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Comparing Campy Cefex with *Campylobacter jejuni/C. coli* Chromogenic Plating Medium for Isolating *Campylobacter jejuni* and *Campylobacter coli* from Raw Poultry

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ABSTRACT

Introduction: Greater than 99% of foodborne campylobacteriosis are caused by *Campylobacter jejuni* and *Campylobacter coli*. Campy-Cefex agar (CCA), listed in the USDA Microbiology Laboratory Guidebook for isolating *Campylobacter* from poultry, lacks differentiation for *C. jejuni* and *C. coli* and allows a substantial number of background microbes to grow. In 2012, a *C. jejuni/C. coli* chromogenic plating medium (CCPM) was developed that differentiates these two *Campylobacter* species from other closely related bacteria with an improved selectivity.

Purpose: To compare CCPM with CCA for isolating *C. jejuni* and *C. coli* artificially inoculated into ground raw chicken and turkey.

Methods: *C. jejuni* (ATCC BAA-1153) and *C. coli* (ATCC 43478) and various microbes used as the background flora were diluted, added to stomacher bags containing raw ground poultry, and hand massaged. Isolation and confirmation of *C. jejuni* and *C. coli* followed the qualitative procedures as outlined in the USDA MLG with the modification of Bolton broth with selective agents added directly to the 140 poultry meat samples at 1:10 dilution.

Results: At low dilution levels (0.70 to 1.50 CFU/g for *C. jejuni* and 0.70 to 0.84 CFU/g for *C. coli*), CCPM isolated these *Campylobacter* sp. 97.5% of the inoculated samples; whereas, CCA detected these microbes in 53.8% of the poultry samples. For inoculation levels ranging from 3 to 8 CFU/g, the incidence of isolation of *C. jejuni* and *C. coli* in the poultry samples were 100.0 and 93.3% for CCPM and CCA, respectively. For CCPM, 83.3% of the positive samples at low detection levels required one colony to be picked for confirmation; whereas, 58.5% of the positive samples on CCA were first picked isolates.

Significance: CCPM isolated *C. jejuni/C. coli* inoculated at low detection levels in raw ground poultry nearly twice the frequency compared with CCA. The ease of isolation (detection and plating efficiency, reduce technician time and fatigue, and cost savings) was substantially better with CCPM.

INTRODUCTION

Campylobacter is regarded a prominent pathogen responsible for food-related gastroenteritis worldwide. Most cases of campylobacteriosis are due to the consumption and handling of raw poultry products and/or cross contamination with these products (1).

Current USDA protocol for isolating *C. jejuni* and *C. coli* from food products, poultry carcass rinses and sponges (carcass and environmental) utilizes Campy Cefex agar (CCA) as the primary medium for direct plating and after enrichment (4). Although CCA is recommended by the USDA, a substantial amount of background microflora contamination occurs coupled with the lack of differentiation make isolating *Campylobacter* difficult. R & F *Campylobacter jejuni/C.coli* Chromogenic Plating Medium (CCPM) was developed to solve these two problems.

The purpose of this investigation is to compare CCA (Acumedia, Lansing, MI) with CCPM (R & F Products, Downers Grove, IL) for isolating *C. jejuni* and *C. coli* artificially inoculated into ground raw poultry samples.

MATERIALS/METHODS

Bacterial strains and inoculum preparation. *C. jejuni* ATCC BAA-1153 and *C. coli* ATCC 43478 were inoculated into ground raw chicken and turkey to evaluate the efficacies of CCA and CCPM. Both *Campylobacter* sp. were separately stored in 10% skim milk at -77°C and thawed.

Microbial cultures used as the background flora included *Salmonella enterica* serovar Typhimurium ATCC 14028, *Klebsiella pneumoniae* ATCC 4352, *Acinetobacter* sp. (isolated from powdered infant formula), *Escherichia coli* ATCC 25922, *Citrobacter freundii* (Isolated from pork sausage), *Pseudomonas aeruginosa* ATCC 15442 and *Enterobacter aerogenes* ATCC 29751. Cultures were stored on brain heart infusion agar slants at 2-8°C and transferred bimonthly.

Inoculum level determination for inoculating raw poultry. *Campylobacter* frozen cultures were transferred by adding 40 µl of each strain to 10 ml of modified Bolton broth and incubated at 42°C for 48 h under microaerophilic conditions. The cultures were serially diluted in saline and appropriate 0.1 ml volumes were spread plated in triplicate on modified Bolton plates and incubated as mentioned previously. Culture broths were stored at 2-8°C for 48 h prior to inoculating the raw poultry. The *Campylobacter* concentrations in the broth determined the dilution scheme needed for inoculating the ground poultry.

Background microbial cultures were grown in 4 ml of brain heart infusion broth at 35°C for 24 h. After incubation, the cultures yielded cell concentration approximately 5.0 X 10^8 which determined the dilution scheme.

Inoculation of ground chicken and turkey. Ground raw chicken and turkey were purchased locally and refrigerated at 2-8°C and tested within 24 h. Three 200 g segments were weighted and placed in separate sterile stomacher bags.

The refrigerated *Campylobacter* cultures were serially diluted in saline and appropriate volumes were added to two of the stomacher bags to achieve high and low target detection levels. In addition, the background microbial cultures were diluted and added to all 3 stomacher bags yielding cell concentrations as follows: *S. enterica* serovar Typhimurium 10^1-10^2 cells/g; *E. coli/E. aerogenes* 10^3-10^4 cells/g; and *Acinetobacter sp./P. aeruginosa/C. freundii/K. pneumoniae* 10^6-10^7 cells/g. The contents of the stomacher bags were hand massaged for at least 3 min.

