International Journal of Food Microbiology 223 (2016) 75–78

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Evaluation of cephamycins as supplements to selective agar for detecting *Campylobacter* spp. in chicken carcass rinses

Jung-Whan Chon [a], Young-Ji Kim [b], Hong-Seok Kim [b], Dong-Hyeon Kim [b], Hyunsook Kim [c], Kwang-Young Song [b], Kidon Sung [a], Kun-Ho Seo [b,*]

[a] Division of Microbiology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079, USA
[b] Center for One Health, College of Veterinary Medicine, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul 05029, Republic of Korea
[c] Department of Food & Nutrition College of Human Ecology, Hanyang University, 222 Wangsimni-ro, Seongdong-gu, Seoul 04763, Republic of Korea

ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form 30 November 2015
Accepted 25 January 2016
Available online 28 January 2016

Keywords:
Campylobacter
Cefotetan
Cefoxitin
ESBL
Chicken

ABSTRACT

Although cefoperazone is the most commonly used antibiotic in *Campylobacter*-selective media, the distribution of cefoperazone-resistant bacteria such as extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* is increasing. Here we evaluated the potential of cephamycins for use as supplements to improve modified charcoal-cefoperazone-deoxycholate agar (mCCDA) by replacing cefoperazone with the same concentrations (32 mg/L) of cefotetan (modified charcoal-cefotetan-deoxycholate agar, mCCtDA) and cefoxitin (modified charcoal-cefoxitin-deoxycholate agar, mCCxDA). In chicken carcass rinse samples, the number of mCCDA plates detecting for *Campylobacter* (18/70, 26%) was significantly lower than that of mCCtDA (42/70, 60%) or mCCxDA plates (40/70, 57%). The number of mCCDA plates (70/70, 100%) that were contaminated with non-*Campylobacter* species was significantly higher than that of mCCtDA (20/70, 29%) or mCCxDA plates (21/70, 30%). The most common competing species identified using mCCDA was ESBL-producing *E. coli*, while *Pseudomonas* species frequently appeared on mCCtDA and mCCxDA.

© 2016 Published by Elsevier B.V.

1. Introduction

Various cephalosporins such as cephalothin and cefazolin were typically used in *Campylobacter*-selective media such as Blaser agar, Butzler agar, and Campy-BAP agar (Corry et al., 1995). However, the 3rd-generation cephalosporin cefoperazone is a superior antibacterial agent than other cephalosporins (Corry et al., 1995; Hutchinson and Bolton, 1984; Karmali et al., 1986). The cefoperazone is commonly used in *Campylobacter*-selective media such as modified charcoal-cefoperazone-deoxycholate agar (mCCDA) and Campy-cefex agar, which are commonly recommended by many food safety authorities (Corry et al., 1995).

However, cefoperazone-resistant bacteria are widespread especially in poultry samples. In studies conducted in Asian and European countries, extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) strains resistant to cefoperazone are the most common interfering bacteria in the selective isolation of *Campylobacter* from raw poultry meat (Chon et al., 2013; Chon et al., 2014; Hayashi et al., 2013; Jasson et al., 2009; Moran et al., 2011; Yoo et al., 2014). The resistant bacteria grew predominately on *Campylobacter* selective agar, leading to false-negatives and making it difficult to isolate suspect *Campylobacter* colonies (Chon et al., 2013; Jasson et al., 2009; Moran et al., 2011; Yoo et al., 2014).

Cephamycins are beta-lactam antibiotics that are more stable than other cephalosporins, because the 7-alpha-methoxy group imparts resistance to beta-lactamases such as ESBL (Birnbaum et al., 1978; Ohya et al., 1983; Rupp and Fey, 2003). Cephamycins are broadly active against gram-negative and gram-positive bacteria, and *Campylobacter* is highly resistant to cephamycins such as cefoxitin (Birnbaum et al., 1978; Lachance et al., 1993). Thus, cephamycins show great potential as antibacterial agents for the detection of *Campylobacter*-selective in the agar. In the present study, we modified mCCDA by replacing cefoperazone with the same concentration of cefotetan or cefoxitin. The isolation rate and selectivity of the modified media for identifying *Campylobacter* in chicken carcass rinses were evaluated by comparing them with those of mCCDA containing cefoperazone.

