Short communication

Improvement of modified charcoal-cefoperazone-deoxycholate agar by addition of potassium clavulanate for detecting *Campylobacter* spp. in chicken carcass rinse

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ARTICLE INFO

ABSTRACT

The presence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) in raw poultry is one of the most common factors that interfere with the isolation of *Campylobacter* by cefoperazone-based selective agar. The performance of modified charcoal-cefoperazone-deoxycholate agar (mCCDA) was improved by addition of an ESBL inhibitor, potassium clavulanate (0.5 mg/L). The ability of the supplemented medium (C-mCCDA) to detect *Campylobacter* species from chicken carcass rinse was compared with that of normal mCCDA. The isolation rate using C-mCCDA was significantly ($p < 0.05$) higher compared with that using mCCDA (C-mCCDA, 67 out of 120; mCCDA, 38 out of 120). Furthermore, the selectivity of the C-mCCDA as assessed by comparing the number of contaminated plates (C-mCCDA, 44 out of 120; mCCDA, 110 out of 120) and growth index (C-mCCDA, 1.76; mCCDA, 2.79) of competing flora was also better ($p < 0.05$) than that of mCCDA.

1. Introduction

Modified charcoal-cefoperazone-deoxycholate agar (mCCDA) along with other selective media has been used to detect *Campylobacter* species in poultry meat samples (Corry et al., 1995; Oyarzabal et al., 2005; Moran et al., 2009). Most of the currently available *Campylobacter* selective media contain several antibiotic agents to eliminate competing flora in poultry carcasses (Corry et al., 1995). Cefoperazone, a third-generation cephalosporin, is the most commonly used antibiotic supplement in many *Campylobacter* media. Most *Campylobacter* selective media such as mCCDA, Campy-Cefex agar, and Karmali agar that are frequently used by food authorities are supplemented with a high concentration of cefoperazone (Corry et al., 1995; Oyarzabal et al., 2005).

However, cefoperazone resistance in bacteria has recently become more widespread, making it difficult to isolate *Campylobacter* spp. from raw poultry meat (Jasson et al., 2009; Moran et al., 2011; Chon et al., 2012a, 2013). Extended-spectrum beta-lactamase (ESBL) is an enzyme produced by bacteria that renders cephalosporin resistance (Warren et al., 2008). In many countries, ESBL-producing *Escherichia coli* (*E. coli*) strains resistant to cefoperazone have been frequently isolated from raw chicken (Warren et al., 2008; Costa et al., 2010; Leverstein-van Hall et al., 2011; Moran et al., 2011). Previous studies have reported that ESBL-producing *E. coli* may overgrow on mCCDA and Campy-Cefex

agar media supplemented with cefoperazone, making it difficult to differentiate and isolate suspected *Campylobacter* colonies (Moran et al., 2011; Chon et al., 2012a, 2012b, 2013). Therefore, elimination of ESBL-producing *E. coli* by using a novel approach could tremendously increase the sensitivity and selectivity of *Campylobacter* selective agar.

In their study, Moran et al. (2011) showed that addition of an ESBL inhibitor, potassium clavulanate, improved the sensitivity and selectivity of Bolton broth containing cefoperazone. Thus far, no published study has discussed the inclusion of potassium clavulanate in cefoperazone-supplemented agar plate medium.

mCCDA is one of the most commonly used selective media for the isolation of *Campylobacter* from poultry meat by many food authorities, including the Food and Drug Administration (FDA) and the International Organization for Standardization (ISO 10272-1:2006) (Odongo et al., 2009; Moran et al., 2009). However, the sensitivity and selectivity of mCCDA has been hampered by widespread growth of ESBL-producing *E. coli* contaminating the medium (Jasson et al., 2009; Moran et al., 2011; Chon et al., 2012a, 2012b). The aim of this study was to evaluate the detection ability of potassium clavulanate-supplemented mCCDA to isolate *Campylobacter* spp. from whole chicken carcass rinse.

2. Materials and methods

2.1. Bacterial strains

A total of 40 strains of *Campylobacter* (*C. jejuni*: NCTC 11168, 5 human isolates, and 14 food isolates; *C. coli*: 8 human isolates and

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12 food isolates) were used in the present study. All clinical strains isolated from human stools were kindly provided by the Korea Centers for Disease Control and Prevention (Cheongwon, South Korea). Food strains were isolated from meat products in our laboratory over 2009 and 2010. All cultures were kept frozen at −70 °C until use; these cultures were streaked onto Columbia blood agar (Oxoid, Hampshire, U.K.) with 5% laked horse blood (Oxoid), and then incubated under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) at 42 °C for 48 h for two passages.

2.2. Preparation of mCCDA and C-mCCDA

mCCDA plates were prepared according to the manufacturer's recommendations obtained with the agar base (Oxoid) and antibiotic supplement (Oxoid). Modified mCCDA plates were prepared by adding 0.5 mg of potassium clavulanate (Sigma, St. Louis, MO) to 1 L of normal mCCDA. Plates were prepared, refrigerated (4 °C), and used within 5 days.