Isolation and confirmation of *C. jejuni* and *C. coli*. The 200 g samples of each trial were sub-divided into 11g segments in stomacher bags. To each, 99 ml of Bolton broth (containing the selective ingredients) were added and the contents mechanically mixed for 2 min. The top of the bag was folded and incubated at 42°C for 48 h under microaerophilic conditions. After incubation, the *Campylobacter* un-inoculated and both inoculated ground poultry enrichment samples were streaked onto CCA and CCPM plates and incubated under microaerophilic conditions at 42°C for 48 h. The plates were analyzed for growth where presumptive positive *C. jejuni/C. coli* colonies appeared yellow-gray, round convex and 1-2 mm in diameter on CCA and salmon colored, round, convex and 1-1.5 mm in diameter on CCPM. Up to 3 presumptive colonies per plate were analyzed using motility and latex agglutination for confirmation.

Table 1. Comparing two selective plating media for isolating *Campylobacter jejuni* and *Campylobacter coli* artificially inoculated at low detection levels in ground turkey and chicken[a].

	Inoculation range	
	Campylobacter jejuni	*Campylobacter coli*
Media	0.78 to 1.5 CFU/g	0.70 to 0.84 CFU/g
CCPM[b]	40/40 (100%)	38/40 (95%)
CCA[c]	21/40 (53%)	22/40 (55%)

[a] Up to 3 presumptive colonies were picked for isolation of *C. jejuni/C. coli* per plating medium for each sample.
[b] R & F® *Campylobacter jejuni/C. coli* Chromogenic Plating Medium.
[c] Campy Cefex Agar.

Table 3. One colony needed for isolating *Campylobacter jejuni* and *Campylobacter coli* inoculated at low detection levels in ground turkey and chicken[a].

Media	First pick isolation/ total positive samples
CCPM[b]	65/78 (83.3%)
CCA[c]	24/41 (58.5%)

[a] Low detection levels for *C. jejuni* and *C. coli* were 0.70 to 1.5 CFU/g and 0.70 to 0.84 CFU/g, respectively.
[b] R & F® *Campylobacter jejuni/C. coli* Chromogenic Plating Medium.
[c] Campy Cefex Agar.

RESULTS/DISCUSSION

Comparison of CCPM and CCA for isolating *C. jejuni* and *C. coli* artificially inoculated at low and high detection levels are presented in Tables 1 and 2. At the low detection levels (0.70 to 1.50 CFU/g for *C. jejuni* and 0.70 to 0.84 CFU/g for *C. coli*), CCPM isolated *C. jejuni/C. coli* from 97.5% of the 11 g samples, whereas, CCA detected these organisms in only 53.8% ofthe samples. These data support the investigations performed by Gharst et al. and Steininger et al. (2,3). Using low numbers of naturally contaminated *Campylobacter* spp. on broiler carcass rinses, 90-100% positive samples were detected with CCPM, whereas, 10% positives were isolated using CCA (2). With raw silo milk and *C. jejuni* and *C. coli* inoculated at just 5 CFU/25 g, Gharst et al (2). reported 5% positive samples on modified cefoperazone charcoal deoxycholate agar (FDA's counterpart to USDA's CCA), but 100% positive samples using CCPM.

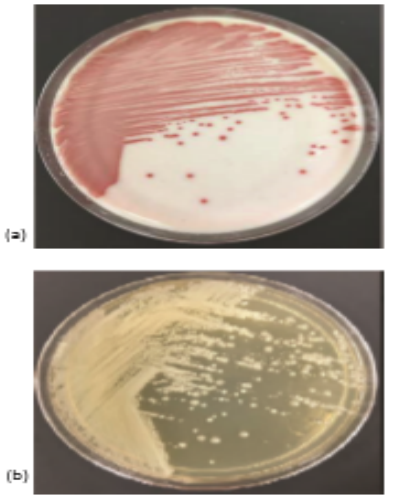
For inoculation levels ranging from 3 to 8 CFU/g, the percent isolation of *C. jejuni/C. coli* in the ground turkey and chicken were 93.3 and 100.0% for CCA and CCPM, respectively (Table 2). At the higher inoculation levels the two media performed similarly.

Another important attribute for any selective/differential plating medium is the ease of isolating for the sought after pathogen which will improve detection and plating efficiency, reduce technician time and fatigue, and ultimately save costs. In this study, Figure 1 shows typical CCA and CCPM plates after incubation which CCPM contained little to no background microflora and CCA usually contained a mixture of colonial morphologies without differentiation. If *C. jejuni/C. coli* can be isolated the vast majority of the time after just examining one presumptive colony/plate, the above mentioned criteria should be accomplished. For CCPM, 83.3% of the positive samples for the low detection levels required only one colony to be picked for a positive result, whereas, only 58.5% of the positive samples on CCA were first pick isolation (Table 3).

Table 2. Comparing two selective plating media for isolating *Campylobacter jejuni* and *Campylobacter coli* artificially inoculated at high detection levels in ground turkey and chicken[a].

	Inoculation range	
	Campylobacter jejuni	*Campylobacter coli*
Media	3.1 to 5.6 CFU/g	7.0 to 8.7 CFU/g
CCPM[b]	40/40 (100%)	28/28 (100%)
CCA[c]	36/40 (90%)	28/28 (100%)

Refer to Table 1.



(a)

(b)

Figure 1. Typical colony isolation of inoculated raw poultry samples struck onto CCPM (a) and CCA (b)

CONCLUSIONS

1. CCPM isolated *C. jejuni/C. coli* inoculated in ground turkey and chicken at low detection levels nearly twice the frequency compared to CCA.
2. The ease of isolating *C. jejuni* and *C. coli* was substantially better with CCPM versus CCA.

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