2. Materials and methods

2.1. Bacterial strains

The *Campylobacter* strains studied here were as follows: 12 *Campylobacter jejuni* strains—ATCC 33560, *CCA_2*, *CCA_4*, *CCA_8*, *Clin_6*, *Clin_7* [human isolates], *DD_3*, *GGD_3*, *GD_1*, *GGD_1*, *E_G2P*, *G_B4P* [chicken isolates] and 13 *Campylobacter coli* (*C. coli*) strains—ATCC 33559, *CCA_1*, *CCA_9*, *CCA_10*, *CCA_11*, *CCA_12* [human isolates], *DD_2*, *GD_2C*,

* Corresponding author.
E-mail address: bracstu3@konkuk.ac.kr (K.-H. Seo).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.01.019
0168-1605/© 2016 Published by Elsevier B.V.

CCH_GDC2, *SD1*, *C_2P3* [chicken isolates], *DDP_3* [porcine isolate], *C_CAM* [canine isolate]). Clinical strains isolated from sporadic cases of food poisoning were kindly provided by the Korea Centers for Disease Control and Prevention (Cheongju, South Korea). All *Campylobacter* strains isolated from animals were from our own repository and were collected from 2009–2011.

A total of 28 non-*Campylobacter* strains [10 ESBL producing *E. coli* (isolated from meats; *e4*, *e6*, *e9*, *e10*, *e12*, *12-3*, *c15*, *c24*, *c47*, and *c52*; kindly provided by Dr. Young-Jo Kim), *E. coli* ATCC 98222, *Salmonella* Enteritidis 22079, *Salmonella* Bredeney KD, *Shigella sonnei* ATCC 9290, *Yersinia enterocolitica* ATCC 27729, *Listeria monocytogenes* H7858, *Citrobacter freundii* ATCC 8090, *Enterobacter durans* ATCC 6056, *Bacillus subtilis* ATCC 6051, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 35984, *Lactobacillus salivarius* ATCC 11741, *Pseudomonas aeruginosa* ATCC 7853, *Pseudomonas aeruginosa* PAO1, *Proteus mirabilis* ATCC 7002, two *Acinetobacter* spp. (*ACB911* and *ACB3*; kindly provided by Dr. Mohamed Nawaz), and *Ochrobactrum anthropi* (*HAC7*; kindly provided by Dr. Mark Hart)] were used in this study.

The *Campylobacter* cultures were streaked onto brain–heart infusion agar (Difco, Sparks, MD, USA) with 5% laked horse blood (BHI-blood agar; Oxoid, Hampshire, UK) followed by incubation at 42 °C for 24–48 h in a microaerobic atmosphere containing 5% O_2, 10% CO_2, and 85% N_2). The non-*Campylobacter* cultures were streaked onto trypticase soy agar with 5% sheep blood (TSA-blood agar; Difco) followed by incubation at 37 °C for 24 h.

2.2. Preparation of standard and modified versions of mCCDA

Standard mCCDA plates were prepared according to the manufacturer's recommendations for the agar base (Oxoid) supplemented with 32 mg/L of cefoperazone (MP Biomedicals, Solon, OH, USA) and 10 mg/L of amphotericin (Sigma-Aldrich, St. Louis, MO, USA). For the preparation of modified charcoal-cefotetan-deoxycholate agar (mCCtDA) and modified charcoal-cefoxitin-deoxycholate agar (mCCxDA), the 32 mg/L of cefotetan (Sigma-Aldrich) or cefoxitin (Sigma-Aldrich) was substituted for cefoperazone in the same plates.

2.3. Recovery of pure Campylobacter cultures on standard and modified mCCDAs

To evaluate the growth of pure *C. jejuni* and *C. coli* strains on selective media, a single colony of each of the 12 *C. jejuni* and 13 *C. coli* strains was picked from blood agar and streaked onto mCCDA, mCCtDA, and mCCxDA plates, followed by incubation at 42 °C for 48 h under microaerobic conditions.

2.4. Growth of non-Campylobacter cultures on standard and modified mCCDAs

Non-*Campylobacter* strains were used to determine the exclusivity of the original and modified media. Approximately 10^4 cells of each strain were inoculated onto three selective agar plates followed by incubation at 37 °C for 72 h and 42 °C for 48 h under microaerobic conditions.