2.3. Isolation of pure strains on mCCDA and C-mCCDA

To evaluate the growth of Campylobacter strains on selective media, approximately 10^4 cells of each of 40 Campylobacter strains suspended in physiological saline were streaked on mCCDA and C-mCCDA plates. All plates were then microaerobically incubated at 42 °C for 48 h.

2.4. Detection of Campylobacter in chicken carcass rinse

A total of 120 whole chickens were purchased from February to May 2012 from six different retail stores (20 samples per retailer) in Seoul, South Korea. All experimental procedures for detecting Campylobacter spp. were performed as described in our previous study (Chon et al., 2013). Briefly, chicken carcasses were placed in 400 mL of buffered peptone water (Oxoid) and rinsed by gentle shaking for 1 min. The rinse fluid (25 mL) was enriched with equal quantity of 2× blood-free Bolton enrichment broth (Oxoid). Each sample was incubated at 42 °C for 48 h under microaerobic conditions. A loopful of the enriched broth was streaked onto mCCDA and C-mCCDA plates, and then incubated microaerobically at 42 °C for 48 h. Suspected colonies were sub-cultured on Columbia blood agar and confirmed by colony PCR, as described by Denis et al. (1999).

2.5. Identification of competing flora on plates

To study the frequent competing flora on two selective media, we identified 20 contaminant colonies from 10 each of mCCDA and C-mCCDA agar plates by using the Vitek 2 GN kit (bioMérieux, Marcy l'Etoile, France). ESBL enzyme production test was performed by using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (2005).

2.6. Data analysis

The effectiveness of a selective agar is determined on the basis of its ability to restrict the overgrowth of competing flora while allowing the growth of target organisms (Ahmed et al., 2012). We compared the number of plates of Campylobacter and competing organisms (non-Campylobacter) in terms of isolation rate and selectivity, as described in our previous studies (Chon et al., 2011, 2012a, 2012b). The number of positive plates for Campylobacter or competing flora was compared using Fisher's exact test. Statistical analysis was carried out using GraphPad Instat software (GraphPad Software, Inc. San Diego, CA).

Selectivity was also evaluated on the basis of the growth index of the competing flora (1, growth of a few colonies; 2, growth of colonies on approximately half of the plate; and 3, growth of colonies on most of the plate), according to the scales described in our previous study (Chon et al., 2012a). The growth index was determined and the average was calculated only in contaminated media with competing flora.

3. Results and discussion

3.1. Recovery of Campylobacter strains on mCCDA and C-mCCDA in pure cultures

We inoculated 40 Campylobacter strains (20 C. jejuni and 20 C. coli) from various sources on two selective media to determine if C-mCCDA is appropriate as a selective agar for the detection of Campylobacter without any adverse effect on the growth of either C. jejuni or C. coli. All tested Campylobacter strains grew on mCCDA and C-mCCDA, indicating identical sensitivity for the two selective media.

Moran et al. (2011) assessed the addition of potassium clavulanate into Bolton broth by using three different concentrations (0.5, 2.0, and 10.0 mg/L) and the optimal concentration was found to be 2.0 mg/L. In preliminary study, we first determined the optimal concentration of potassium clavulanate to be added to mCCDA that would not affect the growth of the Campylobacter strains tested in this study. We found that the growth of some strains was inhibited on mCCDA at 2 mg/L potassium clavulanate (data not shown). Based on further standardization experiments, our results indicate that the optimal concentration of potassium clavulanate to be added to mCCDA is 0.5 mg/L.

3.2. Isolation rate and selectivity of the two media for detecting Campylobacter in chicken carcass rinse

The number of C-mCCDA plates (55.8%, 67 out of 120) positive for Campylobacter was significantly higher ($p < 0.05$) than that of mCCDA plates (31.7%, 38 out of 120, Table 1). Further, fewer ($p < 0.05$) C-mCCDA plates (36.7%, 44 out of 120) were contaminated than mCCDA (91.7%, 110 out of 120) (Table 1). C-mCCDA also exhibited a much lower growth index of competing flora (C-mCCDA, 1.76; mCCDA, 2.79; Table 1). These results indicate that C-mCCDA provides better isolation rate and selectivity than normal mCCDA for detecting Campylobacter in naturally contaminated chicken rinse.

The growth of the colonies on each selective agar is shown in Fig. 1. On mCCDA plate (Fig. 1A), differentiation and picking of suspected colonies was significantly hindered by dense competing microflora occupying the entire plate. Detection of Campylobacter species was much easier on C-mCCDA (Fig. 1B) because of the reduction of the competing microflora.

Table 1

Comparison of the growth of Campylobacter and competing flora on two different media on the basis of the number of positive plates and growth index.

	mCCDA agar	C-mCCDA agar
Number of positive plates for Campylobacter / total number of plates tested (%) [a]	38 / 120 (31.7%) A	67/120 (55.8%) B
Number of positive plates for competing flora / total number of plates tested (%) [a]	110 / 120 (91.7%) A	44/120 (36.7%) B
Growth index of competing flora [b]	2.79	1.76

[a] Different letters (A, B) within a row indicate a significant difference ($p < 0.05$) in the number of positive samples.
[b] 1, growth of a few colonies; 2, growth of colonies on about half of the plate; 3, growth on most of the plate.