. jejuni, excluding possible false positives.

The performance of the three selective media for detecting C. jejuni in ground beef and fresh-cut vegetables is compared in Tables 1 and 2. For ground beef, there were no significant differences (P > 0.05) in the numbers of positives among the Preston agar (54 of 60), mCCDA (54 of 60), and Karmali agar (54 of 60) plates (Table 1).
Meanwhile, for fresh-cut vegetables, Preston agar (15 of 60) performed better than mCCDA (6 of 15) and Karmali agar (11 of 60), although there were no statistical differences (P > 0.05) (Table 2). Preston agar provided better selectivity than the other selective media for all samples, with significant statistical differences (P < 0.05). For ground beef, only 3 plates of Preston agar were contaminated with competing flora but 25 plates of mCCDA and 27 plates of Karmali agar were contaminated (P < 0.05) (Table 1). For fresh-cut vegetables, all of the Karmali and mCCDA plates were contaminated with competing flora, while 39 of 60 plates of Preston agar were contaminated (P < 0.05) (Table 2).

Although it was reported in the previous studies that Karmali agar or mCCDA showed better recovery and selectivity than Preston agar, the evaluation was performed only with human feces (8, 10, 17). It is known that the number of Campylobacter organisms is fairly high and the organisms are under less stress in feces than in food samples (20, 21). On the other hand, Peterz (25) compared Preston agar and mCCDA for their selectivities and sensitivities with chicken liver. In the study, the two media showed similar recovery rates, but contaminants were more inhibited in mCCDA than in Preston agar. However, it is noteworthy that chicken liver is a rather different food matrix and has low background microflora compared with the samples validated in our study. A cefoperazone and activated charcoal combination used in blood-free media such as mCCDA and Karmali agar has been known to provide good recovery rates and to be an effective combination in properly inhibiting normal flora in feces and meat (8, 10). However, blood could be a better toxin neutralizer, especially in vegetables that have a severe and stressful growth environment for Campylobacter (7). Although there was no statistical difference, blood-containing Preston agar detected more positives than blood-free selective media, especially in vegetable samples. Besides, the antibiotic combination in Preston agar could exclude diverse background microflora of fresh meat and vegetables. It was reported that the antimicrobial agents used in mCCDA and Karmali agar were not effective against gram-negative bacteria (8). However, the antibiotics used in Preston agar inhibited not only gram-positive bacteria (rifampin-trimethoprim) but also gram-negative bacteria (polymyxin B) (8). It is possible that this wide spectrum of antibiotic activity made Preston agar more selective than other media.

Table 2. Comparison of plates positive for C. jejuni and competing flora on three selective media with 20 ground beef samples in each trial

<table>
<thead>
<tr>
<th>Trial</th>
<th>Inoculation level (CFU/500 g)</th>
<th>No. of plates with C. jejuni (sensitivity)*</th>
<th>No. of plates with competing flora (selectivity)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mCCDA</td>
<td>Karmali agar</td>
<td>Preston agar</td>
</tr>
<tr>
<td>First</td>
<td>91</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Second</td>
<td>25</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Third</td>
<td>42</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>54 A</td>
<td>54 A</td>
<td>54 A</td>
</tr>
</tbody>
</table>

* Different letters (A, B) within a row indicate a significant difference (P < 0.05) in sensitivities. ** Different letters (C, D) within a row indicate a significant difference (P < 0.05) in selectivities.

Comparison of various medium combinations and the VIDAS Campylobacter. Some of the medium combinations and the VIDAS assay are compared in Table 3. No statistical differences in the numbers of positives were observed between all of the selective agar combinations and the VIDAS assay for ground beef (P > 0.05) (Table 3). In fresh-cut vegetables, however, the VIDAS assay yielded the best results (29 of 60) for the detection of Campylobacter, followed by the three-medium combination of mCCDA, Karmali, and Preston agar (21 of 60) (Table 3). Unlike ground beef, there was a statistical difference between the VIDAS assay and the culture method for fresh-cut vegetables (P < 0.05) when fewer than three media were used, except in the case of the mCCDA and Preston agar combination (19 of 60) (Table 3). Considering that one or two selective media are routinely used for Campylobacter detection, VIDAS Campylobacter could be...

Table 3. Comparison of plates positive for C. jejuni and competing flora on three selective media with 20 fresh-cut vegetable samples in each trial

<table>
<thead>
<tr>
<th>Trial</th>
<th>Inoculation level (CFU/500 g)</th>
<th>No. of plates with C. jejuni (sensitivity)*</th>
<th>No. of plates with competing flora (selectivity)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mCCDA</td>
<td>Karmali agar</td>
<td>Preston agar</td>
</tr>
<tr>
<td>First</td>
<td>750</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Second</td>
<td>635</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Third</td>
<td>690</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>6 A</td>
<td>11 A</td>
<td>15 A</td>
</tr>
</tbody>
</table>

* Different letters (A, B) within a row indicate a significant difference (P < 0.05) in sensitivities. ** Different letters (C, D) within a row indicate a significant difference (P < 0.05) in selectivities.
TABLE 3. Comparison of plates positive for C. jejuni using different medium combinations and the VIDAS assay in 60 samples in three trials

<table>
<thead>
<tr>
<th>Medium or combination or test</th>
<th>Ground beef</th>
<th>Fresh-cut vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCCDA</td>
<td>54 A</td>
<td>6 A</td>
</tr>
<tr>
<td>Karmali</td>
<td>54 A</td>
<td>11 AB</td>
</tr>
<tr>
<td>Preston</td>
<td>54 A</td>
<td>15 AB</td>
</tr>
<tr>
<td>mCCDA + Karmali</td>
<td>54 A</td>
<td>14 AB</td>
</tr>
<tr>
<td>Preston + Karmali</td>
<td>55 A</td>
<td>17 BC</td>
</tr>
<tr>
<td>mCCDA + Preston</td>
<td>55 A</td>
<td>19 BCD</td>
</tr>
<tr>
<td>mCCDA + Karmali + Preston</td>
<td>55 A</td>
<td>21 BCD</td>
</tr>
<tr>
<td>VIDAS Campylobacter</td>
<td>51 A</td>
<td>29 D</td>
</tr>
</tbody>
</table>

a Different letters (A, B, C, D) within a column indicate a significant difference (P < 0.05).

a more reliable tool than the culture method, especially for vegetable samples. The VIDAS assay has not been known to cross-react with other competing bacteria (29), so the detection ability of the assay could be better than that of the culture method, especially in food samples that have high and diverse background microflora. Although VIDAS Campylobacter could not differentiate Campylobacter species, this assay could be used as an effective and sensitive presumptive screening tool, considering its efficiency and good detection ability (24).

The detection ability of these methods could be altered by the experimental conditions. The information generated by this study is limited because only one isolate and one detection protocol were used. If a shortened enrichment time (~20 to 24 h), which has been used in other studies (21, 25), was applied, the effect of background microflora might be much less because the long incubation time (~44 to 48 h) in this study may result in the overgrowth of the competing bacteria.

Although fresh vegetables have been regarded as a minor cause of campylobacteriosis, fresh produce was second only to dairy products or chicken meats as a risk factor in some epidemiological studies conducted in United States or United Kingdom (5, 9). Furthermore, fresh vegetables could be a notable cause of campylobacteriosis illness because of unheated preparation processes, low infectious dose, and cross-contamination (6, 7, 14). However, there have been no appropriate detection methods for Campylobacter, especially in fresh vegetables (7). It is suggested that a more sophisticated and precise detection method should be developed and evaluated in all food types which could cause campylobacteriosis.

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