2.5. Detection of Campylobacter in chicken carcass rinses

Seventy chickens produced by four different integrated broiler chicken operations were purchased from August to September 2014 (10–20 chickens from each brand). Chickens were collected from four retailers located in the *Gwangjin* region of Seoul. Briefly, chicken carcasses were placed in 400 mL of buffered peptone water (Oxoid) and rinsed for 1 min with gentle shaking. The rinse fluid (25 mL) was enriched with an equal volume of 2× blood-free Bolton enrichment broth (Oxoid). Each sample (50 mL) was incubated at 42 °C for 48 h under microaerobic conditions. A loopful of each of the enriched cultures was streaked onto a mCCDA, mCCtDA, or mCCxDA plate, which were then incubated at 42 °C for 48 h under microaerobic condition. Suspective colonies were subcultured on BHI-blood agar and identified using colony PCR (Yamazaki-Matsune et al., 2007).

2.6. Identification of competing flora

We identified 30 contaminating colonies from 10 plates each of mCCDA, mCCtDA, and mCCxDA to determine the frequencies of competing bacteria from growing on each of the selective media. Ten bacterial cultures from each of three selective agar plates were chosen at random from the plates contaminated with non-*Campylobacter* colonies. A single colony was subcultured on tryptic soy agar (Oxoid) or blood agar (Oxoid). Identification of these bacteria was conducted using the Vitek 2 assay (BioMérieux, Marcy l'Etoile, France). The presence of ESBL in the bacterial strains was detected using the disk diffusion method according to the protocol published by the Clinical and Laboratory Standards Institute (2013).

2.7. Data analysis

To determine the isolation rate and selectivity, we compared the number of agar plates positive for *Campylobacter* spp. and non-*Campylobacter*, respectively (Chon et al., 2013). The number of positive plates was compared using Fisher's exact test as implemented in GraphPad InStat software (GraphPad Software, Inc., San Diego, CA, USA). Selectivity was also evaluated according to the growth index of the competing flora (1, growth of a few colonies; 2, growth of colonies on approximately half the plate; and 3, growth of colonies on most of the plate; Chon et al., 2013). The growth index was determined, and the average was calculated only for contaminated media.

3. Results and discussion

3.1. Recovery of Campylobacter strains from mCCDA, mCCtDA, and mCCxDA

To determine if *Campylobacter* strains from various sources grew on modified media, 25 *Campylobacter* strains (12 *C. jejuni* and 13 *C. coli*) were inoculated onto each of the three selective agars. All *Campylobacter* strains grew on mCCDA, mCCtDA, and mCCxDA. Our findings indicate that mCCtDA and mCCxDA support the growth of *Campylobacter* at 32 mg/L of cefotetan and cefoxitin, respectively. It appears that both mCCtDA and mCCxDA may be useful for selective isolation of *Campylobacter*.

3.2. Growth of non-Campylobacter strains from mCCDA, mCCtDA, and mCCxDA

The contamination on mCCDA and other cefoperazone containing-*Campylobacter* media with other microorganism such as *Ochrobactrum* spp., *Acinetobacter* spp., *Proteus* spp., and *Lactobacillus salivarius* as well as *E. coli* and *Pseudomonas* spp., is of great interest when isolating thermophilic *Campylobacter* spp. from fecal, environmental, and food samples (Ahmed et al., 2012; Baylis et al., 2000; Chon et al., 2012; Hu and Kuo, 2011; Vaz et al., 2014; Yoo et al., 2014). The selectivity of *Campylobacter* media by the addition of other antibacterial agents against those competing floras was addressed in this research. We also tested 10 ESBL-producing *E. coli*, generic *E. coli*, and other pathogenic or non-pathogenic bacteria that might grow on *Campylobacter* selective media.

The growth of ESBL-producing strains was inhibited more frequently on mCCtDA and mCCxDA (10 out of 10 strains were inhibited) than on standard mCCDA (0 out of 10 strains were inhibited) (Table 1) in both incubation conditions. mCCDA was more inhibitory against other tested bacteria including *Pseudomonas*, *Acinetobacter*, *Ochrobactrum*, and

Download English Version:

https://daneshyari.com/en/article/4366343

Download Persian Version:

https://daneshyari.com/article/4366343

Daneshyari.com